NOTICE PERTINENT TO THE SEPTEMBER 2009 REVISIONS
OF THE
NIH GUIDELINES FOR RESEARCH INVOLVING
RECOMBINANT DNA MOLECULES
(NIH GUIDELINES)

The amendments to Section III-D-7, Appendix B, and Appendix G-II-C, which were published in the *Federal Register* on September 22, 2009 (74 FR 48275) and became effective on that date, clarify and augment the current biosafety guidance for research with potentially pandemic influenza viruses, and harmonize with the CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories* (5th edition) and other regulatory policies. Section III-D-7 provides guidance regarding the biosafety level containment for research with influenza viruses generated by recombinant methods. In Appendix B, the potentially pandemic influenza viruses human H2N2 (1957-1968), 1918 H1N1, and HPAI H5N1 are classified as Risk Group 3 agents. Appendix G-II-C-5 provides additional biosafety guidance for research with these influenza viruses including Biosafety Level 3 enhanced containment, practices and training, animal containment and occupational health.

Summary of Amendments [Major Actions]:

Page 66.	Appendix D: Major Actions. Addition of Appendices D-116 and D-117

Summary of Amendments [Minor Actions] - Corrections/Updates

Page 1	Corrected the page number for the July 5, 1994 Federal Register notice.
Page 19	Section III-D-7: New -- Addition of experiments that require IBC approval before initiation: Recombinant influenza viruses. New Sections: III-D-7-a, III-D-7-b, III-D-7-c, III-D-7-d.
Page 34	Section V-C: Updated reference to the Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th edition (hyperlinked).
Page 37	Updated reference to the Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th edition (hyperlinked).
Page 40	Appendix B-II-D: Added – "(except those listed in Appendix B-III-D)." Deleted – "as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)."
Page 42	Appendix B-III-D: Addition of specified Orthomyxoviruses to the list of RG3 agents
Page 70	Updated reference to the Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th edition (hyperlinked).
Page 76	Appendix G-II-C-5: Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses.
Page 76	Appendix G-II-C-5-a: Containment, Practices, and Training for Research with Risk Group 3 Influenza Viruses (BL3 Enhanced).
Page 76	Appendix G-II-C-5-a-(1).
Page 76	Appendix G-II-C-5-a-(2).
Page 76	Appendix G-II-C-5-a-(3).
Page 76	Appendix G-II-C-5-a-(4).
Page 76	Appendix G-II-C-5-a-(4).

Page 76	Appendix G-II-C-5-b: Containment for Animal Research.
Page 76	Appendix G-II-C-5-b-1.
Page 76	Appendix G-II-C-5-b-2.
Page 76	Appendix G-II-C-5-b-3.
Page 77	Appendix G-II-C-5-b-4.
Page 77	Appendix G-II-C-5-c: Occupational Health.
Page 77	Appendix G-II-C-5-c-(1).
Page 77	Appendix G-II-C-5-c-(2).
Page 77	Appendix G-II-C-5-c-(3).
Page 77	Appendix G-II-C-5-c-(4).
Page 78	Appendix G-II-C-5-c-(5).
Page 78	Appendix G-II-C-5-c-(6).
Page 83	Updated reference to the Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th edition (hyperlinked).
Various locations	References to Appendix G-III-B were changed to Appendix G-III-A, referencing the 5th edition of the BMBL (hyperlinked).

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NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES (NIH GUIDELINES)

September 2009

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Visit the OBA Web site at:
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For current information on Guidelines, Protocols, Principal Investigators, Meetings, and information about upcoming Gene Therapy Policy Conferences

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health
Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)

These *NIH Guidelines* supersede all earlier versions and shall be in effect until further notice.

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TABLE OF CONTENTS

SECTION I. SCOPE OF THE *NIH GUIDELINES*....8
Section I-A. Purpose....8
Section I-B. Definition of Recombinant DNA Molecules....9
Section I-C. General Applicability....9
Section I-D. Compliance with the *NIH Guidelines*....10
Section I-E. General Definitions....10

SECTION II. SAFETY CONSIDERATIONS 11
Section II-A. Risk Assessment 11
Section II-A-1. Risk Groups 11
Section II-A-2. Criteria for Risk Groups 11
Section II-A-3. Comprehensive Risk Assessment 12
Section II-B. Containment 12

SECTION III. EXPERIMENTS COVERED BY THE *NIH GUIDELINES* 14
Section III-A. Experiments that Require Institutional Biosafety Committee Approval, RAC Review, and NIH Director Approval Before Initiation (See Section IV-C-1-b-(1), Major Actions). 14
Section III-A-1. Major Actions under the *NIH Guidelines* 14
Section III-B. Experiments That Require NIH/OBA and Institutional Biosafety Committee Approval Before Initiation 14
Section III-B-1. Experiments Involving the Cloning of Toxin Molecules with LD_{50} of Less than 100 Nanograms per Kilogram Body Weight 15
Section III-C. Experiments that Require Institutional Biosafety Committee and Institutional Review Board Approvals and RAC Review Before Research Participant Enrollment 15
Section III-C-1. Experiments Involving the Deliberate Transfer of Recombinant DNA, or DNA or RNA Derived from Recombinant DNA, into One or More Human Research Participants 15
Section III-D. Experiments that Require Institutional Biosafety Committee Approval Before Initiation 16
Section III-D-1. Experiments Using Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems (See Section II-A, Risk Assessment) 16
Section III-D-2. Experiments in Which DNA From Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents is Cloned into Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems 16
Section III-D-3. Experiments Involving the Use of Infectious DNA or RNA Viruses or Defective DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems 17
Section III-D-4. Experiments Involving Whole Animals 17
Section III-D-5. Experiments Involving Whole Plants 18
Section III-D-6. Experiments Involving More than 10 Liters of Culture 19
Section III-D-7. Experiments Involving Influenza Viruses 19
Section III-E. Experiments that Require Institutional Biosafety Committee Notice Simultaneous with Initiation 20
Section III-E-1. Experiments Involving the Formation of Recombinant DNA Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus 20
Section III-E-2. Experiments Involving Whole Plants 20
Section III-E-3. Experiments Involving Transgenic Rodents 21
Section III-F. Exempt Experiments 21

SECTION IV. ROLES AND RESPONSIBILITIES 22
Section IV-A. Policy 22
Section IV-B. Responsibilities of the Institution 22
Section IV-B-1. General Information 22
Section IV-B-2. Institutional Biosafety Committee (IBC) 23
Section IV-B-2-a. Membership and Procedures 23
Section IV-B-2-b. Functions 24
Section IV-B-3. Biological Safety Officer (BSO) 25
Section IV-B-4. Plant, Plant Pathogen, or Plant Pest Containment Expert 26
Section IV-B-5. Animal Containment Expert 26
Section IV-B-6. Human Gene Therapy Expertise 26
Section IV-B-7. Principal Investigator (PI) 26
Section IV-B-7-a. General Responsibilities 27
Section IV-B-7-b. Information to Be Submitted by the Principal Investigator to NIH OBA 27
Section IV-B-7-c. Submissions by the Principal Investigator to the Institutional Biosafety Committee 28
Section IV-B-7-d. Responsibilities of the Principal Investigator Prior to Initiating Research 28
Section IV-B-7-e. Responsibilities of the Principal Investigator During the Conduct of the Research.. 28
Section IV-C. Responsibilities of the National Institutes of Health (NIH) 29

Section IV-C-1. NIH Director 29
Section IV-C-1-a. General Responsibilities 29
Section IV-C-1-b. Specific Responsibilities 29
Section IV-C-1-b-(1). Major Actions 29
Section IV-C-1-b-(2). Minor Actions 30
Section IV-C-2. Recombinant DNA Advisory Committee (RAC) 31
Section IV-C-3. Office of Biotechnology Activities (OBA) 32
Section IV-C-4. Other NIH Components 32
Section IV-D. Voluntary Compliance 33
Section IV-D-1. Basic Policy - Voluntary Compliance 33
Section IV-D-2. Institutional Biosafety Committee Approval - Voluntary Compliance 33
Section IV-D-3. Certification of Host-Vector Systems - Voluntary Compliance 33
Section IV-D-4. Requests for Exemptions and Approvals - Voluntary Compliance 33
Section IV-D-5. Protection of Proprietary Data - Voluntary Compliance 33
Section IV-D-5-a. General 33
Section IV-D-5-b. Pre-submission Review 34

SECTION V. FOOTNOTES AND REFERENCES OF SECTIONS I THROUGH IV 34

APPENDIX A. EXEMPTIONS UNDER SECTION III-F-5--SUBLISTS OF NATURAL EXCHANGERS 36

Appendix A-I. Sublist A 36
Appendix A-II. Sublist B 36
Appendix A-III. Sublist C 36
Appendix A-IV. Sublist D 36
Appendix A-V. Sublist E 36
Appendix A-VI. Sublist F 37

APPENDIX B. CLASSIFICATION OF HUMAN ETIOLOGIC AGENTS ON THE BASIS OF HAZARD 37

Appendix B-I. Risk Group 1 (RG1) Agents 37
Appendix B-II. Risk Group 2 (RG2) Agents 38
Appendix B-II-A. Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia 38
Appendix B-II-B. Risk Group 2 (RG2) - Fungal Agents 39
Appendix B-II-C. Risk Group 2 (RG2) - Parasitic Agents 39
Appendix B-II-D. Risk Group 2 (RG2) - Viruses 39
Appendix B-III. Risk Group 3 (RG3) Agents 41
Appendix B-III-A. Risk Group 3 (RG3) - Bacterial Agents Including Rickettsia 41
Appendix B-III-B. Risk Group 3 (RG3) - Fungal Agents 41
Appendix B-III-C. Risk Group 3 (RG3) - Parasitic Agents 41
Appendix B-III-D. Risk Group 3 (RG3) - Viruses and Prions 41
Appendix B-IV. Risk Group 4 (RG4) Agents 42
Appendix B-IV-A. Risk Group 4 (RG4) - Bacterial Agents 42
Appendix B-IV-B. Risk Group 4 (RG4) - Fungal Agents 42
Appendix B-IV-C. Risk Group 4 (RG4) - Parasitic Agents 42
Appendix B-IV-D. Risk Group 4 (RG4) - Viral Agents 42
Appendix B-V. Animal Viral Etiologic Agents in Common Use 43
Appendix B-V-1. Murine Retroviral Vectors 43

APPENDIX C. EXEMPTIONS UNDER SECTION III-F-6 43
Appendix C-I. Recombinant DNA in Tissue Culture 44
Appendix C-I-A. Exceptions 44
Appendix C-II. *Escherichia coli K-12* Host-Vector Systems 44
Appendix C-II-A. Exceptions 44
Appendix C-III. *Saccharomyces* Host-Vector Systems 44
Appendix C-III-A. Exceptions 45
Appendix C-IV. *Bacillus subtilis* or *Bacillus licheniformis* Host-Vector Systems 45
Appendix C-IV-A. Exceptions 45

Appendix C-V. Extrachromosomal Elements of Gram Positive Organisms....45
Appendix C-V-A. Exceptions....46
Appendix C-VI. The Purchase or Transfer of Transgenic Rodents....46
Appendix C-VII. Footnotes and References of Appendix C....46

APPENDIX D. MAJOR ACTIONS TAKEN UNDER THE *NIH GUIDELINES*....47

APPENDIX E. CERTIFIED HOST-VECTOR SYSTEMS (See Appendix I, Biological Containment)....66
Appendix E-I. *Bacillus subtilis*....66
Appendix E-I-A. *Bacillus subtilis* Host-Vector 1 Systems....66
Appendix E-I-B. *Bacillus subtilis* Host-Vector 2 Systems....66
Appendix E-II. Saccharomyces cerevisiae....67
Appendix E-II-A. *Saccharomyces cerevisiae* Host-Vector 2 Systems....67
Appendix E-III. *Escherichia coli*....67
Appendix E-III-A. *Escherichia coli* (EK2) Plasmid Systems....67
Appendix E-III-B. *Escherichia coli* (EK2) Bacteriophage Systems....67
Appendix E-IV. *Neurospora crassa*....67
Appendix E-IV-A. *Neurospora crassa* Host-Vector 1 Systems....67
Appendix E-V. *Streptomyces*....67
Appendix E-V-A. *Streptomyces* Host-Vector 1 Systems....67
Appendix E-VI. *Pseudomonas putida*....68
Appendix E-VI-A. *Pseudomonas putida* Host-Vector 1 Systems....68

APPENDIX F. CONTAINMENT CONDITIONS FOR CLONING OF GENES CODING FOR THE BIOSYNTHESIS OF MOLECULES TOXIC FOR VERTEBRATES....68
Appendix F-I. General Information....68
Appendix F-II. Cloning of Toxin Molecule Genes in *Escherichia coli* K-12....68
Appendix F-III. Cloning of Toxic Molecule Genes in Organisms Other Than *Escherichia coli* K-12.68
Appendix F-IV. Specific Approvals....69

APPENDIX G. PHYSICAL CONTAINMENT....69
Appendix G-I. Standard Practices and Training....69
Appendix G-II. Physical Containment Levels....69
Appendix G-II-A. Biosafety Level 1 (BL1) (See Appendix G-III-M, *Footnotes and References of Appendix G*)....70
Appendix G-II-A-1. Standard Microbiological Practices (BL1)....70
Appendix G-II-A-2. Special Practices (BL1)....70
Appendix G-II-A-3. Containment Equipment (BL1)....70
Appendix G-II-A-4. Laboratory Facilities (BL1)....70
Appendix G-II-B. Biosafety Level 2 (BL2) (See Appendix G-III-N, *Footnotes and References of Appendix G*)....71
Appendix G-II-B-1. Standard Microbiological Practices (BL2)....71
Appendix G-II-B-2. Special Practices (BL2)....71
Appendix G-II-B-3. Containment Equipment (BL2)....72
Appendix G-II-B-4. Laboratory Facilities (BL2)....72
Appendix G-II-C. Biosafety Level 3 (BL3) (See Appendix G-III-P, *Footnotes and References of Appendix G*)....72
Appendix G-II-C-1. Standard Microbiological Practices (BL3)....73
Appendix G-II-C-2. Special Practices (BL3)....73
Appendix G-II-C-2-t. Alternative Selection of Containment Equipment (BL3)....74
Appendix G-II-C-3. Containment Equipment (BL3)....75
Appendix G-II-C-4. Laboratory Facilities (BL3)....75
Appendix G-II-C-5. Biosafety Level 3 Enhanced (BL3 Enhanced) for Research Involving Risk Group 3 Influenza Viruses.....76
Appendix G-II-C-5-a. Containment, Practices, and Training for Research with Risk Group 3 Influenza Viruses (BL3 Enhanced).....76
Appendix G-II-C-5-b. Containment for Animal Research.....76

Appendix G-II-C-5-c. Occupational Health 77
Appendix G-II-D. Biosafety Level 4 (BL4) 78
Appendix G-II-D-1. Standard Microbiological Practices (BL4) 78
Appendix G-II-D-2. Special Practices (BL4) 78
Appendix G-II-D-2-m. Alternative Selection of Containment Equipment (BL4) 79
Appendix G-II-D-3. Containment Equipment (BL4) 79

Appendix G-II-D-4. Laboratory Facilities (BL4) 80
Appendix G-III. Footnotes and References of Appendix G 82

APPENDIX H. SHIPMENT 83
Appendix H-III. Footnotes and References of Appendix H 84

APPENDIX I. BIOLOGICAL CONTAINMENT (See Appendix E, *Certified Host-Vector Systems*) 84
Appendix I-I. Levels of Biological Containment 84
Appendix I-I-A. Host-Vector 1 Systems 84
Appendix I-I-A-1. *Escherichia coli* K-12 Host-Vector 1 Systems (EK1) 84
Appendix I-I-A-2. Other Host-Vector 1 Systems 84
Appendix I-I-B. Host-Vector 2 Systems (EK2) 84
Appendix I-II. Certification of Host-Vector Systems 85
Appendix I-II-A. Responsibility 85
Appendix I-II-B. Data to be Submitted for Certification 85
Appendix I-II-B-1. Host-Vector 1 Systems Other than *Escherichia coli* K-12 85
Appendix I-II-B-2. Host-Vector 2 Systems 86
Appendix I-III. Footnotes and References of Appendix I 86

APPENDIX J. BIOTECHNOLOGY RESEARCH SUBCOMMITTEE 87

APPENDIX K. PHYSICAL CONTAINMENT FOR LARGE SCALE USES OF ORGANISMS CONTAINING RECOMBINANT DNA MOLECULES 88
Appendix K-I. Selection of Physical Containment Levels 88
Appendix K-II. Good Large Scale Practice (GLSP) 88
Appendix K-III. Biosafety Level 1 (BL1) - Large Scale 89
Appendix K-IV. Biosafety Level 2 (BL2) - Large Scale 90
Appendix K-V. Biosafety Level 3 (BL3) - Large Scale 91
Appendix K-VI. Footnotes of Appendix K 95
Appendix K-VII. Definitions to Accompany Containment Grid and Appendix K 95

APPENDIX L. GENE THERAPY POLICY CONFERENCES (GTPCS) 96

APPENDIX M. POINTS TO CONSIDER IN THE DESIGN AND SUBMISSION OF PROTOCOLS FOR THE TRANSFER OF RECOMBINANT DNA MOLECULES INTO ONE OR MORE HUMAN RESEARCH PARTICIPANTS (POINTS TO CONSIDER) 96
Appendix M-I. Requirements for Protocol Submission, Review, and Reporting – Human Gene Transfer Experiments 98
Appendix M-I-A. Requirements for Protocol Submission 98
Appendix M-I-B. RAC Review Requirements 99
Appendix M-I-B-1. Initial RAC Review 99
Appendix M-I-B-2. Public RAC Review and Discussion 99
Appendix M-I-C. Reporting Requirements 100
Appendix M-I-C-1. Initiation of the Clinical Investigation 100
Appendix M-I-C-2. Additional Clinical Trial Sites 100
Appendix M-I-C-3. Annual Reports 100
Appendix M-I-C-4. Safety Reporting 101
Appendix M-I-C-4-a. Safety Reporting: Content and Format 101
Appendix M-I-C-4-b. Safety Reporting: Time frames for Expedited Reports 102

Appendix M-I-C-5. Confidentiality ... 102
Appendix M-I-D. Safety Assessment in Human Gene Transfer Research ... 102
Appendix M-II. Description of the Proposal ... 103
Appendix M-II-A. Objectives and Rationale of the Proposed Research ... 103
Appendix M-II-A-1. Use of Recombinant DNA for Therapeutic Purposes ... 103
Appendix M-II-A-2. Transfer of DNA for Other Purposes ... 103
Appendix M-II-B. Research Design, Anticipated Risks and Benefits ... 103
Appendix M-II-B-1. Structure and Characteristics of the Biological System ... 103
Appendix M-II-B-2. Preclinical Studies, Including Risk-Assessment Studies ... 104
Appendix M-II-B-2-a. Delivery System ... 104
Appendix M-II-B-2-b. Gene Transfer and Expression ... 104
Appendix M-II-B-2-c. Retrovirus Delivery Systems ... 105
Appendix M-II-B-2-d. Non-Retrovirus Delivery/Expression Systems ... 105
Appendix M-II-B-3. Clinical Procedures, Including Research Participant Monitoring ... 105
Appendix M-II-B-4. Public Health Considerations ... 106
Appendix M-II-B-5. Qualifications of Investigators and Adequacy of Laboratory and Clinical Facilities 106
Appendix M-II-C. Selection of the Human Subjects ... 106
Appendix M-III. Informed Consent ... 107
Appendix M-III-A. Communication About the Study to Potential Participants ... 107
Appendix M-III-B. Informed Consent Document ... 107
Appendix M-III-B-1. General Requirements of Human Subjects Research ... 107
Appendix M-III-B-1-a. Description/Purpose of the Study ... 107
Appendix M-III-B-1-b. Alternatives ... 108
Appendix M-III-B-1-c. Voluntary Participation ... 108
Appendix M-III-B-1-d. Benefits ... 108
Appendix M-III-B-1-e. Possible Risks, Discomforts, and Side Effects ... 108
Appendix M-III-B-1-f. Costs ... 108
Appendix M-III-B-2. Specific Requirements of Gene Transfer Research ... 108
Appendix M-III-B-2-a. Reproductive Considerations ... 108
Appendix M-III-B-2-b. Long-Term Follow-Up ... 109
Appendix M-III-B-2-c. Request for Autopsy ... 109
Appendix M-III-B-2-d. Interest of the Media and Others in the Research ... 109
Appendix M-IV. Privacy ... 109
Appendix M-V. Special Issues ... 109
Appendix M-VI. Footnotes of Appendix M ... 110

APPENDIX P. PHYSICAL AND BIOLOGICAL CONTAINMENT FOR RECOMBINANT DNA RESEARCH INVOLVING PLANTS ... 110

Appendix P-I. General Plant Biosafety Levels ... 110
Appendix P-II. Physical Containment Levels ... 111
Appendix P-II-A. Biosafety Level 1 - Plants (BL1-P) ... 111
Appendix P-II-A-1. Standard Practices (BL1-P) ... 111
Appendix P-II-A-1-a. Greenhouse Access (BL1-P) ... 111
Appendix P-II-A-1-b. Records (BL1-P) ... 111
Appendix P-II-A-1-c. Decontamination and Inactivation (BL1-P) ... 111
Appendix P-II-A-1-d. Control of Undesired Species and Motile Macroorganisms (BL1-P) ... 111
Appendix P-II-A-1-e. Concurrent Experiments Conducted in the Greenhouse (BL1-P) ... 111
Appendix P-II-A-2. Facilities (BL1-P) ... 111
Appendix P-II-A-2-a. Definitions (BL1-P) ... 111
Appendix P-II-A-2-b. Greenhouse Design (BL1-P) ... 112
Appendix P-II-B. Biosafety Level 2 - Plants (BL2-P) ... 112
Appendix P-II-B-1. Standard Practices (BL2-P) ... 112
Appendix P-II-B-1-a. Greenhouse Access (BL2-P) ... 112
Appendix P-II-B-1-b. Records (BL2-P) ... 112
Appendix P-II-B-1-c. Decontamination and Inactivation (BL2-P) ... 112
Appendix P-II-B-1-d. Control of Undesired Species and Motile Macroorganisms (BL2-P) ... 112
Appendix P-II-B-1-e. Concurrent Experiments Conducted in the Greenhouse (BL2-P) ... 113
Appendix P-II-B-1-f. Signs (BL2-P) ... 113

Appendix P-II-B-1-g. Transfer of Materials (BL2-P) ... 113
Appendix P-II-B-1-h. Greenhouse Practices Manual (BL2-P) ... 113
Appendix P-II-B-2. Facilities (BL2-P) ... 113
Appendix P-II-B-2-a. Definitions (BL2-P) ... 113
Appendix P-II-B-2-b. Greenhouse Design (BL2-P) ... 113
Appendix P-II-B-2-c. Autoclaves (BL2-P) ... 114
Appendix P-II-B-2-d. Supply and Exhaust Air Ventilation Systems (BL2-P) ... 114
Appendix P-II-B-2-e. Other (BL2-P) ... 114
Appendix P-II-C. Biosafety Level 3 - Plants (BL3-P) ... 114
Appendix P-II-C-1. Standard Practices (BL3-P) ... 114
Appendix P-II-C-1-a. Greenhouse Access (BL3-P) ... 114
Appendix P-II-C-1-b. Records (BL3-P) ... 114
Appendix P-II-C-1-c. Decontamination and Inactivation (BL3-P) ... 114
Appendix P-II-C-1-d. Control of Undesired Species and Motile Macroorganisms (BL3-P) ... 114
Appendix P-II-C-1-e. Concurrent Experiments Conducted in the Greenhouse (BL3-P) ... 115
Appendix P-II-C-1-f. Signs (BL3-P) ... 115
Appendix P-II-C-1-g. Transfer of Materials (BL3-P) ... 115
Appendix P-II-C-1-h. Greenhouse Practices Manual (BL3-P) ... 115
Appendix P-II-C-1-i. Protective Clothing (BL3-P) ... 115
Appendix P-II-C-1-j. Other (BL3-P) ... 115
Appendix P-II-C-2. Facilities (BL3-P) ... 116
Appendix P-II-C-2-a. Definitions (BL3-P) ... 116
Appendix P-II-C-2-b. Greenhouse Design (BL3-P) ... 116
Appendix P-II-C-2-c. Autoclaves (BL3-P) ... 116
Appendix P-II-C-2-d. Supply and Exhaust Air Ventilation Systems (BL3-P) ... 116
Appendix P-II-C-2-e. Other (BL3-P) ... 117
Appendix P-II-D. Biosafety Level 4 - Plants (BL4-P) ... 117
Appendix P-II-D-1. Standard Practices (BL4-P) ... 117
Appendix P-II-D-1-a. Greenhouse Access (BL4-P) ... 117
Appendix P-II-D-1-b. Records (BL4-P) ... 117
Appendix P-II-D-1-c. Decontamination and Inactivation (BL4-P) ... 118
Appendix P-II-D-1-d. Control of Undesired Species and Motile Macroorganisms (BL4-P) ... 118
Appendix P-II-D-1-e. Concurrent Experiments Conducted in the Greenhouse (BL4-P) ... 118
Appendix P-II-D-1-f. Signs (BL4-P) ... 118
Appendix P-II-D-1-g. Transfer of Materials (BL4-P) ... 118
Appendix P-II-D-1-h. Greenhouse Practices Manual (BL4-P) ... 119
Appendix P-II-D-1-i. Protective Clothing (BL4-P) ... 119
Appendix P-II-D-2. Facilities (BL4-P) ... 119
Appendix P-II-D-2-a. Greenhouse Design (BL4-P) ... 119
Appendix P-II-D-2-b. Autoclaves (BL4-P) ... 119
Appendix P-II-D-2-c. Supply and Exhaust Air Ventilation Systems (BL4-P) ... 120
Appendix P-II-D-2-d. Other (BL4-P) ... 120
Appendix P-III. Biological Containment Practices ... 120
Appendix P-III-A. Biological Containment Practices (Plants) ... 120
Appendix P-III-B. Biological Containment Practices (Microorganisms) ... 121
Appendix P-III-C. Biological Containment Practices (Macroorganisms) ... 121

APPENDIX Q. PHYSICAL AND BIOLOGICAL CONTAINMENT FOR RECOMBINANT DNA RESEARCH INVOLVING ANIMALS ... 121

Appendix Q-I. General Considerations ... 121
Appendix Q-I-A. Containment Levels ... 121
Appendix Q-I-B. Disposal of Animals (BL1-N through BL4-N) ... 122
Appendix Q-II. Physical and Biological Containment Levels ... 122
Appendix Q-II-A. Biosafety Level 1 - Animals (BL1-N) ... 122
Appendix Q-II-A-1. Standard Practices (BL1-N) ... 122
Appendix Q-II-A-1-a. Animal Facility Access (BL1-N) ... 122
Appendix Q-II-A-1-b. Other (BL1-N) ... 122
Appendix Q-II-A-2. Animal Facilities (BL1-N) ... 122

Appendix Q-II-B. Biosafety Level 2 - Animals (BL2-N) (See Appendix Q-III-A, *Footnotes and References for Appendix Q*) ... 122
Appendix Q-II-B-1. Standard Practices (BL2-N) ... 122
Appendix Q-II-B-1-a. Animal Facility Access (BL2-N) ... 122
Appendix Q-II-B-1-b. Decontamination and Inactivation (BL2-N) ... 123
Appendix Q-II-B-1-c. Signs (BL2-N) ... 123
Appendix Q-II-B-1-d. Protective Clothing (BL2-N) ... 123
Appendix Q-II-B-1-e. Records (BL2-N) ... 123
Appendix Q-II-B-1-f. Transfer of Materials (BL2-N) ... 123
Appendix Q-II-B-1-g. Other (BL2-N) ... 124
Appendix Q-II-B-2. Animal Facilities (BL2-N) ... 124
Appendix Q-II-C. Biosafety Level 3 - Animals (BL3-N) (See Appendix Q-III-B, *Footnotes and References for Appendix Q*) ... 125
Appendix Q-II-C-1. Standard Practices (BL3-N) ... 125
Appendix Q-II-C-1-a. Animal Facility Access (BL3-N) ... 125
Appendix Q-II-C-1-b. Decontamination and Inactivation (BL3-N) ... 125
Appendix Q-II-C-1-c. Signs (BL3-N) ... 125
Appendix Q-II-C-1-d. Protective Clothing (BL3-N) ... 125
Appendix Q-II-C-1-e. Records (BL3-N) ... 126
Appendix Q-II-C-1-f. Transfer of Materials (BL3-N) ... 126
Appendix Q-II-C-1-g. Other (BL3-N) ... 126
Appendix Q-II-C-2. Animal Facilities (BL3-N) ... 127
Appendix Q-II-D. Biosafety Level 4 - Animals (BL4-N) (See Appendix Q-III-C, *Footnotes and References for Appendix Q*) ... 128
Appendix Q-II-D-1. Standard Practices (BL4-N) ... 128
Appendix Q-II-D-1-a. Animal Facility Access (BL4-N) ... 128
Appendix Q-II-D-1-b. Decontamination and Inactivation (BL4-N) ... 129
Appendix Q-II-D-1-c. Signs (BL4-N) ... 130
Appendix Q-II-D-1-d. Protective Clothing (BL4-N) ... 130
Appendix Q-II-D-1-e. Records (BL4-N) ... 130
Appendix Q-II-D-1-f. Transfer of Materials (BL4-N) ... 130
Appendix Q-II-D-1-g. Other (BL4-N) ... 131
Appendix Q-II-D-2. Animal Facilities (BL4-N) ... 132
Appendix Q-III. Footnotes and References for Appendix Q ... 133

****

LIST OF TABLES

Appendix B - Table 1. Basis for the Classification of Biohazardous Agents by Risk Group ... 37

Appendix G - Table 1. Possible Alternate Combinations Of Physical And Biological Containment Safeguards ... 81

Appendix K - Table 1. Comparison of Good Large Scale Practice (GLSP) and Biosafety Level (BL) - Large Scale (LS) Practice ... 94

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SECTION I. SCOPE OF THE *NIH GUIDELINES*

Section I-A. Purpose

The purpose of the *NIH Guidelines* is to specify practices for constructing and handling: (i) recombinant deoxyribonucleic acid (DNA) molecules, and (ii) organisms and viruses containing recombinant DNA molecules.

Section I-A-1. Any recombinant DNA experiment, which according to the *NIH Guidelines* requires approval by NIH, must be submitted to NIH or to another Federal agency that has jurisdiction for review and approval. Once approvals, or other applicable clearances, have been obtained from a Federal agency other than NIH (whether the experiment is referred to that agency by NIH or sent directly there by the submitter), the experiment may proceed without the necessity for NIH review or approval. (See exception in Section I-A-1-a regarding requirement for human gene transfer protocol registration.)

Section I-A-1-a. For experiments involving the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into human research participants (human gene transfer), no research participant shall be enrolled (see definition of enrollment in Section I-E-7) until the RAC review process has been completed (see Appendix M-I-B, *RAC Review Requirements*); Institutional Biosafety Committee (IBC) approval (from the clinical

trial site) has been obtained; Institutional Review Board approval has been obtained; and all applicable regulatory authorization(s) have been obtained.

For a clinical trial site that is added after the RAC review process, no research participant shall be enrolled (see definition of enrollment in Section I-E-7) at the clinical trial site until the following documentation has been submitted to NIH OBA: (1) IBC approval (from the clinical trial site); (2) Institutional Review Board approval; (3) Institutional Review Board-approved informed consent document; and (4) curriculum vitae of the principal investigator(s) (no more than two pages in biographical sketch format); and (5) NIH grant number(s) if applicable.

Section I-B. Definition of Recombinant DNA Molecules

In the context of the *NIH Guidelines*, recombinant DNA molecules are defined as either: (i) molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above.

Synthetic DNA segments which are likely to yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent) are considered as equivalent to their natural DNA counterpart. If the synthetic DNA segment is not expressed *in vivo* as a biologically active polynucleotide or polypeptide product, it is exempt from the *NIH Guidelines*.

Genomic DNA of plants and bacteria that have acquired a transposable element, even if the latter was donated from a recombinant vector no longer present, are not subject to the *NIH Guidelines* unless the transposon itself contains recombinant DNA.

Section I-C. General Applicability

Section I-C-1. The *NIH Guidelines* are applicable to:

Section I-C-1-a. All recombinant DNA research within the United States (U.S.) or its territories that is within the category of research described in either Section I-C-1-a-(1) or Section I-C-1-a-(2).

Section I-C-1-a-(1). Research that is conducted at or sponsored by an institution that receives any support for recombinant DNA research from NIH, including research performed directly by NIH. An individual who receives support for research involving recombinant DNA must be associated with or sponsored by an institution that assumes the responsibilities assigned in the *NIH Guidelines*.

Section I-C-1-a-(2). Research that involves testing in humans of materials containing recombinant DNA developed with NIH funds, if the institution that developed those materials sponsors or participates in those projects. Participation includes research collaboration or contractual agreements, not mere provision of research materials.

Section I-C-1-b. All recombinant DNA research performed abroad that is within the category of research described in either Section I-C-1-b-(1) or Section I-C-1-b-(2).

Section I-C-1-b-(1). Research supported by NIH funds.

Section I-C-1-b-(2). Research that involves testing in humans of materials containing recombinant DNA developed with NIH funds, if the institution that developed those materials sponsors or participates in those projects. Participation includes research collaboration or contractual agreements, not mere provision of research materials.

Section I-C-1-b-(3). If the host country has established rules for the conduct of recombinant DNA research, then the research must be in compliance with those rules. If the host country does not have such rules, the proposed research must be reviewed and approved by an NIH-approved Institutional Biosafety Committee or equivalent review body and accepted in writing by an appropriate national governmental authority of the host country. The safety practices that are employed abroad must be reasonably consistent with the *NIH Guidelines*.

Section I-D. Compliance with the *NIH Guidelines*

As a condition for NIH funding of recombinant DNA research, institutions shall ensure that such research conducted at or sponsored by the institution, irrespective of the source of funding, shall comply with the *NIH Guidelines*.

Information concerning noncompliance with the *NIH Guidelines* may be brought forward by any person. It should be delivered to both NIH/OBA and the relevant institution. The institution, generally through the Institutional Biosafety Committee, shall take appropriate action. The institution shall forward a complete report of the incident recommending any further action to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985, 301-496-9838/301-496-9839 (fax) (for non-USPS mail, use zip code 20817).

In cases where NIH proposes to suspend, limit, or terminate financial assistance because of noncompliance with the *NIH Guidelines*, applicable DHHS and Public Health Service procedures shall govern.

The policies on compliance are as follows:

Section I-D-1. All NIH-funded projects involving recombinant DNA techniques must comply with the *NIH Guidelines*. Non-compliance may result in: (i) suspension, limitation, or termination of financial assistance for the noncompliant NIH-funded research project and of NIH funds for other recombinant DNA research at the institution, or (ii) a requirement for prior NIH approval of any or all recombinant DNA projects at the institution.

Section I-D-2. All non-NIH funded projects involving recombinant DNA techniques conducted at or sponsored by an institution that receives NIH funds for projects involving such techniques must comply with the *NIH Guidelines*. Noncompliance may result in: (i) suspension, limitation, or termination of NIH funds for recombinant DNA research at the institution, or (ii) a requirement for prior NIH approval of any or all recombinant DNA projects at the institution.

Section I-E. General Definitions

The following terms, which are used throughout the *NIH Guidelines*, are defined as follows:

Section I-E-1. An "institution" is any public or private entity (including Federal, state, and local government agencies).

Section I-E-2. An "Institutional Biosafety Committee" is a committee that: (i) meets the requirements for membership specified in Section IV-B-2, *Institutional Biosafety Committee (IBC)*, and (ii) reviews, approves, and oversees projects in accordance with the responsibilities defined in Section IV-B-2, *Institutional Biosafety Committee (IBC)*.

Section I-E-3. The "Office of Biotechnology Activities (OBA)" is the office within the NIH that is responsible for: (i) reviewing and coordinating all activities relating to the *NIH Guidelines*, and (ii) performing other duties as defined in Section IV-C-3, *Office of Biotechnology Activities (OBA)*.

Section I-E-4. The "Recombinant DNA Advisory Committee" is the public advisory committee that advises the Department of Health and Human Services (DHHS) Secretary, the DHHS Assistant Secretary for Health, and the NIH Director concerning recombinant DNA research. The RAC shall be constituted as specified in Section IV-C-2, *Recombinant DNA Advisory Committee (RAC)*.

Section I-E-5. The "NIH Director" is the Director of the National Institutes of Health, or any other officer or employee of NIH to whom authority has been delegated.

Section I-E-6. "Deliberate release" is defined as a planned introduction of recombinant DNA-containing microorganisms, plants, or animals into the environment.

Section I-E-7. "Enrollment" is the process of obtaining informed consent from a potential research participant, or a designated legal guardian of the participant, to undergo a test or procedure associated with the gene transfer experiment.

Section I-E-8. A "serious adverse event" is any event occurring at any dose that results in any of the following outcomes: death, a life-threatening event, in-patient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization also may be considered a serious adverse event when, upon the basis of appropriate medical judgment, they may jeopardize the human gene transfer research subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Section I-E-9. An adverse event is "associated with the use of a gene transfer product" when there is a reasonable possibility that the event may have been caused by the use of that product.

Section I-E-10. An "unexpected serious adverse event" is any serious adverse event for which the specificity or severity is not consistent with the risk information available in the current investigator's brochure.

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SECTION II. SAFETY CONSIDERATIONS

Section II-A. Risk Assessment

Section II-A-1. Risk Groups

Risk assessment is ultimately a subjective process. The investigator must make an initial risk assessment based on the Risk Group (RG) of an agent (see Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*). Agents are classified into four Risk Groups (RGs) according to their relative pathogenicity for healthy adult humans by the following criteria: (1) Risk Group 1 (RG1) agents are not associated with disease in healthy adult humans. (2) Risk Group 2 (RG2) agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available. (3) Risk Group 3 (RG3) agents are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available. (4) Risk Group 4 (RG4) agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available.

Section II-A-2. Criteria for Risk Groups

Classification of agents in Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*, is based on the potential effect of a biological agent on a healthy human adult and does not account for instances in which an individual may have increased susceptibility to such agents, e.g., preexisting diseases, medications, compromised immunity, pregnancy or breast feeding (which may increase exposure of infants to some agents).

Personnel may need periodic medical surveillance to ascertain fitness to perform certain activities; they may also need to be offered prophylactic vaccines and boosters (see Section IV-B-1-f, *Responsibilities of the Institution, General Information*).

Section II-A-3. Comprehensive Risk Assessment

In deciding on the appropriate containment for an experiment, the initial risk assessment from Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*, should be followed by a thorough consideration of the agent itself and how it is to be manipulated. Factors to be considered in determining the level of containment include agent factors such as: virulence, pathogenicity, infectious dose, environmental stability, route of spread, communicability, operations, quantity, availability of vaccine or treatment, and gene product effects such as toxicity, physiological activity, and allergenicity. Any strain that is known to be more hazardous than the parent (wild-type) strain should be considered for handling at a higher containment level. Certain attenuated strains or strains that have been demonstrated to have irreversibly lost known virulence factors may qualify for a reduction of the containment level compared to the Risk Group assigned to the parent strain (see Section V-B, *Footnotes and References of Sections I-IV*).

A final assessment of risk based on these considerations is then used to set the appropriate containment conditions for the experiment (see Section II-B, *Containment*). The containment level required may be equivalent to the Risk Group classification of the agent or it may be raised or lowered as a result of the above considerations. The Institutional Biosafety Committee must approve the risk assessment and the biosafety containment level for recombinant DNA experiments described in Sections III-A, *Experiments that Require Institutional Biosafety Committee Approval, RAC Review, and NIH Director Approval Before Initiation*; III-B, *Experiments that Require NIH/OBA and Institutional Biosafety Committee Approval Before Initiation*; III-C, *Experiments that Require Institutional Biosafety Committee and Institutional Review Board Approvals and NIH/OBA Registration Before Initiation*; III-D, *Experiments that Require Institutional Biosafety Committee Approval Before Initiation*.

Careful consideration should be given to the types of manipulation planned for some higher Risk Group agents. For example, the RG2 dengue viruses may be cultured under the Biosafety Level (BL) 2 containment (see Section II-B); however, when such agents are used for animal inoculation or transmission studies, a higher containment level is recommended. Similarly, RG3 agents such as Venezuelan equine encephalomyelitis and yellow fever viruses should be handled at a higher containment level for animal inoculation and transmission experiments.

Individuals working with human immunodeficiency virus (HIV), hepatitis B virus (HBV) or other bloodborne pathogens should consult the applicable Occupational Safety and Health Administration (OSHA) regulation, 29 CFR 1910.1030, and OSHA publication 3127 (1996 revised). BL2 containment is recommended for activities involving all blood-contaminated clinical specimens, body fluids, and tissues from all humans, or from HIV- or HBV-infected or inoculated laboratory animals. Activities such as the production of research-laboratory scale quantities of HIV or other bloodborne pathogens, manipulating concentrated virus preparations, or conducting procedures that may produce droplets or aerosols, are performed in a BL2 facility using the additional practices and containment equipment recommended for BL3. Activities involving industrial scale volumes or preparations of concentrated HIV are conducted in a BL3 facility, or BL3 Large Scale if appropriate, using BL3 practices and containment equipment.

Exotic plant pathogens and animal pathogens of domestic livestock and poultry are restricted and may require special laboratory design, operation and containment features not addressed in *Biosafety in Microbiological and Biomedical Laboratories* (see Section V-C, *Footnotes and References of Sections I through IV*). For information regarding the importation, possession, or use of these agents see Sections V-G and V-H, *Footnotes and References of Sections I through IV*.

Section II-B. Containment

Effective biological safety programs have been operative in a variety of laboratories for many years. Considerable information already exists about the design of physical containment facilities and selection of laboratory procedures applicable to organisms carrying recombinant DNA (see Section V-B, *Footnotes and References of Sections I-IV*). The existing programs rely upon mechanisms that can be divided into two categories: (i) a set of standard practices that are generally used in microbiological laboratories; and (ii) special procedures, equipment, and laboratory installations that provide physical barriers that are applied in varying degrees according to the estimated biohazard. Four biosafety levels are described in Appendix G, *Physical Containment*. These biosafety levels consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities appropriate for the operations performed and are based on the potential

hazards imposed by the agents used and for the laboratory function and activity. Biosafety Level 4 provides the most stringent containment conditions, Biosafety Level 1 the least stringent.

Experiments involving recombinant DNA lend themselves to a third containment mechanism, namely, the application of highly specific biological barriers. Natural barriers exist that limit either: (i) the infectivity of a vector or vehicle (plasmid or virus) for specific hosts, or (ii) its dissemination and survival in the environment. Vectors, which provide the means for recombinant DNA and/or host cell replication, can be genetically designed to decrease, by many orders of magnitude, the probability of dissemination of recombinant DNA outside the laboratory (see Appendix I, *Biological Containment*).

Since these three means of containment are complementary, different levels of containment can be established that apply various combinations of the physical and biological barriers along with a constant use of standard practices. Categories of containment are considered separately in order that such combinations can be conveniently expressed in the *NIH Guidelines*.

Physical containment conditions within laboratories, described in Appendix G, *Physical Containment*, may not always be appropriate for all organisms because of their physical size, the number of organisms needed for an experiment, or the particular growth requirements of the organism. Likewise, biological containment for microorganisms described in Appendix I, *Biological Containment*, may not be appropriate for all organisms, particularly higher eukaryotic organisms. However, significant information exists about the design of research facilities and experimental procedures that are applicable to organisms containing recombinant DNA that is either integrated into the genome or into microorganisms associated with the higher organism as a symbiont, pathogen, or other relationship. This information describes facilities for physical containment of organisms used in non-traditional laboratory settings and special practices for limiting or excluding the unwanted establishment, transfer of genetic information, and dissemination of organisms beyond the intended location, based on both physical and biological containment principles. Research conducted in accordance with these conditions effectively confines the organism.

For research involving plants, four biosafety levels (BL1-P through BL4-P) are described in Appendix P, *Physical and Biological Containment for Recombinant DNA Research Involving Plants*. BL1-P is designed to provide a moderate level of containment for experiments for which there is convincing biological evidence that precludes the possibility of survival, transfer, or dissemination of recombinant DNA into the environment, or in which there is no recognizable and predictable risk to the environment in the event of accidental release. BL2-P is designed to provide a greater level of containment for experiments involving plants and certain associated organisms in which there is a recognized possibility of survival, transmission, or dissemination of recombinant DNA containing organisms, but the consequence of such an inadvertent release has a predictably minimal biological impact. BL3-P and BL4-P describe additional containment conditions for research with plants and certain pathogens and other organisms that require special containment because of their recognized potential for significant detrimental impact on managed or natural ecosystems. BL1-P relies upon accepted scientific practices for conducting research in most ordinary greenhouse or growth chamber facilities and incorporates accepted procedures for good pest control and cultural practices. BL1-P facilities and procedures provide a modified and protected environment for the propagation of plants and microorganisms associated with the plants and a degree of containment that adequately controls the potential for release of biologically viable plants, plant parts, and microorganisms associated with them. BL2-P and BL3-P rely upon accepted scientific practices for conducting research in greenhouses with organisms infecting or infesting plants in a manner that minimizes or prevents inadvertent contamination of plants within or surrounding the greenhouse. BL4-P describes facilities and practices known to provide containment of certain exotic plant pathogens.

For research involving animals, which are of a size or have growth requirements that preclude the use of conventional primary containment systems used for small laboratory animals, four biosafety levels (BL1-N through BL4-N) are described in Appendix Q, *Physical and Biological Containment for Recombinant DNA Research Involving Animals*. BL1-N describes containment for animals that have been modified by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant DNA-modified microorganisms and is designed to eliminate the possibility of sexual transmission of the modified genome or transmission of recombinant DNA-derived viruses known to be transmitted from animal parent to offspring only by sexual reproduction. Procedures, practices, and facilities follow classical methods of avoiding genetic exchange between animals. BL2-N describes containment which is used for transgenic animals associated with recombinant DNA-derived organisms and is designed to eliminate the possibility of vertical or horizontal transmission. Procedures, practices, and facilities follow classical methods of avoiding genetic exchange between animals or controlling arthropod transmission. BL3-N

and BL4-N describe higher levels of containment for research with certain transgenic animals involving agents which pose recognized hazard.

In constructing the *NIH Guidelines*, it was necessary to define boundary conditions for the different levels of physical and biological containment and for the classes of experiments to which they apply. These definitions do not take into account all existing and anticipated information on special procedures that will allow particular experiments to be conducted under different conditions than indicated here without affecting risk. Individual investigators and Institutional Biosafety Committees are urged to devise simple and more effective containment procedures and to submit recommended changes in the *NIH Guidelines* to permit the use of these procedures.

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SECTION III. EXPERIMENTS COVERED BY THE *NIH GUIDELINES*

This section describes six categories of experiments involving recombinant DNA: (i) those that require Institutional Biosafety Committee (IBC) approval, RAC review, and NIH Director approval before initiation (see Section III-A), (ii) those that require NIH/OBA and Institutional Biosafety Committee approval before initiation (see Section III-B), (iii) those that require Institutional Biosafety Committee and Institutional Review Board approvals and RAC review before research participant enrollment (see Section III-C), (iv) those that require Institutional Biosafety Committee approval before initiation (see Section III-D), (v) those that require Institutional Biosafety Committee notification simultaneous with initiation (see Section III-E), and (vi) those that are exempt from the *NIH Guidelines* (see Section III-F).

Note: *If an experiment falls into Sections III-A, III-B, or III-C and one of the other sections, the rules pertaining to Sections III-A, III-B, or III-C shall be followed*. If an experiment falls into Section III-F and into either Sections III-D or III-E as well, the experiment is considered exempt from the *NIH Guidelines*.

Any change in containment level, which is different from those specified in the *NIH Guidelines*, may not be initiated without the express approval of NIH/OBA (see Section IV-C-1-b-(2) and its subsections, *Minor Actions*).

Section III-A. Experiments that Require Institutional Biosafety Committee Approval, RAC Review, and NIH Director Approval Before Initiation (See Section IV-C-1-b-(1), Major Actions).

Section III-A-1. Major Actions under the *NIH Guidelines*

Experiments considered as *Major Actions* under the *NIH Guidelines* cannot be initiated without submission of relevant information on the proposed experiment to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax), the publication of the proposal in the *Federal Register* for 15 days of comment, review by RAC, and specific approval by NIH. The containment conditions or stipulation requirements for such experiments will be recommended by RAC and set by NIH at the time of approval. Such experiments require Institutional Biosafety Committee approval before initiation. Specific experiments already approved are included in Appendix D, *Major Actions Taken under the NIH Guidelines*, which may be obtained from the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

Section III-A-1-a. The deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally (see Section V-B, *Footnotes and References of Sections I-IV*), if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture, will be reviewed by RAC.

Section III-B. Experiments That Require NIH/OBA and Institutional Biosafety Committee Approval Before Initiation

Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH/OBA. The containment conditions for such experiments will be determined by NIH/OBA in consultation with *ad hoc* experts. Such experiments require Institutional Biosafety Committee approval before initiation (see Section IV-B-2-b-(1), *Institutional Biosafety Committee*).

Section III-B-1. Experiments Involving the Cloning of Toxin Molecules with LD_{50} of Less than 100 Nanograms per Kilogram Body Weight

Deliberate formation of recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, and *Shigella dysenteriae* neurotoxin). Specific approval has been given for the cloning in *Escherichia coli* K-12 of DNA containing genes coding for the biosynthesis of toxic molecules which are lethal to vertebrates at 100 nanograms to 100 micrograms per kilogram body weight. Specific experiments already approved under this section may be obtained from the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

Section III-C. Experiments that Require Institutional Biosafety Committee and Institutional Review Board Approvals and RAC Review Before Research Participant Enrollment

Section III-C-1. Experiments Involving the Deliberate Transfer of Recombinant DNA, or DNA or RNA Derived from Recombinant DNA, into One or More Human Research Participants

For an experiment involving the deliberate transfer of recombinant DNA, or DNA or RNA derived from recombinant DNA, into human research participants (human gene transfer), no research participant shall be enrolled (see definition of enrollment in Section I-E-7) until the RAC review process has been completed (see Appendix M-I-B, *RAC Review Requirements*).

In its evaluation of human gene transfer proposals, the RAC will consider whether a proposed human gene transfer experiment presents characteristics that warrant public RAC review and discussion (See Appendix M-I-B-2). The process of public RAC review and discussion is intended to foster the safe and ethical conduct of human gene transfer experiments. Public review and discussion of a human gene transfer experiment (and access to relevant information) also serves to inform the public about the technical aspects of the proposal, meaning and significance of the research, and any significant safety, social, and ethical implications of the research.

Public RAC review and discussion of a human gene transfer experiment may be: (1) initiated by the NIH Director; or (2) initiated by the NIH OBA Director following a recommendation to NIH OBA by: (a) three or more RAC members; or (b) a Federal agency other than NIH. After a human gene transfer experiment is reviewed by the RAC at a regularly scheduled meeting, NIH OBA will send a letter, unless NIH OBA determines that there are exceptional circumstances, within 10 working days to the NIH Director, the Principal Investigator, the sponsoring institution, and other DHHS components, as appropriate, summarizing the RAC recommendations.

For a clinical trial site that is added after the RAC review process, no research participant shall be enrolled (see definition of enrollment in Section I-E-7) at the clinical trial site until the following documentation has been submitted to NIH OBA: (1) Institutional Biosafety Committee approval (from the clinical trial site); (2) Institutional Review Board approval; (3) Institutional Review Board-approved informed consent document; (4) curriculum vitae of the principal investigator(s) (no more than two pages in biographical sketch format); and (5) NIH grant number(s) if applicable.

In order to maintain public access to information regarding human gene transfer protocols (including protocols that are not publicly reviewed by the RAC), NIH OBA will maintain the documentation described in Appendices M-I through M-V. The information provided in response to Appendix M should not contain any confidential commercial information or trade secrets, enabling all aspects of RAC review to be open to the public.

Note: For specific directives concerning the use of retroviral vectors for gene delivery, consult Appendix B-V-1, *Murine Retroviral Vectors.*

Section III-D. Experiments that Require Institutional Biosafety Committee Approval Before Initiation

Prior to the initiation of an experiment that falls into this category, the Principal Investigator must submit a registration document to the Institutional Biosafety Committee which contains the following information: (i) the source(s) of DNA; (ii) the nature of the inserted DNA sequences; (iii) the host(s) and vector(s) to be used; (iv) if an attempt will be made to obtain expression of a foreign gene, and if so, indicate the protein that will be produced; and (v) the containment conditions that will be implemented as specified in the *NIH Guidelines*. For experiments in this category, the registration document shall be dated, signed by the Principal Investigator, and filed with the Institutional Biosafety Committee. The Institutional Biosafety Committee shall review and approve all experiments in this category prior to their initiation. Requests to decrease the level of containment specified for experiments in this category will be considered by NIH (see Section IV-C-1-b-(2)-(c), *Minor Actions*).

Section III-D-1. Experiments Using Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems (See Section II-A, Risk Assessment)

Section III-D-1-a. Experiments involving the introduction of recombinant DNA into Risk Group 2 agents will usually be conducted at Biosafety Level (BL) 2 containment. Experiments with such agents will usually be conducted with whole animals at BL2 or BL2-N (Animals) containment.

Section III-D-1-b. Experiments involving the introduction of recombinant DNA into Risk Group 3 agents will usually be conducted at BL3 containment. Experiments with such agents will usually be conducted with whole animals at BL3 or BL3-N containment.

Section III-D-1-c. Experiments involving the introduction of recombinant DNA into Risk Group 4 agents shall be conducted at BL4 containment. Experiments with such agents shall be conducted with whole animals at BL4 or BL4-N containment.

Section III-D-1-d. Containment conditions for experiments involving the introduction of recombinant DNA into restricted agents shall be set on a case-by-case basis following NIH/OBA review. A U.S. Department of Agriculture - Animal and Plant Health Inspection Service (USDA/APHIS) permit is required for work with plant or animal pathogens (see Section V-G and V-M, *Footnotes and References of Sections I-IV*). Experiments with such agents shall be conducted with whole animals at BL4 or BL4-N containment.

Section III-D-2. Experiments in Which DNA From Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents is Cloned into Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems

Section III-D-2-a. Experiments in which DNA from Risk Group 2 or Risk Group 3 agents (see Section II-A, *Risk Assessment)* is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment. Experiments in which DNA from Risk Group 4 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment after demonstration that only a totally and irreversibly defective fraction of the agent's genome is present in a given recombinant. In the absence of such a demonstration, BL4 containment shall be used. The Institutional Biosafety Committee may approve the specific lowering of containment for particular experiments to BL1. Many experiments in this category are exempt from the *NIH Guidelines* (see Section III-F, *Exempt Experiments*). Experiments involving the formation of recombinant DNA for certain genes coding for molecules toxic for vertebrates require NIH/OBA approval (see Section III-B-1, *Experiments Involving the Cloning of Toxin Molecules with* LD_{50} *of Less than 100 Nanograms Per Kilogram Body Weight*) or shall be conducted under NIH specified conditions as described in Appendix F, *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*.

Section III-D-2-b. Containment conditions for experiments in which DNA from restricted agents is transferred into nonpathogenic prokaryotes or lower eukaryotes shall be determined by NIH/OBA following a case-by-case review (see Section V-L, *Footnotes and References of Sections I-IV*). A U.S. Department of Agriculture permit is required for work with plant or animal pathogens (see Section V-G, *Footnotes and References of Sections I-IV*).

Section III-D-3. Experiments Involving the Use of Infectious DNA or RNA Viruses or Defective DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems

Caution: Special care should be used in the evaluation of containment levels for experiments which are likely to either enhance the pathogenicity (e.g., insertion of a host oncogene) or to extend the host range (e.g., introduction of novel control elements) of viral vectors under conditions that permit a productive infection. In such cases, serious consideration should be given to increasing physical containment by at least one level.

Note: Recombinant DNA or RNA molecules derived therefrom, which contain less than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family (see Section V-J, *Footnotes and References of Sections I-IV*) being considered identical (see Section V-K, *Footnotes and References of Sections I-IV*), are considered defective and may be used in the absence of helper under the conditions specified in Section III-E-1, *Experiments Involving the Formation of Recombinant DNA Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus*.

Section III-D-3-a. Experiments involving the use of infectious or defective Risk Group 2 viruses (see Appendix B-II, *Risk Group 2 Agents*) in the presence of helper virus may be conducted at BL2.

Section III-D-3-b. Experiments involving the use of infectious or defective Risk Group 3 viruses (see Appendix B-III-D, *Risk Group 3 (RG3) - Viruses and Prions*) in the presence of helper virus may be conducted at BL3.

Section III-D-3-c. Experiments involving the use of infectious or defective Risk Group 4 viruses (see Appendix B-IV-D, *Risk Group 4 (RG4) - Viral Agents*) in the presence of helper virus may be conducted at BL4.

Section III-D-3-d. Experiments involving the use of infectious or defective restricted poxviruses (see Sections V-A and V-L, *Footnotes and References of Sections I-IV*) in the presence of helper virus shall be determined on a case-by-case basis following NIH/OBA review. A U.S. Department of Agriculture permit is required for work with plant or animal pathogens (see Section V-G, *Footnotes and References of Sections I-IV*).

Section III-D-3-e. Experiments involving the use of infectious or defective viruses in the presence of helper virus which are not covered in Sections III-D-3-a through III-D-3-d may be conducted at BL1.

Section III-D-4. Experiments Involving Whole Animals

This section covers experiments involving whole animals in which the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant DNA-modified microorganisms tested on whole animals. For the latter, other than viruses which are only vertically transmitted, the experiments may *not* be conducted at BL1-N containment. A minimum containment of BL2 or BL2-N is required.

Caution - Special care should be used in the evaluation of containment conditions for some experiments with transgenic animals. For example, such experiments might lead to the creation of novel mechanisms or increased transmission of a recombinant pathogen or production of undesirable traits in the host animal. In such cases, serious consideration should be given to increasing the containment conditions.

Section III-D-4-a. Recombinant DNA, or DNA or RNA molecules derived therefrom, from any source except for greater than two-thirds of eukaryotic viral genome may be transferred to any non-human vertebrate or any invertebrate organism and propagated under conditions of physical containment comparable to BL1 or BL1-N and appropriate to the organism under study (see Section V-B, *Footnotes and References of Sections I-IV*). Animals that contain sequences from viral vectors, which do not lead to transmissible infection either directly or indirectly as a result of complementation or recombination in animals, may be propagated under conditions of physical containment comparable to BL1 or BL1-N and appropriate to the organism under study. Experiments involving the introduction of other sequences from eukaryotic viral genomes into animals are covered under Section III-D-4-b, *Experiments Involving Whole Animals*. For experiments involving recombinant DNA-modified Risk Groups 2, 3, 4, or restricted organisms, see Sections V-A, V-G, and V-L, *Footnotes and References of Sections I-IV*. It is important that the investigator demonstrate that the fraction of the viral genome being utilized does not lead to productive infection. A U.S. Department of Agriculture permit is required for work with plant or animal pathogens (see Section V-G, *Footnotes and References of Sections I-IV*).

Section III-D-4-b. For experiments involving recombinant DNA, or DNA or RNA derived therefrom, involving whole animals, including transgenic animals, and not covered by Sections III-D-1, *Experiments Using Human or Animal Pathogens (Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems*, or III-D-4-a, *Experiments Involving Whole Animals*, the appropriate containment shall be determined by the Institutional Biosafety Committee.

Section III-D-4-c. Exceptions under Section III-D-4, *Experiments Involving Whole Animals*

Section III-D-4-c-(1). Experiments involving the generation of transgenic rodents that require BL1 containment are described under Section III-E-3, *Experiments Involving Transgenic Rodents*.

Section III-D-4-c-(2). The purchase or transfer of transgenic rodents is exempt from the *NIH Guidelines* under Section III-F, *Exempt Experiments* (see Appendix C-VI, *The Purchase or Transfer of Transgenic Rodents*).

Section III-D-5. Experiments Involving Whole Plants

Experiments to genetically engineer plants by recombinant DNA methods, to use such plants for other experimental purposes (e.g., response to stress), to propagate such plants, or to use plants together with microorganisms or insects containing recombinant DNA, may be conducted under the containment conditions described in Sections III-D-5-a through III-D-5-e. If experiments involving whole plants are not described in Section III-D-5 and do not fall under Sections III-A, III-B, III-D or III-F, they are included in Section III-E.

NOTE - For recombinant DNA experiments falling under Sections III-D-5-a through III-D-5-d, physical containment requirements may be reduced to the next lower level by appropriate biological containment practices, such as conducting experiments on a virus with an obligate insect vector in the absence of that vector or using a genetically attenuated strain.

Section III-D-5-a. BL3-P (Plants) or BL2-P + biological containment is recommended for experiments involving most exotic (see Section V-M, *Footnotes and References of Sections I-IV*) infectious agents with recognized potential for serious detrimental impact on managed or natural ecosystems when recombinant DNA techniques are associated with whole plants.

Section III-D-5-b. BL3-P or BL2-P + biological containment is recommended for experiments involving plants containing cloned genomes of readily transmissible exotic (see Section V-M, *Footnotes and References of Sections I-IV*) infectious agents with recognized potential for serious detrimental effects on managed or natural ecosystems in which there exists the possibility of reconstituting the complete and functional genome of the infectious agent by genomic complementation *in planta.*

Section III-D-5-c. BL4-P containment is recommended for experiments with a small number of readily transmissible exotic (see Section V-M, *Footnotes and References of Sections I-IV*) infectious agents, such as the soybean rust fungus (*Phakospora pachyrhizi*) and maize streak or other viruses in the presence of their specific arthropod vectors, that have the potential of being serious pathogens of major U.S. crops.

Section III-D-5-d. BL3-P containment is recommended for experiments involving sequences encoding potent vertebrate toxins introduced into plants or associated organisms. Recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD_{50} of <100 nanograms per kilogram body weight fall under Section III-B-1, *Experiments Involving the Cloning of Toxin Molecules with LD_{50} of Less than 100 Nanograms Per Kilogram Body Weight*, and require NIH/OBA and Institutional Biosafety Committee approval before initiation.

Section III-D-5-e. BL3-P or BL2-P + biological containment is recommended for experiments with microbial pathogens of insects or small animals associated with plants if the recombinant DNA-modified organism has a recognized potential for serious detrimental impact on managed or natural ecosystems.

Section III-D-6. Experiments Involving More than 10 Liters of Culture

The appropriate containment will be decided by the Institutional Biosafety Committee. Where appropriate, Appendix K, *Physical Containment for Large Scale Uses of Organisms Containing Recombinant DNA Molecules*, shall be used. Appendix K describes containment conditions Good Large Scale Practice through BL3-Large Scale.

Section III-D-7. Experiments Involving Influenza Viruses

Experiments with influenza viruses generated by recombinant methods (e.g., generation by reverse genetics of chimeric viruses with reassorted segments, introduction of specific mutations) shall be conducted at the biosafety level containment corresponding to the risk group of the virus that was the source of the majority of segments in the recombinant virus (e.g., experiments with viruses containing a majority of segments from a RG3 virus shall be conducted at BL3). Experiments with influenza viruses containing genes or segments from 1918-1919 H1N1 (1918 H1N1), human H2N2 (1957-1968) and highly pathogenic avian influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1) shall be conducted at BL3 enhanced containment (see Appendix G-II-C-5, Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses) unless indicated below.

Section III-D-7-a. Human H2N2 (1957-1968). Experiments with influenza viruses containing the H2 hemagglutinin (HA) segment shall be conducted at BL3 enhanced (see Appendix G-II-C-5, Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses). Experiments with the H2 HA gene in cold-adapted, live attenuated vaccine strains (e.g., A/Ann Arbor/6/60 H2N2) may be conducted at BL2 containment provided segments with mutations conferring temperature sensitivity and attenuation are not altered in the recombinant virus. Experiments with Risk Group 2 influenza viruses containing genes from human H2N2 other than the HA gene can be worked on at BL2.

Section III-D-7-b. Highly Pathogenic Avian Influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1). Experiments involving influenza viruses containing a majority of genes and/or segments from a HPAI H5N1 influenza virus shall be conducted at BL3 enhanced containment, (see Appendix G-II-C-5, Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses). Experiments involving influenza viruses containing a minority of genes and/or segments from a HPAI H5N1 influenza virus shall be conducted at BL3 enhanced unless a risk assessment performed by the IBC determines that they can be conducted safely at biosafety level 2 and after they have been excluded pursuant to 9 CFR 121.3(e). OBA is available to IBCs to provide consultation with the RAC and influenza virus experts when risk assessments are being made to determine the appropriate biocontainment for experiments with influenza viruses containing a minority of gene/segments from HPAI H5N1. Such experiments may be performed at BL3 enhanced containment or containment may be lowered to biosafety level 2, the level of containment for most research with other influenza viruses. (USDA/APHIS regulations and decisions on lowering containment also apply.) In deciding to lower containment, the IBC should consider whether, in at least two animal models (e.g., ferret, mouse, Syrian golden hamster, cotton rat, non-human primates), there is evidence that the resulting influenza virus shows reduced replication and virulence compared to the parental RG3 virus at relevant doses. This should be determined by measuring biological indices appropriate for the specific animal model (e.g., severe weight loss, elevated temperature, mortality or neurological symptoms).

Section III-D-7-c. 1918 H1N1. Experiments involving influenza viruses containing any gene or segment from 1918 H1N1 shall be conducted at BL3 enhanced containment (see Appendix G-II-C-5, Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses).

Section III-D-7-d. Antiviral Susceptibility and Containment. The availability of antiviral drugs as preventive and therapeutic measures is an important safeguard for experiments with 1918 H1N1, HPAI H5N1, and human H2N2 (1957-1968). If an influenza virus containing genes from one of these viruses is resistant to both classes of current antiviral agents, adamantanes and neuraminidase inhibitors, higher containment may be required based on the risk assessment considering transmissibility to humans, virulence, pandemic potential, alternative antiviral agents if available, etc.

Experiments with 1918 H1N1, human H2N2 (1957-1968) or HPAI H5N1 that are designed to create resistance to neuraminidase inhibitors or other effective antiviral agents (including investigational antiviral agents being developed for influenza) would be subject to Section III-A-1 (Major Actions) and require RAC review and NIH Director approval. As per Section I-A-1 of the *NIH Guidelines,* if the agent is a Select Agent, the NIH will defer to the appropriate Federal agency (HHS or USDA Select Agent Divisions) on such experiments.

Section III-E. Experiments that Require Institutional Biosafety Committee Notice Simultaneous with Initiation

Experiments not included in Sections III-A, III-B, III-C, III-D, III-F, and their subsections are considered in Section III-E. All such experiments may be conducted at BL1 containment. For experiments in this category, a registration document (see Section III-D, *Experiments that Require Institutional Biosafety Committee Approval Before Initiation*) shall be dated and signed by the investigator and filed with the local Institutional Biosafety Committee at the time the experiment is initiated. The Institutional Biosafety Committee reviews and approves all such proposals, but Institutional Biosafety Committee review and approval prior to initiation of the experiment is not required (see Section IV-A, *Policy*). For example, experiments in which all components derived from non-pathogenic prokaryotes and non-pathogenic lower eukaryotes fall under Section III-E and may be conducted at BL1 containment.

Section III-E-1. Experiments Involving the Formation of Recombinant DNA Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus

Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family being considered identical [see Section V-J, *Footnotes and References of Sections I-IV]*) may be propagated and maintained in cells in tissue culture using BL1 containment. For such experiments, it must be demonstrated that the cells lack helper virus for the specific Families of defective viruses being used. If helper virus is present, procedures specified under Section III-D-3, *Experiments Involving the Use of Infectious Animal or Plant DNA or RNA Viruses or Defective Animal or Plant DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems*, should be used. The DNA may contain fragments of the genome of viruses from more than one Family but each fragment shall be less than two-thirds of a genome.

Section III-E-2. Experiments Involving Whole Plants

This section covers experiments involving recombinant DNA-modified whole plants, and/or experiments involving recombinant DNA-modified organisms associated with whole plants, except those that fall under Section III-A, III-B, III-D, or III-F. It should be emphasized that knowledge of the organisms and judgment based on accepted scientific practices should be used in all cases in selecting the appropriate level of containment. For example, if the genetic modification has the objective of increasing pathogenicity or converting a non-pathogenic organism into a pathogen, then a higher level of containment may be appropriate depending on the organism, its mode of dissemination, and its target organisms. By contrast, a lower level of containment may be appropriate for small animals associated with many types of recombinant DNA-modified plants.

Section III-E-2-a. BL1-P is recommended for all experiments with recombinant DNA-containing plants and plant-associated microorganisms not covered in Section III-E-2-b or other sections of the *NIH Guidelines*. Examples of such experiments are those involving recombinant DNA-modified plants that are not noxious weeds or that cannot interbreed with noxious weeds in the immediate geographic area, and experiments involving whole plants and recombinant DNA-modified non-exotic (see Section V-M, *Footnotes and References of Sections I-IV*) microorganisms that have no recognized potential for rapid and widespread dissemination or for serious detrimental impact on managed or natural ecosystems (e.g., *Rhizobium* spp. and *Agrobacterium* spp.).

Section III-E-2-b. BL2-P or BL1-P + biological containment is recommended for the following experiments:

Section III-E-2-b-(1). Plants modified by recombinant DNA that are noxious weeds or can interbreed with noxious weeds in the immediate geographic area.

Section III-E-2-b-(2). Plants in which the introduced DNA represents the complete genome of a non-exotic infectious agent (see Section V-M, *Footnotes and References of Sections I-IV*).

Section III-E-2-b-(3). Plants associated with recombinant DNA-modified non-exotic microorganisms that have a recognized potential for serious detrimental impact on managed or natural ecosystems (see Section V-M, *Footnotes and References of Sections I-IV*).

Section III-E-2-b-(4). Plants associated with recombinant DNA-modified exotic microorganisms that have no recognized potential for serious detrimental impact on managed or natural ecosystems (see Section V-M, *Footnotes and References of Sections I-IV*).

Section III-E-2-b-(5). Experiments with recombinant DNA-modified arthropods or small animals associated with plants, or with arthropods or small animals with recombinant DNA-modified microorganisms associated with them if the recombinant DNA-modified microorganisms have no recognized potential for serious detrimental impact on managed or natural ecosystems (see Section V-M, *Footnotes and References of Sections I-IV*).

Section III-E-3. Experiments Involving Transgenic Rodents

This section covers experiments involving the generation of rodents in which the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic rodents). Only experiments that require BL1 containment are covered under this section; experiments that require BL2, BL3, or BL4 containment are covered under Section III-D-4, *Experiments Involving Whole Animals*.

Section III-F. Exempt Experiments

The following recombinant DNA molecules are exempt from the *NIH Guidelines* and registration with the Institutional Biosafety Committee is not required:

Section III-F-1. Those that are not in organisms or viruses.

Section III-F-2. Those that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.

Section III-F-3. Those that consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well established physiological means.

Section III-F-4. Those that consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).

Section III-F-5. Those that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the NIH Director with advice of the RAC after appropriate notice and opportunity for public comment (see Section IV-C-1-b-(1)-(c), *Major Actions*). See Appendices A-I through A-VI, *Exemptions Under Section III-F-5--Sublists of Natural Exchangers*, for a list of natural exchangers that are exempt from the *NIH Guidelines*.

Section III-F-6. Those that do not present a significant risk to health or the environment (see Section IV-C-1-b-(1)-(c), *Major Actions*), as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment. See Appendix C, *Exemptions under Section III-F-6* for other classes of experiments which are exempt from the *NIH Guidelines*.

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SECTION IV. ROLES AND RESPONSIBILITIES

Section IV-A. Policy

The safe conduct of experiments involving recombinant DNA depends on the individual conducting such activities. The *NIH Guidelines* cannot anticipate every possible situation. Motivation and good judgment are the key essentials to protection of health and the environment. The *NIH Guidelines* are intended to assist the institution, Institutional Biosafety Committee, Biological Safety Officer, and the Principal Investigator in determining safeguards that should be implemented. The *NIH Guidelines* will never be complete or final since all conceivable experiments involving recombinant DNA cannot be foreseen. Therefore, *it is the responsibility of the institution and those associated with it to adhere to the intent of the NIH Guidelines as well as to their specifics.* Each institution (and the Institutional Biosafety Committee acting on its behalf) is responsible for ensuring that all recombinant DNA research conducted at or sponsored by that institution is conducted in compliance with the *NIH Guidelines*. General recognition of institutional authority and responsibility properly establishes accountability for safe conduct of the research at the local level. The following roles and responsibilities constitute an administrative framework in which safety is an essential and integral part of research involving recombinant DNA molecules. Further clarifications and interpretations of roles and responsibilities will be issued by NIH as necessary.

Section IV-B. Responsibilities of the Institution

Section IV-B-1. General Information

Each institution conducting or sponsoring recombinant DNA research which is covered by the *NIH Guidelines* is responsible for ensuring that the research is conducted in full conformity with the provisions of the *NIH Guidelines*. In order to fulfill this responsibility, the institution shall:

Section IV-B-1-a. Establish and implement policies that provide for the safe conduct of recombinant DNA research and that ensure compliance with the *NIH Guidelines*. As part of its general responsibilities for implementing the *NIH Guidelines*, the institution may establish additional procedures, as deemed necessary, to govern the institution and its components in the discharge of its responsibilities under the *NIH Guidelines*. Such procedures may include: (i) statements formulated by the institution for the general implementation of the *NIH Guidelines*, and (ii) any additional precautionary steps the institution deems appropriate.

Section IV-B-1-b. Establish an Institutional Biosafety Committee that meets the requirements set forth in Section IV-B-2-a and carries out the functions detailed in Section IV-B-2-b.

Section IV-B-1-c. Appoint a Biological Safety Officer (who is also a member of the Institutional Biosafety Committee) if the institution: (i) conducts recombinant DNA research at Biosafety Level (BL) 3 or BL4, or (ii) engages in large-scale (greater than 10 liters) research. The Biological Safety Officer carries out the duties specified in Section IV-B-3.

Section IV-B-1-d. Appoint at least one individual with expertise in plant, plant pathogen, or plant pest containment principles (who is a member of the Institutional Biosafety Committee) if the institution conducts recombinant DNA research that requires Institutional Biosafety Committee approval in accordance with Appendix P, *Physical and Biological Containment for Recombinant DNA Research Involving Plants*.

Section IV-B-1-e. Appoint at least one individual with expertise in animal containment principles (who is a member of the Institutional Biosafety Committee) if the institution conducts recombinant DNA research that requires Institutional Biosafety Committee approval in accordance with Appendix Q, *Physical and Biological Containment for Recombinant DNA Research Involving Animals*.

Section IV-B-1-f. Ensure that when the institution participates in or sponsors recombinant DNA research involving human subjects: (i) the Institutional Biosafety Committee has adequate expertise and training (using *ad hoc* consultants as deemed necessary), (ii) all aspects of Appendix M have been appropriately addressed by the Principal Investigator; and (iii) no research participant shall be enrolled (see definition of enrollment in Section I-E-7) in a human gene transfer experiment until the RAC review process has been completed (see Appendix M-I-B, *RAC Review Requirements*), Institutional Biosafety Committee approval has been obtained,

Institutional Review Board approval has been obtained, and all applicable regulatory authorizations have been obtained. Institutional Biosafety Committee approval must be obtained from each institution at which recombinant DNA material will be administered to human subjects (as opposed to each institution involved in the production of vectors for human application and each institution at which there is *ex vivo* transduction of recombinant DNA material into target cells for human application).

Section IV-B-1-g. Assist and ensure compliance with the *NIH Guidelines* by Principal Investigators conducting research at the institution as specified in Section IV-B-7.

Section IV-B-1-h. Ensure appropriate training for the Institutional Biosafety Committee Chair and members, Biological Safety Officer and other containment experts (when applicable), Principal Investigators, and laboratory staff regarding laboratory safety and implementation of the *NIH Guidelines*. The Institutional Biosafety Committee Chair is responsible for ensuring that Institutional Biosafety Committee members are appropriately trained. The Principal Investigator is responsible for ensuring that laboratory staff are appropriately trained. The institution is responsible for ensuring that the Principal Investigator has sufficient training; however, this responsibility may be delegated to the Institutional Biosafety Committee.

Section IV-B-1-i. Determine the necessity for health surveillance of personnel involved in connection with individual recombinant DNA projects; and if appropriate, conduct a health surveillance program for such projects. The institution shall establish and maintain a health surveillance program for personnel engaged in large-scale research or production activities involving viable organisms containing recombinant DNA molecules which require BL3 containment at the laboratory scale. The institution shall establish and maintain a health surveillance program for personnel engaged in animal research involving viable recombinant DNA-containing microorganisms that require BL3 or greater containment in the laboratory. The *Laboratory Safety Monograph* discusses various components of such a program (e.g., records of agents handled, active investigation of relevant illnesses, and the maintenance of serial serum samples for monitoring serologic changes that may result from the employees' work experience). Certain medical conditions may place a laboratory worker at increased risk in any endeavor where infectious agents are handled. Examples cited in the *Laboratory Safety Monograph* include gastrointestinal disorders and treatment with steroids, immunosuppressive drugs, or antibiotics. Workers with such disorders or treatment should be evaluated to determine whether they should be engaged in research with potentially hazardous organisms during their treatment or illness. Copies of the *Laboratory Safety Monograph* are available from the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

Section IV-B-1-j. Report any significant problems, violations of the *NIH Guidelines*, or any significant research-related accidents and illnesses to NIH/OBA within thirty days, unless the institution determines that a report has already been filed by the Principal Investigator or Institutional Biosafety Committee. Reports shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

Section IV-B-2. Institutional Biosafety Committee (IBC)

The institution shall establish an Institutional Biosafety Committee whose responsibilities need not be restricted to recombinant DNA. The Institutional Biosafety Committee shall meet the following requirements:

Section IV-B-2-a. Membership and Procedures

Section IV-B-2-a-(1). The Institutional Biosafety Committee must be comprised of no fewer than five members so selected that they collectively have experience and expertise in recombinant DNA technology and the capability to assess the safety of recombinant DNA research and to identify any potential risk to public health or the environment. At least two members shall not be affiliated with the institution (apart from their membership on the Institutional Biosafety Committee) and who represent the interest of the surrounding community with respect to health and protection of the environment (e.g., officials of state or local public health or environmental protection agencies, members of other local governmental bodies, or persons active in medical, occupational health, or environmental concerns in the community). The Institutional Biosafety Committee shall include at least one individual with expertise in plant, plant pathogen, or plant pest containment principles when experiments utilizing Appendix P, *Physical and Biological Containment for Recombinant DNA Research*

Involving Plants, require prior approval by the Institutional Biosafety Committee. The Institutional Biosafety Committee shall include at least one scientist with expertise in animal containment principles when experiments utilizing Appendix Q, *Physical and Biological Containment for Recombinant DNA Research Involving Animals*, require Institutional Biosafety Committee prior approval. When the institution conducts recombinant DNA research at BL3, BL4, or Large Scale (greater than 10 liters), a Biological Safety Officer is mandatory and shall be a member of the Institutional Biosafety Committee (see Section IV-B-3, *Biological Safety Officer*). When the institution participates in or sponsors recombinant DNA research involving human research participants, the institution must ensure that: (i) the Institutional Biosafety Committee has adequate expertise and training (using *ad hoc* consultants as deemed necessary); (ii) all aspects of Appendix M have been appropriately addressed by the Principal Investigator; (iii) no research participant shall be enrolled (see definition of enrollment in Section I-E-7) in a human gene transfer experiment until the RAC review process has been completed (see Appendix M-I-B, *RAC Review Requirements*); and (iv) final IBC approval is granted only after the RAC review process has been completed (see Appendix M-I-B, *RAC Review Requirements*). Institutional Biosafety Committee approval must be obtained from the institution at which recombinant DNA material will be administered to human research participants (rather than the site involved in manufacturing gene transfer products).

Note: Individuals, corporations, and institutions not otherwise covered by the *NIH Guidelines*, are encouraged to adhere to the standards and procedures set forth in Sections I through IV (see Section IV-D, *Voluntary Compliance*. The policy and procedures for establishing an Institutional Biosafety Committee under *Voluntary Compliance*, are specified in Section IV-D-2, *Institutional Biosafety Committee Approval*).

Section IV-B-2-a-(2). In order to ensure the competence necessary to review and approve recombinant DNA activities, it is recommended that the Institutional Biosafety Committee: (i) include persons with expertise in recombinant DNA technology, biological safety, and physical containment; (ii) include or have available as consultants persons knowledgeable in institutional commitments and policies, applicable law, standards of professional conduct and practice, community attitudes, and the environment, and (iii) include at least one member representing the laboratory technical staff.

Section IV-B-2-a-(3). The institution shall file an annual report with NIH/OBA which includes: (i) a roster of all Institutional Biosafety Committee members clearly indicating the Chair, contact person, Biological Safety Officer (if applicable), plant expert (if applicable), animal expert (if applicable), human gene therapy expertise or *ad hoc* consultant(if applicable); and (ii) biographical sketches of all Institutional Biosafety Committee members (including community members).

Section IV-B-2-a-(4). No member of an Institutional Biosafety Committee may be involved (except to provide information requested by the Institutional Biosafety Committee) in the review or approval of a project in which he/she has been or expects to be engaged or has a direct financial interest.

Section IV-B-2-a-(5). The institution, that is ultimately responsible for the effectiveness of the Institutional Biosafety Committee, may establish procedures that the Institutional Biosafety Committee shall follow in its initial and continuing review and approval of applications, proposals, and activities.

Section IV-B-2-a-(6). When possible and consistent with protection of privacy and proprietary interests, the institution is encouraged to open its Institutional Biosafety Committee meetings to the public.

Section IV-B-2-a-(7). Upon request, the institution shall make available to the public all Institutional Biosafety Committee meeting minutes and any documents submitted to or received from funding agencies which the latter are required to make available to the public. If public comments are made on Institutional Biosafety Committee actions, the institution shall forward both the public comments and the Institutional Biosafety Committee's response to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

Section IV-B-2-b. Functions

On behalf of the institution, the Institutional Biosafety Committee is responsible for:

Section IV-B-2-b-(1). Reviewing recombinant DNA research conducted at or sponsored by the institution for compliance with the *NIH Guidelines* as specified in Section III, *Experiments Covered by the NIH Guidelines*, and approving those research projects that are found to conform with the *NIH Guidelines*. This review shall include: (i) independent assessment of the containment levels required by the *NIH Guidelines* for the proposed research; (ii) assessment of the facilities, procedures, practices, and training and expertise of personnel involved in recombinant DNA research; (iii) ensuring that all aspects of Appendix M have been appropriately addressed by the Principal Investigator; (iv) ensuring that no research participant is enrolled (see definition of enrollment in Section I-E-7) in a human gene transfer experiment until the RAC review process has been completed (see Appendix M-I-B, *RAC Review Requirements*), Institutional Biosafety Committee approval (from the clinical trial site) has been obtained, Institutional Review Board approval has been obtained, and all applicable regulatory authorizations have been obtained; (v) for human gene transfer protocols selected for public RAC review and discussion, consideration of the issues raised and recommendations made as a result of this review and consideration of the Principal Investigator's response to the RAC recommendations; (vi) ensuring that final IBC approval is granted only after the RAC review process has been completed (see Appendix M-I-B, *RAC Review Requirements*); and (vii) ensuring compliance with all surveillance, data reporting, and adverse event reporting requirements set forth in the *NIH Guidelines*.

Section IV-B-2-b-(2). Notifying the Principal Investigator of the results of the Institutional Biosafety Committee's review and approval.

Section IV-B-2-b-(3). Lowering containment levels for certain experiments as specified in Section III-D-2-a, *Experiments in which DNA from Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents is Cloned into Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems*.

Section IV-B-2-b-(4). Setting containment levels as specified in Sections III-D-4-b, *Experiments Involving Whole Animals*, and III-D-5, *Experiments Involving Whole Plants*.

Section IV-B-2-b-(5). Periodically reviewing recombinant DNA research conducted at the institution to ensure compliance with the *NIH Guidelines*.

Section IV-B-2-b-(6). Adopting emergency plans covering accidental spills and personnel contamination resulting from recombinant DNA research.

Note: The *Laboratory Safety Monograph* describes basic elements for developing specific procedures dealing with major spills of potentially hazardous materials in the laboratory, including information and references about decontamination and emergency plans. The NIH and the Centers for Disease Control and Prevention are available to provide consultation and direct assistance, if necessary, as posted in the *Laboratory Safety Monograph*. The institution shall cooperate with the state and local public health departments by reporting any significant research-related illness or accident that may be hazardous to the public health.

Section IV-B-2-b-(7). Reporting any significant problems with or violations of the *NIH Guidelines* and any significant research-related accidents or illnesses to the appropriate institutional official and NIH/OBA within 30 days, unless the Institutional Biosafety Committee determines that a report has already been filed by the Principal Investigator. Reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

Section IV-B-2-b-(8). The Institutional Biosafety Committee may not authorize initiation of experiments which are not explicitly covered by the *NIH Guidelines* until NIH (with the advice of the RAC when required) establishes the containment requirement.

Section IV-B-2-b-(9). Performing such other functions as may be delegated to the Institutional Biosafety Committee under Section IV-B-2, *Institutional Biosafety Committee*.

Section IV-B-3. Biological Safety Officer (BSO)

Section IV-B-3-a. The institution shall appoint a Biological Safety Officer if it engages in large-scale research or production activities involving viable organisms containing recombinant DNA molecules.

Section IV-B-3-b. The institution shall appoint a Biological Safety Officer if it engages in recombinant DNA research at BL3 or BL4. The Biological Safety Officer shall be a member of the Institutional Biosafety Committee.

Section IV-B-3-c. The Biological Safety Officer's duties include, but are not be limited to:

Section IV-B-3-c-(1). Periodic inspections to ensure that laboratory standards are rigorously followed;

Section IV-B-3-c-(2). Reporting to the Institutional Biosafety Committee and the institution any significant problems, violations of the *NIH Guidelines*, and any significant research-related accidents or illnesses of which the Biological Safety Officer becomes aware unless the Biological Safety Officer determines that a report has already been filed by the Principal Investigator;

Section IV-B-3-c-(3). Developing emergency plans for handling accidental spills and personnel contamination and investigating laboratory accidents involving recombinant DNA research;

Section IV-B-3-c-(4). Providing advice on laboratory security;

Section IV-B-3-c-(5). Providing technical advice to Principal Investigators and the Institutional Biosafety Committee on research safety procedures.

Note: See the *Laboratory Safety Monograph* for additional information on the duties of the Biological Safety Officer.

Section IV-B-4. Plant, Plant Pathogen, or Plant Pest Containment Expert

When the institution conducts recombinant DNA research that requires Institutional Biosafety Committee approval in accordance with Appendix P, *Physical and Biological Containment for Recombinant DNA Research Involving Plants*, the institution shall appoint at least one individual with expertise in plant, plant pathogen, or plant pest containment principles (who is a member of the Institutional Biosafety Committee).

Section IV-B-5. Animal Containment Expert

When the institution conducts recombinant DNA research that requires Institutional Biosafety Committee approval in accordance with Appendix Q, *Physical and Biological Containment for Recombinant DNA Research Involving Animals*, the institution shall appoint at least one individual with expertise in animal containment principles (who is a member of the Institutional Biosafety Committee).

Section IV-B-6. Human Gene Therapy Expertise

When the institution participates in or sponsors recombinant DNA research involving human subjects, the institution must ensure that: (i) the Institutional Biosafety Committee has adequate expertise and training (using *ad hoc* consultants as deemed necessary) and (ii) all aspects of Appendix M, *Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA Molecules into One or More Human Subjects (Points to Consider)* , have been appropriately addressed by the Principal Investigator prior to submission to NIH/OBA.

Section IV-B-7. Principal Investigator (PI)

On behalf of the institution, the Principal Investigator is responsible for full compliance with the *NIH Guidelines* in the conduct of recombinant DNA research. A Principal Investigator engaged in human gene transfer research may delegate to another party, such as a corporate sponsor, the reporting functions set forth in Appendix M, with written notification to the NIH OBA of the delegation and of the name(s), address, telephone, and fax numbers of the contact. The Principal Investigator is responsible for ensuring that the reporting requirements are fulfilled and will be held accountable for any reporting lapses.

Section IV-B-7-a. General Responsibilities

As part of this general responsibility, the Principal Investigator shall:

Section IV-B-7-a-(1). Initiate or modify no recombinant DNA research which requires Institutional Biosafety Committee approval prior to initiation (see Sections III-A, III-B, III-C, III-D, and III-E, *Experiments Covered by the NIH Guidelines*) until that research or the proposed modification thereof has been approved by the Institutional Biosafety Committee and has met all other requirements of the *NIH Guidelines*;

Section IV-B-7-a-(2). Determine whether experiments are covered by Section III-E, *Experiments that Require Institutional Biosafety Committee Notice Simultaneous with Initiation*, and ensure that the appropriate procedures are followed;

Section IV-B-7-a-(3). Report any significant problems, violations of the *NIH Guidelines*, or any significant research-related accidents and illnesses to the Biological Safety Officer (where applicable), Greenhouse/Animal Facility Director (where applicable), Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities (if applicable) within 30 days. Reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax);

Section IV-B-7-a-(4). Report any new information bearing on the *NIH Guidelines* to the Institutional Biosafety Committee and to NIH/OBA (reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax);

Section IV-B-7-a-(5). Be adequately trained in good microbiological techniques;

Section IV-B-7-a-(6). Adhere to Institutional Biosafety Committee approved emergency plans for handling accidental spills and personnel contamination; and

Section IV-B-7-a-(7). Comply with shipping requirements for recombinant DNA molecules (see Appendix H, *Shipment*, for shipping requirements and the *Laboratory Safety Monograph* for technical recommendations).

Section IV-B-7-b. Information to Be Submitted by the Principal Investigator to NIH OBA

The Principal Investigator shall:

Section IV-B-7-b-(1). Submit information to NIH/OBA for certification of new host-vector systems;

Section IV-B-7-b-(2). Petition NIH/OBA, with notice to the Institutional Biosafety Committee, for proposed exemptions to the *NIH Guidelines*;

Section IV-B-7-b-(3). Petition NIH/OBA, with concurrence of the Institutional Biosafety Committee, for approval to conduct experiments specified in Sections III-A-1, *Major Actions Under the NIH Guidelines*, and III-B, *Experiments that Require NIH/OBA and Institutional Biosafety Committee Approval Before Initiation*;

Section IV-B-7-b-(4). Petition NIH/OBA for determination of containment for experiments requiring case-by-case review; and

Section IV-B-7-b-(5). Petition NIH/OBA for determination of containment for experiments not covered by the *NIH Guidelines*.

Section IV-B-7-b-(6). Ensure that all aspects of Appendix M have been appropriately addressed prior to submission of a human gene transfer experiment to NIH OBA, and provide a letter signed by the Principal Investigator(s) on institutional letterhead acknowledging that the documentation being submitted to NIH OBA complies with the requirements set forth in Appendix M. No research participant shall be enrolled (see definition of enrollment in Section I-E-7) in a human gene transfer experiment until the RAC review process has been

completed (see Appendix M-I-B, *RAC Review Requirements*); IBC approval (from the clinical trial site) has been obtained; Institutional Review Board (IRB) approval has been obtained; and all applicable regulatory authorization(s) have been obtained.

For a clinical trial site that is added after the RAC review process, no research participant shall be enrolled (see definition of enrollment in Section I-E-7) at the clinical trial site until the following documentation has been submitted to NIH OBA: (1) IBC approval (from the clinical trial site); (2) IRB approval; (3) IRB-approved informed consent document; (4) curriculum vitae of the principal investigator(s) (no more than two pages in biographical sketch format); and (5) NIH grant number(s) if applicable.

Section IV-B-7-c. Submissions by the Principal Investigator to the Institutional Biosafety Committee

The Principal Investigator shall:

Section IV-B-7-c-(1). Make an initial determination of the required levels of physical and biological containment in accordance with the *NIH Guidelines*;

Section IV-B-7-c-(2). Select appropriate microbiological practices and laboratory techniques to be used for the research;

Section IV-B-7-c-(3). Submit the initial research protocol and any subsequent changes (e.g., changes in the source of DNA or host-vector system), if covered under Sections III-A, III-B, III-C, III-D, or III-E (*Experiments Covered by the NIH Guidelines*), to the Institutional Biosafety Committee for review and approval or disapproval; and

Section IV-B-7-c-(4). Remain in communication with the Institutional Biosafety Committee throughout the conduct of the project.

Section IV-B-7-d. Responsibilities of the Principal Investigator Prior to Initiating Research

The Principal Investigator shall:

Section IV-B-7-d-(1). Make available to all laboratory staff the protocols that describe the potential biohazards and the precautions to be taken;

Section IV-B-7-d-(2). Instruct and train laboratory staff in: (i) the practices and techniques required to ensure safety, and (ii) the procedures for dealing with accidents; and

Section IV-B-7-d-(3). Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection).

Section IV-B-7-e. Responsibilities of the Principal Investigator During the Conduct of the Research

The Principal Investigator shall:

Section IV-B-7-e-(1). Supervise the safety performance of the laboratory staff to ensure that the required safety practices and techniques are employed;

Section IV-B-7-e-(2). Investigate and report any significant problems pertaining to the operation and implementation of containment practices and procedures in writing to the Biological Safety Officer (where applicable), Greenhouse/Animal Facility Director (where applicable), Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities (if applicable) (reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax);

Section IV-B-7-e-(3). Correct work errors and conditions that may result in the release of recombinant DNA materials; and

Section IV-B-7-e-(4). Ensure the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., purity and genotypic and phenotypic characteristics).

Section IV-B-7-e-(5). Comply with reporting requirements for human gene transfer experiments conducted in compliance with the *NIH Guidelines* (see Appendix M-I-C, *Reporting Requirements*).

Section IV-C. Responsibilities of the National Institutes of Health (NIH)

Section IV-C-1. NIH Director

The NIH Director is responsible for: (i) establishing the *NIH Guidelines*, (ii) overseeing their implementation, and (iii) their final interpretation. The NIH Director has responsibilities under the *NIH Guidelines* that involve OBA and RAC. OBA's responsibilities under the *NIH Guidelines* are administrative. Advice from RAC is primarily scientific, technical, and ethical. In certain circumstances, there is specific opportunity for public comment with published response prior to final action.

Section IV-C-1-a. General Responsibilities

The NIH Director is responsible for:

Section IV-C-1-a-(1). Promulgating requirements as necessary to implement the *NIH Guidelines*;

Section IV-C-1-a-(2). Establishing and maintaining RAC to carry out the responsibilities set forth in Section IV-C-2, *Recombinant DNA Advisory Committee* (RAC membership is specified in its charter and in Section IV-C-2);

Section IV-C-1-a-(3). Establishing and maintaining NIH/OBA to carry out the responsibilities defined in Section IV-C-3, *Office of Biotechnology Activities*;

Section IV-C-1-a-(4). Conducting and supporting training programs in laboratory safety for Institutional Biosafety Committee members, Biological Safety Officers and other institutional experts (if applicable), Principal Investigators, and laboratory staff.

Section IV-C-1-a-(5). Establishing and convening Gene Therapy Policy Conferences as described in Appendix L, *Gene Therapy Policy Conferences.*

Section IV-C-1-b. Specific Responsibilities

In carrying out the responsibilities set forth in this section, the NIH Director, or a designee shall weigh each proposed action through appropriate analysis and consultation to determine whether it complies with the *NIH Guidelines* and presents no significant risk to health or the environment.

Section IV-C-1-b-(1). Major Actions

To execute *Major Actions*, the NIH Director shall seek the advice of RAC and provide an opportunity for public and Federal agency comment. Specifically, the Notice of Meeting and *Proposed Actions* shall be published in the *Federal Register* at least 15 days before the RAC meeting. The NIH Director's decision/recommendation (at his/her discretion) may be published in the *Federal Register* for 15 days of comment before final action is taken. The NIH Director's final decision/recommendation, along with responses to public comments, shall be published in the *Federal Register*. The RAC and Institutional Biosafety Committee Chairs shall be notified of the following decisions:

Section IV-C-1-b-(1)-(a). Changing containment levels for types of experiments that are specified in the *NIH Guidelines* when a *Major Action* is involved;

Section IV-C-1-b-(1)-(b). Assigning containment levels for types of experiments that are not explicitly considered in the *NIH Guidelines* when a *Major Action* is involved;

Section IV-C-1-b-(1)-(c). Promulgating and amending a list of classes of recombinant DNA molecules to be exempt from the *NIH Guidelines* because they consist entirely of DNA segments from species that exchange DNA by known physiological processes or otherwise do not present a significant risk to health or the environment;

Section IV-C-1-b-(1)-(d). Permitting experiments specified by Section III-A, *Experiments that Require Institutional Biosafety Committee Approval, RAC Review, and NIH Director Approval Before Initiation*;

Section IV-C-1-b-(1)-(e). Certifying new host-vector systems with the exception of minor modifications of already certified systems (the standards and procedures for certification are described in Appendix I-II, *Certification of Host-Vector Systems*). Minor modifications constitute (e.g., those of minimal or no consequence to the properties relevant to containment); and

Section IV-C-1-b-(1)-(f). Adopting other changes in the *NIH Guidelines*.

Section IV-C-1-b-(2). Minor Actions

NIH/OBA shall carry out certain functions as delegated to it by the NIH Director (see Section IV-C-3, *Office of Biotechnology Activities*). *Minor Actions* (as determined by NIH/OBA in consultation with the RAC Chair and one or more RAC members, as necessary) will be transmitted to RAC and Institutional Biosafety Committee Chairs:

Section IV-C-1-b-(2)-(a). Changing containment levels for experiments that are specified in Section III, *Experiments Covered by the NIH Guidelines* (except when a *Major Action* is involved);

Section IV-C-1-b-(2)-(b). Assigning containment levels for experiments not explicitly considered in the *NIH Guidelines*;

Section IV-C-1-b-(2)-(c). Revising the *Classification of Etiologic Agents* for the purpose of these *NIH Guidelines* (see Section V-A, *Footnotes and References of Sections I-IV*).

Section IV-C-1-b-(2)-(d). Interpreting the *NIH Guidelines* for experiments to which the *NIH Guidelines* do not specifically assign containment levels;

Section IV-C-1-b-(2)-(e). Setting containment under Sections III-D-1-d, *Experiments Using Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems*, and III-D-2-b, *Experiments in which DNA from Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents is Cloned into Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems*;

Section IV-C-1-b-(2)-(f). Approving minor modifications of already certified host-vector systems (the standards and procedures for such modifications are described in Appendix I-II, *Certification of Host-Vector Systems*);

Section IV-C-1-b-(2)-(g). Decertifying already certified host-vector systems;

Section IV-C-1-b-(2)-(h). Adding new entries to the list of molecules toxic for vertebrates (see Appendix F, *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*); and

Section IV-C-1-b-(2)-(i). Determining appropriate containment conditions for experiments according to case precedents developed under Section IV-C-1-b-(2)-(c).

Section IV-C-2. Recombinant DNA Advisory Committee (RAC)

The RAC is responsible for carrying out the functions specified in the *NIH Guidelines*, as well as others specified in its charter or assigned by the Secretary of Health and Human Services or the NIH Director. The RAC membership and procedures, in addition to those set forth in the *NIH Guidelines*, are specified in the charter for the RAC which is filed as provided in the General Services Administration Federal Advisory Committee Management regulations, 41 CFR part 101-6, and is available on the OBA web site, http://www4.od.nih.gov/oba/rac/RACcharter2002.pdf. In the event of a conflict between the *NIH Guidelines* and the charter, the charter shall control.

The RAC will consist of not less than 15 voting members, including the Chair, appointed under the procedures of the NIH and the Department of Health and Human Services. The maximum number of voting members will be established in the charter of the RAC. At least a majority of the voting members must be knowledgeable in relevant scientific fields, e.g., molecular genetics, molecular biology, recombinant DNA research, including clinical gene transfer research. At least 4 members of the RAC must be knowledgeable in fields such as public health, laboratory safety, occupational health, protection of human subjects of research, the environment, ethics, law, public attitudes or related fields. Representatives of the Federal agencies listed in the charter shall serve as non-voting members. Nominations for RAC members may be submitted to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax).

All meetings of the RAC shall be announced in the *Federal Register*, including tentative agenda items, 15 days before the meeting. Final agendas, if modified, shall be available at least 72 hours before the meeting. No item defined as a *Major Action* under Section IV-C-1-b-(1) may be added to an agenda following *Federal Register* publication.

RAC shall be responsible for:

Section IV-C-2-a. Advising the NIH Director on the following actions: (1) Adopting changes in the *NIH Guidelines*. (2) Assigning containment levels, changing containment levels, and approving experiments considered as *Major Actions* under the *NIH Guidelines*, i.e., the deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally, if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture. (3) Promulgating and amending lists of classes of recombinant DNA molecules to be exempt from the *NIH Guidelines* because they consist entirely of DNA segments from species that exchange DNA by known physiological processes or otherwise do not present a significant risk to health or the environment. (4) Certifying new host-vector systems.

Section IV-C-2-b. Identifying novel human gene transfer experiments deserving of public discussion by the full RAC;

Section IV-C-2-c. Transmitting to the NIH Director specific comments/ recommendations about: (i) a specific human gene transfer experiment, or (ii) a category of human gene transfer experiments;

Section IV-C-2-d. Publicly reviewing human gene transfer clinical trial data and relevant information evaluated and summarized by NIH/OBA in accordance with the annual data reporting requirements;

Section IV-C-2-e. Identifying broad scientific, safety, social, and ethical issues relevant to gene therapy research as potential Gene Therapy Policy Conference topics;

Section IV-C-2-f. Identifying novel social and ethical issues relevant to specific human applications of gene transfer and recommending appropriate modifications to the *Points to Consider* that will provide guidance in the preparation of relevant Informed Consent documents; and

Section IV-C-2-g. Identifying novel scientific and safety issues relevant to specific human applications of gene transfer and recommending appropriate modifications to the *Points to Consider* that will provide guidance in the design and submission of human gene transfer clinical trials.

Section IV-C-3. Office of Biotechnology Activities (OBA)

OBA shall serve as a focal point for information on recombinant DNA activities and provide advice to all within and outside NIH including institutions, Biological Safety Officers, Principal Investigators, Federal agencies, state and local governments, and institutions in the private sector. OBA shall carry out such other functions as may be delegated to it by the NIH Director. OBA's responsibilities include (but are not limited to) the following:

Section IV-C-3-a. Serving as the focal point for public access to summary information pertaining to human gene transfer experiments;

Section IV-C-3-b. Serving as the focal point for data management of human gene transfer experiments;

Section IV-C-3-c. Administering the annual data reporting requirements (and subsequent review) for human gene transfer experiments (see Appendix M-I-C, *Reporting Requirements*);

Section IV-C-3-d. Transmitting comments/recommendations arising from public RAC discussion of a novel human gene transfer experiment to the NIH Director. RAC recommendations shall be forwarded to the Principal Investigator, the sponsoring institution, and other DHHS components, as appropriate.

Section IV-C-3-e. Collaborating with Principal Investigators, Institutional Biosafety Committees, Institutional Review Boards, and other DHHS components (including FDA and the Office for Human Research Protections), to ensure human gene transfer experiment registration compliance in accordance with Appendix M-I, *Requirements for Protocol Submission, Review, and Reporting-Human Gene Transfer Experiments* of the *NIH Guidelines*.

Section IV-C-3-f. Administering Gene Therapy Policy Conferences as deemed appropriate by the NIH Director (see Appendix L, *Gene Therapy Policy Conferences*).

Section IV-C-3-g. Reviewing and approving experiments in conjunction with *ad hoc* experts involving the cloning of genes encoding for toxin molecules that are lethal for vertebrates at an LD_{50} of less than or equal to 100 nanograms per kilogram body weight in organisms other than *Escherichia coli* K-12 (see Section III-B-1, *Experiments Involving the Cloning of Toxin Molecules with LD_{50} of Less than 100 Nanograms Per Kilogram Body Weight*, Appendix F, *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*);

Section IV-C-3-h. Serving as the executive secretary of RAC;

Section IV-C-3-i. Publishing in the *Federal Register*:

Section IV-C-3-i-(1). Announcements of RAC meetings and tentative agendas at least 15 days in advance (Note: If the agenda for a RAC meeting is modified, OBA shall make the revised agenda available to anyone upon request in advance of the meeting);

Section IV-C-3-i-(2). Announcements of Gene Therapy Policy Conferences and tentative agendas at least 15 days in advance;

Section IV-C-3-i-(3). Proposed *Major Actions* (see Section IV-C-1-b-(1), *Major Actions*) at least 15 days prior to the RAC meeting; and

Section IV-C-3-j. Reviewing and approving the membership of an institution's Institutional Biosafety Committee, and where it finds the Institutional Biosafety Committee meets the requirements set forth in Section IV-B-2, *Institutional Biosafety Committee (IBC)*, giving its approval to the Institutional Biosafety Committee membership.

Section IV-C-4. Other NIH Components

Other NIH components shall be responsible for certifying maximum containment (BL4) facilities, inspecting them periodically, and inspecting other recombinant DNA facilities as deemed necessary.

Section IV-D. Voluntary Compliance

Section IV-D-1. Basic Policy - Voluntary Compliance

Individuals, corporations, and institutions not otherwise covered by the *NIH Guidelines* are encouraged to follow the standards and procedures set forth in Sections I through IV. In order to simplify discussion, references hereafter to "institutions" are intended to encompass corporations and individuals who have no organizational affiliation. For purposes of complying with the *NIH Guidelines*, an individual intending to carry out research involving recombinant DNA is encouraged to affiliate with an institution that has an Institutional Biosafety Committee approved under the *NIH Guidelines*.

Since commercial organizations have special concerns, such as protection of proprietary data, some modifications and explanations of the procedures are provided in Sections IV-D-2 through IV-D-5-b, *Voluntary Compliance*, in order to address these concerns.

Section IV-D-2. Institutional Biosafety Committee Approval - Voluntary Compliance

It should be emphasized that employment of an Institutional Biosafety Committee member solely for purposes of membership on the Institutional Biosafety Committee does not itself make the member an institutionally affiliated member. Except for the unaffiliated members, a member of an Institutional Biosafety Committee for an institution not otherwise covered by the *NIH Guidelines* may participate in the review and approval of a project in which the member has a direct financial interest so long as the member has not been, and does not expect to be, engaged in the project. Section IV-B-2-a-(4), *Institutional Biosafety Committee*, is modified to that extent for purposes of these institutions.

Section IV-D-3. Certification of Host-Vector Systems - Voluntary Compliance

A host-vector system may be proposed for certification by the NIH Director in accordance with the procedures set forth in Appendix I-II, *Certification of Host-Vector Systems*. In order to ensure protection for proprietary data, any public notice regarding a host-vector system which is designated by the institution as proprietary under Section IV-D, *Voluntary Compliance*, will be issued only after consultation with the institution as to the content of the notice.

Section IV-D-4. Requests for Exemptions and Approvals - Voluntary Compliance

Requests for exemptions or other approvals as required by the *NIH Guidelines* should be submitted based on the procedures set forth in Sections I through IV. In order to ensure protection for proprietary data, any public notice regarding a request for an exemption or other approval which is designated by the institution as proprietary under Section IV-D-5-a, *Voluntary Compliance*, will be issued only after consultation with the institution as to the content of the notice.

Section IV-D-5. Protection of Proprietary Data - Voluntary Compliance

Section IV-D-5-a. General

In general, the Freedom of Information Act requires Federal agencies to make their records available to the public upon request. However, this requirement does not apply to, among other things, "trade secrets and commercial or financial information that is obtained from a person and that is privileged or confidential." Under 18 U.S.C. 1905, it is a criminal offense for an officer or employee of the U.S. or any Federal department or agency to publish, divulge, disclose, or make known "in any manner or to any extent not authorized by law any information coming to him in the course of his employment or official duties or by reason of any examination or investigation made by, or return, report or record made to or filed with, such department or agency or officer or employee thereof, which information concerns or relates to the trade secrets, (or) processes...of any person, firm, partnership, corporation, or association." This provision applies to all employees of the Federal Government, including special Government employees. Members of RAC are "special Government employees."

In submitting to NIH for purposes of voluntary compliance with the *NIH Guidelines*, an institution may designate those items of information which the institution believes constitute trade secrets, privileged, confidential, commercial, or financial information. If NIH receives a request under the Freedom of Information Act for information so designated, NIH will promptly contact the institution to secure its views as to whether the information (or some portion) should be released. If NIH decides to release this information (or some portion) in response to a Freedom of Information request or otherwise, the institution will be advised and the actual release will be delayed in accordance with 45 Code of Federal Regulations, Section 5.65(d) and (e).

Section IV-D-5-b. Pre-submission Review

Any institution not otherwise covered by the *NIH Guidelines*, which is considering submission of data or information voluntarily to NIH, may request pre-submission review of the records involved to determine if NIH will make all or part of the records available upon request under the Freedom of Information Act.

A request for pre-submission review should be submitted to NIH/OBA along with the records involved to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). These records shall be clearly marked as being the property of the institution on loan to NIH solely for the purpose of making a determination under the Freedom on Information Act. NIH/OBA will seek a determination from the responsible official under DHHS regulations (45 CFR Part 5) as to whether the records involved, (or some portion) will be made available to members of the public under the Freedom of Information Act. Pending such a determination, the records will be kept separate from NIH/OBA files, will be considered records of the institution and not NIH/OBA, and will not be received as part of NIH/OBA files. No copies will be made of such records.

NIH/OBA will inform the institution of the NIH Freedom of Information Officer's determination and follow the institution's instructions as to whether some or all of the records involved are to be returned to the institution or to become a part of NIH/OBA files. If the institution instructs NIH/OBA to return the records, no copies or summaries of the records will be made or retained by DHHS, NIH, or OBA. The NIH Freedom of Information Officer's determination will represent that official's judgment at the time of the determination as to whether the records involved (or some portion) would be exempt from disclosure under the Freedom on Information Act if at the time of the determination the records were in NIH/OBA files and a request was received for such files under the Freedom of Information Act.

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SECTION V. FOOTNOTES AND REFERENCES OF SECTIONS I THROUGH IV

Section V-A. The NIH Director, with advice of the RAC, may revise the classification for the purposes of the *NIH Guidelines* (see Section IV-C-1-b-(2)-(e), *Minor Actions*). The revised list of organisms in each risk group is reprinted in Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*.

Section V-B. Section III, *Experiments Covered by the NIH Guidelines*, describes a number of places where judgments are to be made. In all these cases, the Principal Investigator shall make the judgment on these matters as part of his/her responsibility to "make the initial determination of the required levels of physical and biological containment in accordance with the *NIH Guidelines*" (see Section IV-B-7-c-(1)). For cases falling under Sections III-A through III-E, *Experiments Covered by the NIH Guidelines*, this judgment is to be reviewed and approved by the Institutional Biosafety Committee as part of its responsibility to make an "independent assessment of the containment levels required by the *NIH Guidelines* for the proposed research" (see Section IV-B-2-b-(1), *Institutional Biosafety Committee*). The Institutional Biosafety Committee may refer specific cases to NIH/OBA as part of NIH/OBA's functions to "provide advice to all within and outside NIH" (see Section IV-C-3,). NIH/OBA may request advice from the RAC as part of the RAC's responsibility for "interpreting the *NIH Guidelines* for experiments to which the *NIH Guidelines* do not specifically assign containment levels" (see Section IV-C-1-b-(2)-(f), *Minor Actions*).

Section V-C. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and the National Institutes of Health. *Biosafety in Microbiological and Biomedical Laboratories*, 5th Edition, 2007. Copies are available from: Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20401-0001, Phone (202) 512-1800 [http://www.gpo.gov/].

Section V-D. *Classification of Etiologic Agents on the Basis of Hazard*, 4th Edition, July 1974, U.S. Department of Health, Education, and Welfare, Public Health Service, Centers for Disease Control, Office of Biosafety, Atlanta, Georgia 30333.

Section V-E. Chin, James ed., *Control of Communicable Diseases Manual*, 17th Edition, 2000. ISBN: 087553-242-X, American Public Health Association, 800 I Street, N.W., Washington, D.C. Phone: (202) 777-2742.

Section V-F. *World Health Organization Laboratory Biosafety Manual*, 2nd edition. 1993. WHO Albany, NY. Copies are available from: WHO Publication Centre, USA, (Q Corp) 49 Sheridan Avenue, Albany, New York 12210; Phone: (518) 436-9686 (Order # 1152213).

Section V-G. A U.S. Department of Agriculture permit, required for import and interstate transport of plant and animal pathogens, may be obtained from the U.S. Department of Agriculture, ATTN: Animal and Plant Health Inspection Service (APHIS), Veterinary Services, National Center for Import-Export, Products Program, 4700 River Road, Unit 40, Riverdale, Maryland 20737. Phone: (301) 734-8499; Fax: (301) 734-8226.

Section V-H. American Type Culture Collection Catalogues of plant viruses, animal viruses, cells, bacteria, fungi, etc. are available from American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209. Phone: (703) 365-2700.

Section V-I. U.S. Department of Labor, Occupational Safety and Health Administration, 29 CFR 1910.1030, *Bloodborne Pathogens*. See also*, Exposure to Bloodborne Pathogens,* OSHA 3127, 1996 (Revised).

Section V-J. As classified in the *Virus Taxonomy: The Classification and Nomenclature of Viruses. The Seventh Report of the International Committee on Taxonomy of Viruses*, Academic Press, 2000 (0123702003) San Diego, CA.

Section V-K. *i.e.*, the total of all genomes within a family shall not exceed two-thirds of the genome.

Section V-L. Organisms including alastrim, smallpox (variola) and whitepox may not be studied in the United States except at specified facilities. All activities, including storage of variola and whitepox, are restricted to the single national facility (World Health Organization Collaborating Center for Smallpox Research, Centers for Disease Control and Prevention, Atlanta, Georgia).

Section V-M. In accordance with accepted scientific and regulatory practices of the discipline of plant pathology, an exotic plant pathogen (e.g., virus, bacteria, or fungus) is one that is unknown to occur within the U.S. (see Section V-G, *Footnotes and References of Sections I-IV*). Determination of whether a pathogen has a potential for serious detrimental impact on managed (agricultural, forest, grassland) or natural ecosystems should be made by the Principal Investigator and the Institutional Biosafety Committee, in consultation with scientists knowledgeable of plant diseases, crops, and ecosystems in the geographic area of the research.

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APPENDIX A. EXEMPTIONS UNDER SECTION III-F-5--SUBLISTS OF NATURAL EXCHANGERS

Certain specified recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent are exempt from these *NIH Guidelines* (see Section III-F-5, *Exempt Experiments*). Institutional Biosafety Committee registration is not required for these exempt experiments. A list of such exchangers will be prepared and periodically revised by the NIH Director with advice from the RAC after appropriate notice and opportunity for public comment (see Section IV-C-1-b-(1)-(c), *NIH Director--Specific Responsibilities*). For a list of natural exchangers that are exempt from the *NIH Guidelines*, see Appendices A-I through A-VI, *Exemptions Under Section III-F-5 Sublists of Natural Exchangers*. Section III-F-5, *Exempt Experiments*, describes recombinant DNA molecules that are: (1) composed entirely of DNA segments from one or more of the organisms within a sublist, and (2) to be propagated in any of the organisms within a sublist (see *Classification of Bergey's Manual of Determinative Bacteriology*; 8th edition, R. E. Buchanan and N. E. Gibbons, editors, Williams and Wilkins Company; Baltimore, Maryland 1984). Although these experiments are exempt, it is recommended that they be performed at the appropriate biosafety level for the host or recombinant organism (see *Biosafety in Microbiological and Biomedical Laboratories*, 5th edition, 2007, U.S. DHHS, Public Health Service, Centers for Disease Control and Prevention, Atlanta, Georgia, and NIH Office of Biosafety, Bethesda, Maryland).

Appendix A-I. Sublist A

Genus Escherichia
Genus *Shigella*
Genus *Salmonella* - including *Arizona*
Genus *Enterobacter*
Genus *Citrobacter* - including *Levinea*
Genus *Klebsiella* - including *oxytoca*
Genus *Erwinia*
Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas fluorescens, and Pseudomonas mendocina
Serratia marcescens
Yersinia enterocolitica

Appendix A-II. Sublist B

Bacillus subtilis
Bacillus licheniformis
Bacillus pumilus
Bacillus globigii
Bacillus niger
Bacillus nato
Bacillus amyloliquefaciens
Bacillus aterrimus

Appendix A-III. Sublist C

Streptomyces aureofaciens
Streptomyces rimosus
Streptomyces coelicolor

Appendix A-IV. Sublist D

Streptomyces griseus
Streptomyces cyaneus
Streptomyces venezuelae

Appendix A-V. Sublist E

One way transfer of *Streptococcus mutans* or *Streptococcus lactis* DNA into *Streptococcus sanguis*

Appendix A-VI. Sublist F

Streptococcus sanguis
Streptococcus pneumoniae
Streptococcus faecalis
Streptococcus pyogenes
Streptococcus mutans

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APPENDIX B. CLASSIFICATION OF HUMAN ETIOLOGIC AGENTS ON THE BASIS OF HAZARD

This appendix includes those biological agents known to infect humans as well as selected animal agents that may pose theoretical risks if inoculated into humans. Included are lists of representative genera and species known to be pathogenic; mutated, recombined, and non-pathogenic species and strains are not considered. Non-infectious life cycle stages of parasites are excluded.

This appendix reflects the current state of knowledge and should be considered a resource document. Included are the more commonly encountered agents and is not meant to be all-inclusive. Information on agent risk assessment may be found in the *Agent Summary Statements* of the CDC/NIH publication, *Biosafety in Microbiological and Biomedical Laboratories* (see Sections V-C, V-D, V-E, and V-F, *Footnotes and References of Sections I through IV*. Further guidance on agents not listed in Appendix B may be obtained through: Centers for Disease Control and Prevention, Biosafety Branch, Atlanta, Georgia 30333, Phone: (404) 639-3883, Fax: (404) 639-2294; National Institutes of Health, Division of Safety, Bethesda, Maryland 20892, Phone: (301) 496-1357; National Animal Disease Center, U.S. Department of Agriculture, Ames, Iowa 50010, Phone: (515) 862-8258.

A special committee of the American Society for Microbiology will conduct an annual review of this appendix and its recommendation for changes will be presented to the Recombinant DNA Advisory Committee as proposed amendments to the *NIH Guidelines*.

Appendix B - Table 1. Basis for the Classification of Biohazardous Agents by Risk Group (RG)

Risk Group	Description
Risk Group 1 (RG1)	Agents that are not associated with disease in healthy adult humans
Risk Group 2 (RG2)	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available
Risk Group 3 (RG3)	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available (high individual risk but low community risk)
Risk Group 4 (RG4)	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available (high individual risk and high community risk)

Appendix B-I. Risk Group 1 (RG1) Agents

RG1 agents are not associated with disease in healthy adult humans. Examples of RG1 agents include asporogenic *Bacillus subtilis* or *Bacillus licheniformis* (see Appendix C-IV-A, *Bacillus subtilis* or *Bacillus licheniformis* Host-Vector Systems, Exceptions); adeno- associated virus (AAV) types 1 through 4; and recombinant AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and are produced in the absence of a helper virus. A strain of *Escherichia coli* (see Appendix C-II-A, *Escherichia coli* K-12 Host Vector Systems, Exceptions) is an RG1 agent if it (1) does not possess a complete lipopolysaccharide (*i.e.*, lacks the O antigen); and (2) does not carry any active virulence factor (*e.g.*, toxins) or colonization factors and does not carry any genes encoding these factors.

Those agents not listed in Risk Groups (RGs) 2, 3 and 4 are not automatically or implicitly classified in RG1; a risk assessment must be conducted based on the known and potential properties of the agents and their relationship to agents that are listed.

Appendix B-II. Risk Group 2 (RG2) Agents

RG2 agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available.

Appendix B-II-A. Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia

--*Acinetobacter baumannii* (formerly *Acinetobacter calcoaceticus*)
--*Actinobacillus*
--*Actinomyces pyogenes* (formerly *Corynebacterium pyogenes*)
--*Aeromonas hydrophila*
--*Amycolata autotrophica*
--*Archanobacterium haemolyticum* (formerly *Corynebacterium haemolyticum*)
--*Arizona hinshawii* - all serotypes
--*Bacillus anthracis*
--*Bartonella henselae, B. quintana, B. vinsonii*
--*Bordetella* including *B. pertussis*
--*Borrelia recurrentis, B. burgdorferi*
--*Burkholderia* (formerly *Pseudomonas* species) except those listed in Appendix B-III-A (RG3))
--*Campylobacter coli, C. fetus, C. jejuni*
--*Chlamydia psittaci*, *C. trachomatis*, *C. pneumoniae*
--*Clostridium botulinum*, *Cl. chauvoei, Cl. haemolyticum, Cl. histolyticum, Cl. novyi, Cl. septicum, Cl. tetani*
--*Corynebacterium diphtheriae, C. pseudotuberculosis, C. renale*
--*Dermatophilus congolensis*
--*Edwardsiella tarda*
--*Erysipelothrix rhusiopathiae*
--*Escherichia coli* - all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen, including *E. coli* O157:H7
--*Haemophilus ducreyi, H. influenzae*
--*Helicobacter pylori*
--*Klebsiella* - all species except *K. oxytoca* (RG1)
--*Legionella* including *L. pneumophila*
--*Leptospira interrogans* - all serotypes
--*Listeria*
--*Moraxella*
--*Mycobacterium* (except those listed in Appendix B-III-A (RG3)) including *M. avium* complex, *M. asiaticum, M. bovis* BCG vaccine strain, *M. chelonei, M. fortuitum, M. kansasii, M. leprae, M. malmoense, M. marinum, M. paratuberculosis, M. scrofulaceum, M. simiae, M. szulgai, M. ulcerans, M. xenopi*
--*Mycoplasma*, except *M. mycoides* and *M. agalactiae* which are restricted animal pathogens
--*Neisseria gonorrhoeae*, *N. meningitidis*
--*Nocardia asteroides, N. brasiliensis, N. otitidiscaviarum, N. transvalensis*
--*Rhodococcus equi*
--*Salmonella* including *S. arizonae, S. cholerasuis, S. enteritidis, S. gallinarum-pullorum, S. meleagridis, S. paratyphi,* A, B, C, *S. typhi*, *S. typhimurium*
--*Shigella* including *S. boydii, S. dysenteriae,* type 1, *S. flexneri, S. sonnei*
--*Sphaerophorus necrophorus*
--*Staphylococcus aureus*
--*Streptobacillus moniliformis*
--*Streptococcus* including *S. pneumoniae, S. pyogenes*
--*Treponema pallidum, T. carateum*
--*Vibrio cholerae, V. parahemolyticus, V. vulnificus*
--*Yersinia enterocolitica*

Appendix B-II-B. Risk Group 2 (RG2) - Fungal Agents

--*Blastomyces dermatitidis*
--*Cladosporium bantianum, C. (Xylohypha) trichoides*
--*Cryptococcus neoformans*
--*Dactylaria galopava (Ochroconis gallopavum)*
--*Epidermophyton*
--*Exophiala (Wangiella) dermatitidis*
--*Fonsecaea pedrosoi*
--*Microsporum*
--*Paracoccidioides braziliensis*
--*Penicillium marneffei*
--*Sporothrix schenckii*
--*Trichophyton*

Appendix B-II-C. Risk Group 2 (RG2) - Parasitic Agents

--*Ancylostoma* human hookworms including *A. duodenale, A. ceylanicum*
--*Ascaris* including *Ascaris lumbricoides suum*
--*Babesia* including *B. divergens, B. microti*
--*Brugia* filaria worms including *B. malayi, B. timori*
--*Coccidia*
--*Cryptosporidium* including *C. parvum*
--*Cysticercus cellulosae* (hydatid cyst, larva of *T. solium*)
--*Echinococcus* including *E. granulosis, E. multilocularis, E. vogeli*
--*Entamoeba histolytica*
--*Enterobius*
--*Fasciola* including *F. gigantica, F. hepatica*
--*Giardia* including *G. lamblia*
--*Heterophyes*
--*Hymenolepis* including *H. diminuta, H. nana*
--*Isospora*
--*Leishmania* including *L. braziliensis, L. donovani, L. ethiopia, L. major, L. mexicana, L. peruvania, L. tropica*
--*Loa loa* filaria worms
--*Microsporidium*
--*Naegleria fowleri*
--*Necator* human hookworms including *N. americanus*
--*Onchocerca* filaria worms including, *O. volvulus*
--*Plasmodium* including simian species, *P. cynomologi, P. falciparum, P. malariae, P. ovale, P. vivax*
--*Sarcocystis* including *S. sui hominis*
--*Schistosoma* including *S. haematobium, S. intercalatum, S. japonicum, S. mansoni, S. mekongi*
--*Strongyloides* including *S. stercoralis*
--*Taenia solium*
--*Toxocara* including *T. canis*
--*Toxoplasma* including *T. gondii*
--*Trichinella spiralis*
--*Trypanosoma* including *T. brucei brucei, T. brucei gambiense, T. brucei rhodesiense, T. cruzi*
--*Wuchereria bancrofti* filaria worms

Appendix B-II-D. Risk Group 2 (RG2) - Viruses

Adenoviruses, human - all types

Alphaviruses (Togaviruses) - Group A Arboviruses
--Eastern equine encephalomyelitis virus
--Venezuelan equine encephalomyelitis vaccine strain TC-83
--Western equine encephalomyelitis virus

Arenaviruses
--Lymphocytic choriomeningitis virus (non-neurotropic strains)
--Tacaribe virus complex
--Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)

Bunyaviruses
--Bunyamwera virus
--Rift Valley fever virus vaccine strain MP-12
--Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)

Caliciviruses

Coronaviruses

Flaviviruses (Togaviruses) - Group B Arboviruses
--Dengue virus serotypes 1, 2, 3, and 4
--Yellow fever virus vaccine strain 17D
--Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)

Hepatitis A, B, C, D, and E viruses

Herpesviruses - except Herpesvirus simiae (Monkey B virus) (see Appendix B-IV-D, *Risk Group 4 (RG4) - Viral Agents*)
--Cytomegalovirus
--Epstein Barr virus
--*Herpes simplex* types 1 and 2
--*Herpes zoster*
--Human herpesvirus types 6 and 7

Orthomyxoviruses
--Influenza viruses types A, B, and C (except those listed in Appendix B-III-D, *Risk Group 3 (RG3) - Viruses and Prions*)
--Tick-borne orthomyxoviruses

Papovaviruses
--All human papilloma viruses

Paramyxoviruses
--Newcastle disease virus
--Measles virus
--Mumps virus
--Parainfluenza viruses types 1, 2, 3, and 4
--Respiratory syncytial virus

Parvoviruses
--Human parvovirus (B19)

Picornaviruses
--Coxsackie viruses types A and B
--Echoviruses - all types
--Polioviruses - all types, wild and attenuated
--Rhinoviruses - all types

Poxviruses - all types except Monkeypox virus (see Appendix B-III-D, *Risk Group 3 (RG3) - Viruses and Prions*) and restricted poxviruses including Alastrim, Smallpox, and Whitepox (see Section V-L, *Footnotes and References of Sections I through IV*)

Reoviruses - all types including Coltivirus, human Rotavirus, and Orbivirus (Colorado tick fever virus)

Rhabdoviruses
--Rabies virus - all strains
--Vesicular stomatitis virus - laboratory adapted strains including VSV-Indiana, San Juan, and Glasgow

Togaviruses (see Alphaviruses and Flaviviruses)
--Rubivirus (rubella)

Appendix B-III. Risk Group 3 (RG3) Agents

RG3 agents are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available.

Appendix B-III-A. Risk Group 3 (RG3) - Bacterial Agents Including Rickettsia

--*Bartonella*
--*Brucella* including *B. abortus, B. canis*, *B. suis*
--*Burkholderia (Pseudomonas) mallei, B. pseudomallei*
--*Coxiella burnetii*
--*Francisella tularensis*
--*Mycobacterium bovis* (except BCG strain, see Appendix B-II-A, *Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia*), *M. tuberculosis*
--*Pasteurella multocida* type B -"buffalo" and other virulent strains
--Rickettsia akari, R. australis, R. canada, R. conorii, R. prowazekii, R. rickettsii, R, siberica, R. tsutsugamushi, R. typhi (R. mooseri)
--*Yersinia pestis*

Appendix B-III-B. Risk Group 3 (RG3) - Fungal Agents

--*Coccidioides immitis* (sporulating cultures; contaminated soil)
--*Histoplasma capsulatum, H. capsulatum var.. duboisii*

Appendix B-III-C. Risk Group 3 (RG3) - Parasitic Agents

None

Appendix B-III-D. Risk Group 3 (RG3) - Viruses and Prions

Alphaviruses (Togaviruses) - Group A Arboviruses
--Semliki Forest virus
--St. Louis encephalitis virus
--Venezuelan equine encephalomyelitis virus (except the vaccine strain TC-83, see Appendix B-II-D (RG2))
--Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)

Arenaviruses
--Flexal
--Lymphocytic choriomeningitis virus (LCM) (neurotropic strains)

Bunyaviruses
--Hantaviruses including Hantaan virus
--Rift Valley fever virus

Flaviviruses (Togaviruses) - Group B Arboviruses
--Japanese encephalitis virus
--Yellow fever virus
--Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)

Orthomyxoviruses
-- Influenza viruses 1918-1919 H1N1 (1918 H1N1), human H2N2 (1957-1968), and highly pathogenic avian influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1).

Poxviruses
--Monkeypox virus

Prions
--Transmissible spongioform encephalopathies (TME) agents (Creutzfeldt-Jacob disease and kuru agents)(see Section V-C, *Footnotes and References of Sections I through IV*, for containment instruction)

Retroviruses
--Human immunodeficiency virus (HIV) types 1 and 2
--Human T cell lymphotropic virus (HTLV) types 1 and 2
--Simian immunodeficiency virus (SIV)

Rhabdoviruses
--Vesicular stomatitis virus

Appendix B-IV. Risk Group 4 (RG4) Agents

RG4 agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available.

Appendix B-IV-A. Risk Group 4 (RG4) - Bacterial Agents

None

Appendix B-IV-B. Risk Group 4 (RG4) - Fungal Agents

None

Appendix B-IV-C. Risk Group 4 (RG4) - Parasitic Agents

None

Appendix B-IV-D. Risk Group 4 (RG4) - Viral Agents

Arenaviruses
--Guanarito virus
--Lassa virus

--Junin virus
--Machupo virus
--Sabia

Bunyaviruses (Nairovirus)
--Crimean-Congo hemorrhagic fever virus

Filoviruses
--Ebola virus
--Marburg virus

Flaviruses (Togaviruses) - Group B Arboviruses
--Tick-borne encephalitis virus complex including Absetterov, Central European encephalitis, Hanzalova, Hypr, Kumlinge, Kyasanur Forest disease, Omsk hemorrhagic fever, and Russian spring-summer encephalitis viruses

Herpesviruses (alpha)
--Herpesvirus simiae (Herpes B or Monkey B virus)

Paramyxoviruses
--Equine morbillivirus

Hemorrhagic fever agents and viruses as yet undefined

Appendix B-V. Animal Viral Etiologic Agents in Common Use

The following list of animal etiologic agents is appended to the list of human etiologic agents. None of these agents is associated with disease in healthy adult humans; they are commonly used in laboratory experimental work.

A containment level appropriate for RG1 human agents is recommended for their use. For agents that are infectious to human cells, e.g., amphotropic and xenotropic strains of murine leukemia virus, a containment level appropriate for RG2 human agents is recommended.

Baculoviruses

Herpesviruses
--Herpesvirus ateles
--Herpesvirus saimiri
--Marek's disease virus
--Murine cytomegalovirus

Papovaviruses
--Bovine papilloma virus
--Polyoma virus
--Shope papilloma virus
--Simian virus 40 (SV40)

Retroviruses
--Avian leukosis virus
--Avian sarcoma virus
--Bovine leukemia virus
--Feline leukemia virus
--Feline sarcoma virus
--Gibbon leukemia virus
--Mason-Pfizer monkey virus
--Mouse mammary tumor virus
--Murine leukemia virus
--Murine sarcoma virus
--Rat leukemia virus

Appendix B-V-1. Murine Retroviral Vectors

Murine retroviral vectors to be used for human transfer experiments (less than 10 liters) that contain less than 50% of their respective parental viral genome and that have been demonstrated to be free of detectable replication competent retrovirus can be maintained, handled, and administered, under BL1 containment.

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APPENDIX C. EXEMPTIONS UNDER SECTION III-F-6

Section III-F-6 states that exempt from these *NIH Guidelines* are "those that do not present a significant risk to health or the environment (see Section IV-C-1-b-(1)-(c), *NIH Director--Specific Responsibilities*), as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment. See Appendix C, *Exemptions under Sections III-F-6*, for other classes of experiments which are exempt from the *NIH Guidelines*." The following classes of experiments are exempt under Section III-F-6:

Appendix C-I. Recombinant DNA in Tissue Culture

Recombinant DNA molecules containing less than one-half of any eukaryotic viral genome (all viruses from a single family being considered identical -- see Appendix C-VII-E, *Footnotes and References of Appendix C*), that are propagated and maintained in cells in tissue culture are exempt from these *NIH Guidelines* with the exceptions listed in Appendix C-I-A.

Appendix C-I-A. Exceptions

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in Section III-A which require Institutional Biosafety Committee approval, RAC review, and NIH Director approval before initiation, (ii) experiments described in Section III-B which require NIH/OBA and Institutional Biosafety Committee approval before initiation, (iii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*, and Sections V-G and V-L, *Footnotes and References of Sections I through IV*) or cells known to be infected with these agents, (iv) experiments involving the deliberate introduction of genes coding for the biosynthesis of molecules that are toxic for vertebrates (see Appendix F, *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*), and (v) whole plants regenerated from plant cells and tissue cultures are covered by the exemption provided they remain axenic cultures even though they differentiate into embryonic tissue and regenerate into plantlets.

Appendix C-II. *Escherichia coli K-12* Host-Vector Systems

Experiments which use *Escherichia coli* K-12 host-vector systems, with the exception of those experiments listed in Appendix C-II-A, are exempt from the *NIH Guidelines* provided that: (i) the *Escherichia coli* host does not contain conjugation proficient plasmids or generalized transducing phages; or (ii) lambda or lambdoid or Ff bacteriophages or non-conjugative plasmids (see Appendix C-VII-B, *Footnotes and References of Appendix C*) shall be used as vectors. However, experiments involving the insertion into *Escherichia coli* K-12 of DNA from prokaryotes that exchange genetic information (see Appendix C-VII-C, *Footnotes and References of Appendix C*) with *Escherichia coli* may be performed with any *Escherichia coli* K-12 vector (e.g., conjugative plasmid). When a non-conjugative vector is used, the *Escherichia coli* K-12 host may contain conjugation-proficient plasmids either autonomous or integrated, or generalized transducing phages. For these exempt laboratory experiments, Biosafety Level (BL) 1 physical containment conditions are recommended. For large-scale fermentation experiments, the appropriate physical containment conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques; the Institutional Biosafety Committee can specify higher containment if deemed necessary.

Appendix C-II-A. Exceptions

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in Section III-A which require Institutional Biosafety Committee approval, RAC review, and NIH Director approval before initiation, (ii) experiments described in Section III-B which require NIH/OBA and Institutional Biosafety Committee approval before initiation, (iii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*, and Sections V-G and V-L, *Footnotes and References of Sections I through IV*) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-D-2 with prior Institutional Biosafety Committee review and approval, (iv) large-scale experiments (e.g., more than 10 liters of culture), and (v) experiments involving the cloning of toxin molecule genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F, *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*).

Appendix C-III. *Saccharomyces* Host-Vector Systems

Experiments involving *Saccharomyces cerevisiae* and *Saccharomyces uvarum* host-vector systems, with the exception of experiments listed in Appendix C-III-A, are exempt from the *NIH Guidelines*. For these exempt experiments, BL1 physical containment is recommended. For large-scale fermentation experiments, the appropriate physical containment conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques; the Institutional Biosafety Committee can specify higher containment if deemed necessary.

Appendix C-III-A. Exceptions

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in Section III-A which require Institutional Biosafety Committee approval, RAC review, and NIH Director approval before initiation, (ii) experiments described in Section III-B which require NIH/OBA and Institutional Biosafety Committee approval before initiation, (iii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*, and Sections V-G and V-L, *Footnotes and References of Sections I through IV*) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-D-2 with prior Institutional Biosafety Committee review and approval, (iv) large-scale experiments (e.g., more than 10 liters of culture), and (v) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F, *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*).

Appendix C-IV. *Bacillus subtilis* or *Bacillus licheniformis* Host-Vector Systems

Any asporogenic *Bacillus subtilis* or asporogenic *Bacillus licheniformis* strain which does not revert to a spore-former with a frequency greater than 10^{-7} may be used for cloning DNA with the exception of those experiments listed in Appendix C-IV-A, *Exceptions*. For these exempt laboratory experiments, BL1 physical containment conditions are recommended. For large-scale fermentation experiments, the appropriate physical containment conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques; the Institutional Biosafety Committee can specify higher containment if it deems necessary.

Appendix C-IV-A. Exceptions

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in Section III-A which require Institutional Biosafety Committee approval, RAC review, and NIH Director approval before initiation, (ii) experiments described in Section III-B which require NIH/OBA and Institutional Biosafety Committee approval before initiation, (iii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*, and Sections V-G and V-L, *Footnotes and References of Sections I through IV*) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-D-2 with prior Institutional Biosafety Committee review and approval, (iv) large-scale experiments (e.g., more than 10 liters of culture), and (v) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F, *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*).

Appendix C-V. Extrachromosomal Elements of Gram Positive Organisms

Recombinant DNA molecules derived entirely from extrachromosomal elements of the organisms listed below (including shuttle vectors constructed from vectors described in Appendix C), propagated and maintained in organisms listed below are exempt from these *NIH Guidelines*.

Bacillus amyloliquefaciens
Bacillus amylosacchariticus
Bacillus anthracis
Bacillus aterrimus
Bacillus brevis
Bacillus cereus
Bacillus globigii
Bacillus licheniformis
Bacillus megaterium
Bacillus natto
Bacillus niger
Bacillus pumilus
Bacillus sphaericus
Bacillus stearothermophilis
Bacillus subtilis
Bacillus thuringiensis

Clostridium acetobutylicum
Lactobacillus casei
Listeria grayi
Listeria monocytogenes
Listeria murrayi
Pediococcus acidilactici
Pediococcus damnosus
Pediococcus pentosaceus
Staphylococcus aureus
Staphylcoccus carnosus
Staphylococcus epidermidis
Streptococcus agalactiae
Streptococcus anginosus
Streptococcus avium
Streptococcus cremoris
Streptococcus dorans
Streptococcus equisimilis
Streptococcus faecalis
Streptococcus ferus
Streptococcus lactis
Streptococcus ferns
Streptococcus mitior
Streptococcus mutans
Streptococcus pneumoniae
Streptococcus pyogenes
Streptococcus salivarious
Streptococcus sanguis
Streptococcus sobrinus
Streptococcus thermophylus

Appendix C-V-A. Exceptions

The following categories are not exempt from the *NIH Guidelines*: (i) experiments described in Section III-A which require Institutional Biosafety Committee approval, RAC review, and NIH Director approval before initiation, (ii) experiments described in Section III-B which require NIH/OBA and Institutional Biosafety Committee approval before initiation, (iii) experiments involving DNA from Risk Groups 3, 4, or restricted organisms (see Appendix B, *Classification of Human Etiologic Agents on the Basis of Hazard*, and Sections V-G and V-L, *Footnotes and References of Sections I through IV*) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-D-2 with prior Institutional Biosafety Committee review and approval, (iv) large-scale experiments (e.g., more than 10 liters of culture), and (v) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F, *Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates*).

Appendix C-VI. The Purchase or Transfer of Transgenic Rodents

The purchase or transfer of transgenic rodents for experiments that require BL1 containment (See Appendix G-III-M, *Footnotes and References of Appendix G*) are exempt from the *NIH Guidelines*.

Appendix C-VII. Footnotes and References of Appendix C

Appendix C-VII-A. The NIH Director, with advice of the RAC, may revise the classification for the purposes of these *NIH Guidelines* (see Section IV-C-1-b-(2)-(b), *Minor Actions*). The revised list of organisms in each Risk Group is located in Appendix B.

Appendix C-VII-B. A subset of non-conjugative plasmid vectors are poorly mobilizable (e.g., pBR322, pBR313). Where practical, these vectors should be employed.

Appendix C-VII-C. Defined as observable under optimal laboratory conditions by transformation, transduction, phage infection, and/or conjugation with transfer of phage, plasmid, and/or chromosomal genetic information. Note that this definition of exchange may be less stringent than that applied to exempt organisms under Section III-F-5, *Exempt Experiments*.

Appendix C-VII-D. As classified in the *Third Report of the International Committee on Taxonomy of Viruses: Classification and Nomenclature of Viruses*, R. E. F. Matthews (ed.), Intervirology 12 (129-296), 1979.

Appendix C-VII-E. i.e., the total of all genomes within a Family shall not exceed one-half of the genome.

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APPENDIX D. MAJOR ACTIONS TAKEN UNDER THE *NIH GUIDELINES*

As noted in the subsections of Section IV-C-1-b-(1), the Director, NIH, may take certain actions with regard to the *NIH Guidelines* after the issues have been considered by the RAC. Some of the actions taken to date include the following:

Appendix D-1. Permission is granted to clone foot and mouth disease virus in the EK1 host-vector system consisting of *E. coli* K-12 and the vector pBR322, all work to be done at the Plum Island Animal Disease Center.

Appendix D-2. Certain specified clones derived from segments of the foot and mouth disease virus may be transferred from Plum Island Animal Disease Center to the facilities of Genentech, Inc., of South San Francisco, California. Further development of the clones at Genentech, Inc., has been approved under BL1 + EK1 conditions.

Appendix D-3. The Rd strain of *Hemophilus influenzae* can be used as a host for the propagation of the cloned Tn 10 tet R gene derived from *E. coli* K-12 employing the non-conjugative *Hemophilus* plasmid, pRSF0885, under BL1 conditions.

Appendix D-4. Permission is granted to clone certain subgenomic segments of foot and mouth disease virus in HV1 *Bacillus subtilis* and *Saccharomyces cerevisiae* host-vector systems under BL1 conditions at Genentech, Inc., South San Francisco, California.

Appendix D-5. Permission is granted to Dr. Ronald Davis of Stanford University to field test corn plants modified by recombinant DNA techniques under specified containment conditions.

Appendix D-6. Permission is granted to clone in *E. coli* K-12 under BL1 physical containment conditions subgenomic segments of rift valley fever virus subject to conditions which have been set forth by the RAC.

Appendix D-7. Attenuated laboratory strains of *Salmonella typhimurium* may be used under BL1 physical containment conditions to screen for the *Saccharomyces cerevisiae* pseudouridine synthetase gene. The plasmid YEp13 will be employed as the vector.

Appendix D-8. Permission is granted to transfer certain clones of subgenomic segments of foot and mouth disease virus from Plum Island Animal Disease Center to the laboratories of Molecular Genetics, Inc., Minnetonka, Minnesota, and to work with these clones under BL1 containment conditions. Approval is contingent upon review of data on infectivity testing of the clones by a working group of the RAC.

Appendix D-9. Permission is granted to Dr. John Sanford of Cornell University to field test tomato and tobacco plants transformed with bacterial (*E.coli* K-12) and yeast DNA using pollen as a vector.

Appendix D-10. Permission is granted to Drs. Steven Lindow and Nickolas Panopoulos of the University of California, Berkeley, to release under specified conditions *Pseudomonas syringae*, pathovars (pv.) *syringae,* and *Erwinia herbicola* carrying *in vitro* generated deletions of all or part of the genes involved in ice nucleation.

Appendix D-11. Agracetus of Middleton, Wisconsin, may field test under specified conditions disease resistant tobacco plants prepared by recombinant DNA techniques.

Appendix D-12. Eli Lilly and Company of Indianapolis, Indiana, may conduct large-scale experiments and production involving *Cephalosporium acremonium* strain LU4-79-6 under less than Biosafety Level 1 - Large Scale (BL1-LS) conditions.

Appendix D-13. Drs. W. French Anderson, R. Michael Blaese, and Steven Rosenberg of the NIH, Bethesda, Maryland, can conduct experiments in which a bacterial gene coding for neomycin phosphotransferase will be inserted into a portion of the tumor infiltrating lymphocytes (TIL) of cancer patients using a retroviral vector, N2. The marked TIL then will be combined with unmarked TIL, and reinfused into the patients. This experiment is an addition to an ongoing adoptive immunotherapy protocol in which TIL are isolated from a patient's tumor, grown in culture in the presence of interleukin-2, and reinfused into the patient. The marker gene will be used to detect TIL at various time intervals following reinfusion.

Approval is based on the following four stipulations: (I) there will be no limitation of the number of patients in the continuing trial; (ii) the patients selected will have a life expectancy of about 90 days; (iii) the patients give fully informed consent to participate in the trial; and (iv) the investigators will provide additional data before inserting a gene for therapeutic purposes. (Protocol #8810-001)

Appendix D-14. U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) may conduct certain experiments involving products of a yellow fever virus originating from the 17-D yellow fever clone at the Biosafety Level 3 containment level using HEPA filters and vaccination of laboratory personnel.

In addition, USAMRIID may conduct certain experiments involving vaccine studies of Venezuelan equine encephalitis virus at the Biosafety Level 3 containment level using HEPA filters and vaccination of laboratory personnel.

Appendix D-15. Drs. R. Michael Blaese and W. French Anderson of the NIH, Bethesda, Maryland, can conduct experiments in which a gene coding for adenosine deaminase (ADA) will be inserted into T lymphocytes of patients with severe combined immunodeficiency disease, using a retroviral vector, LASN. Following insertion of the gene, these T lymphocytes will be reinfused into the patients. The patients will then be followed for evidence of clinical improvement in the disease state, and measurement of multiple parameters of immune function by laboratory testing.

Approval is based on the following two stipulations: (I) that intraperitoneal administration of transduced T lymphocytes not be used before clearance by the Chair of the Recombinant DNA Advisory Committee; and (ii) that the number of research patients be limited to 10 at this time.

In addition to the conditions outlined in the initial approval, patients may be given a supplement of a CD-34+-enriched peripheral blood lymphocytes (PBL) which have been placed in culture conditions that favor progenitor cell growth. This enriched population of cells will be transduced with the retroviral vector, G1NaSvAd. G1NaSvAd is similar to LASN, yet distinguishable by PCR. LASN has been used to transduce peripheral blood T lymphocytes with the ADA gene. Lymphocytes and myeloid cells will be isolated from patients over time and assayed for the presence of the LASN or G1NaSvAd vectors. The primary objectives of this protocol are to transduce CD 34+ peripheral blood cells with the adenosine deaminase gene, administer these cells to patients, and determine if such cells can differentiate into lymphoid and myeloid cells *in vivo*. There is a potential for benefit to the patients in that these hematopoietic progenitor cells may survive longer, and divide to yield a broader range of gene-corrected cells. (Protocol #9007-002)

Appendix D-16. Dr. Steven A. Rosenberg of the National Institutes of Health, Bethesda, Maryland, can conduct experiments on patients with advanced melanoma who have failed all effective therapy. These patients will be treated with escalating doses of autologous tumor infiltrating lymphocytes (TIL) transduced with a gene coding for tumor necrosis factor (TNF). Escalating numbers of transduced TIL will be administered at three weekly intervals along with the administration of interleukin-2 (IL-2). The objective is to evaluate the toxicity and possible therapeutic efficacy of the administration of tumor infiltrating lymphocytes (TIL) transduced with the gene coding for TNF. (Protocol #9007-003)

Appendix D-17. Dr. Malcolm K. Brenner of St. Jude Children's Research Hospital of Memphis, Tennessee, can conduct experiments on patients with acute myelogenous leukemia (AML). Using the LNL6 retroviral vector, the autologous bone marrow cells will be transduced with the gene coding for neomycin resistance. The purpose of this gene marking experiment is to determine whether the source of relapse after autologous bone marrow transplantation for acute myelogenous leukemia is residual malignant cells in the harvested marrow or reoccurrence of tumor in the patient. Determining the source of relapse should indicate whether or not purging of the bone marrow is a necessary procedure. (Protocol #9102-004)

Appendix D-18. Dr. Malcolm K. Brenner of St. Jude Children's Research Hospital of Memphis, Tennessee, can conduct experiments on pediatric patients with Stage D (disseminated) neuroblastoma who are being treated with high-dose carboplatin and etoposide in either phase I/II or phase II trials. All the patients in these studies will be subjected to bone marrow transplantation since it will allow them to be exposed to chemoradiation that would be lethal were it not for the availability of stored autologous marrow for rescue. The bone marrow cells of these patients will be transduced with the gene coding for neomycin resistance using the LNL6 vector. The purpose of this gene marking study is to determine whether the source of relapse after autologous bone marrow transplantation is residual malignant cells in the harvested marrow or residual disease in the patient. Secondly, it is hoped to determine the contribution of marrow autographs to autologous reconstitution. (Protocol #9105-005/9105-006)

Appendix D-19. Dr. Albert B. Deisseroth of the MD Anderson Cancer Center of Houston, Texas, can conduct experiments on patients with chronic myelogenous leukemia who have been reinduced into a second chronic phase or blast cells. The patients in these studies will receive autologous bone marrow transplantation. Using the LNL6 vector, the bone marrow cells will be transduced with the gene coding for neomycin resistance. The purpose of these marking studies is to determine if the origin of relapse arises from residual leukemic cells in the patients or from viable leukemic cells remaining in the bone marrow used for autologous transplantation. (Protocol #9105-007)

Appendix D-20. Drs. Fred D. Ledley and Savio L. C. Woo of Baylor College of Medicine of Houston, Texas, can conduct experiments on pediatric patients with acute hepatic failure who are identified as candidates for hepatocellular transplantation. Using the LNL6 vector, the hepatocytes will be transduced with the gene coding for neomycin resistance. The purpose of using a genetic marker is to demonstrate the pattern of engraftment of transplanted hepatocytes and to help determine the success or failure of engraftment. (Protocol #9105-008)

Appendix D-21. Dr. Steven A. Rosenberg of the National Institutes of Health, Bethesda, Maryland, can conduct experiments on patients with advanced melanoma, renal cell cancer, and colon carcinoma who have failed all effective therapy. In an attempt to increase these patients' immune responses to the tumor, the tumor necrosis factor gene or the interleukin-2 gene will be introduced into a tumor cell line established from the patient. These gene-modified autologous tumor cells will then be injected into the thigh of the patient. To further utilize the immune system of the patient to fight the tumor, stimulated lymphocytes will be cultured from either the draining regional lymph nodes or the injected tumor itself. The patients will be evaluated for antitumor effects engendered by the injection of the gene modified tumor cells themselves as well as after the infusion of the cultured lymphocytes. (Protocol #9110-010/9110-011)

Appendix D-22. Dr. James M. Wilson of the University of Michigan Medical Center of Ann Arbor, Michigan, can conduct experiments on three patients with the homozygous form of familial hypercholesterolemia. Both children and adults will be eligible for this therapy. In an attempt to correct the basic genetic defect in this disease, the gene coding for the low-density lipoprotein (LDL) receptor will be introduced into liver cells taken from the patient. The gene-corrected hepatocytes will then be infused into the portal circulation of the patient through an indwelling catheter. The patients will be evaluated for engraftment of the these treated hepatocytes through a series of metabolic studies; three months after gene therapy, a liver biopsy will be taken and analyzed for the presence of recombinant derived RNA and DNA to document the presence of the gene coding for the normal LDL receptor. (Protocol #9110-012)

Appendix D-23. Dr. Michael T. Lotze of the University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, can conduct experiments on 20 patients with metastatic melanoma who have failed conventional therapy. A gene transfer experiment will be performed, transducing the patients' tumor infiltrating lymphocytes (TILs) with the gene for neomycin resistance. Through the use of this gene marking technique, it is proposed to determine how long TIL cells can be detected *in vivo* in the peripheral blood of the patients, and how the administration of interleukin-2 and interleukin-4 affects localization and survival of TIL cells in tumor sites. (Protocol #9105-009)

Appendix D-24. Dr. Gary J. Nabel of the University of Michigan Medical School, Ann Arbor, Michigan, can conduct gene therapy experiments on twelve patients with melanoma or adenocarcinoma. Patient population will be limited to adults over the age of 18 and female patients must be postmenopausal or have undergone tubal ligation or orchiectomy. The patient's immune response will be stimulated by the introduction of a gene encoding for a Class I MHC protein, HLA-B7, in order to enhance tumor regression. DNA/liposome-mediated transfection techniques will be used to directly transfer this foreign gene into tumor cells. HLA-B7 expression will be confirmed *in vivo*, and the immune response stimulated by the expression of this antigen will be characterized. These experiments will be analyzed for their efficacy in treating cancer. (Protocol #9202-013)

Appendix D-25. Kenneth Cornetta of Indiana University, Indianapolis, Indiana, can conduct gene transfer experiments on up to 10 patients with acute myelogenous leukemia (AML) and up to 10 patients with acute lymphocytic leukemia (ALL). The patient population will be limited to persons between 18 and 65 years of age. Using the LNL-6 vector, autologous bone marrow cells will be marked with the neomycin resistance gene. Gene marked and untreated bone marrow cells will be reinfused at the time of bone marrow transplantation. Patients will then be monitored for evidence of the neomycin resistance gene in peripheral blood and bone marrow cells in order to determine whether relapse of their disease is a result of residual malignant cells remaining in the harvested marrow or inadequate ablation of the tumor cells by chemotherapeutic agents. Determining the source of relapse may indicate whether or not purging of the bone marrow is a necessary procedure for these leukemia patients. Further studies will be performed in order to determine the percentage of leukemic cells that contain the LNL-6 vector and the clonality of the marked cells. (Protocol #9202-014)

Appendix D-26. Dr. James S. Economou of the University of California, Los Angeles, can conduct gene transfer experiments on 20 patients with metastatic melanoma and 20 patients with renal cell carcinoma. These patients will be treated with various combinations of tumor-infiltrating lymphocytes and peripheral blood leukocytes, including CD8 and CD4 subsets of both types of cells. These effector cell populations will be given in combination with interleukin-2 (IL-2) in the melanoma patients and IL-2 plus alpha interferon in the renal cell carcinoma patients. The effector cells will be transduced with the neomycin resistance gene using either the LNL6 or G1N retroviral vectors. This "genetic marking" of the tumor-infiltrating lymphocytes and peripheral blood lymphocytes is designed to answer questions about the trafficking of these cells, their localization to tumors, and their *in vivo* life span. (Protocol #9202-015)

Appendix D-27. Drs. Philip Greenberg and Stanley R. Riddell of the Fred Hutchinson Cancer Research Center, Seattle, Washington, may conduct gene transfer experiments on 15 human immunodeficiency virus (HIV) seropositive patients (18-45 years old) undergoing allogeneic bone marrow transplantation for non-Hodgkin's lymphoma and 15 HIV-seropositive patients (18-50 years old) who do not have acquired immunodeficiency syndrome (AIDS)-related lymphoma and who are not undergoing bone marrow transplantation to evaluate the safety and efficacy of HIV-specific cytotoxic T lymphocyte (CTL) therapy. CTL will be transduced with a retroviral vector (HyTK) encoding a gene that is a fusion product of the hygromycin phosphotransferase gene (HPH) and the herpes simplex virus thymidine kinase (HSV-TK) gene. This vector will deliver both a marker gene and an ablatable gene in these T cell clones in the event that patients develop side effects as a consequence of CTL therapy. Data will be correlated over time, looking at multiple parameters of HIV disease activity. The objectives of these studies include evaluating the safety and toxicity of CTL therapy, determining the duration of *in vivo* survival of HIV-specific CTL clones, and determining if ganciclovir therapy can eradicate genetically modified, adoptively transferred CTL cells. (Protocol #9202-017)

Appendix D-28. Dr. Malcolm Brenner of St. Jude Children's Research Hospital, Memphis, Tennessee, can conduct gene therapy experiments on twelve patients with relapsed/refractory neuroblastoma who have relapsed after receiving autologous bone marrow transplant. In an attempt to stimulate the patient's immune response, the gene coding for Interleukin-2 (IL-2) will be used to transduce tumor cells, and these gene-modified cells will be injected subcutaneously in a Phase 1 dose escalation trial. Patients will be evaluated for an anti-tumor response. (Protocol #9206-018)

Appendix D-29. Drs. Edward Oldfield, Kenneth Culver, Zvi Ram, and R. Michael Blaese of the National Institutes of Health, Bethesda, Maryland, can conduct gene therapy experiments on ten patients with primary malignant brain tumors and ten patients with lung cancer, breast cancer, malignant melanoma, or renal cell carcinoma who have brain metastases. The patient population will be limited to adults over the age of 18.

Patients will be divided into two groups based on the surgical accessibility of their lesions. Both surgically accessible and surgically inaccessible lesions will receive intra-tumoral injections of the retroviral Herpes simplex thymidine kinase (HS-tk) vector-producer cell line, G1TkSvNa, using a guided stereotaxic approach. Surgically accessible lesions will be excised seven days after stereotaxic injection, and the tumor bed will be infiltrated with the HS-tk producer cells. The removed tumor will be evaluated for the efficiency of transduction. Ganciclovir (GCV) will be administered beginning on the fifth postoperative day. In the case of surgically inaccessible lesions, the patients will receive intravenous therapy with GCV seven days after receiving the intra-tumoral injections of the retroviral HS-tk vector-producer cells. (Protocol #9206-019)

Appendix D-30. Dr. Albert D. Deisseroth of MD Anderson Cancer Center, Houston, Texas, can conduct gene transfer experiments on ten patients who have developed blast crisis or accelerated phase chronic myelogenous leukemia (CML). The retroviral vectors G1N and LNL6 which code for neomycin resistance will be used to transduce autologous peripheral blood and bone marrow cells that have been removed and stored at the time of cytogenetic remission or re-induction of chronic phase in Philadelphia chromosome positive CML patients. Following reinduction of the chronic phase of CML and preparative chemotherapy, patients will be infused with the transduced autologous cells.

This protocol is designed to determine the cause of relapse of CML. If polyclonal CML neomycin marked blastic cells appear at the time of relapse, their presence will indicate that relapse arises from the leukemic CML blast cells present in the autologous cells infused following chemotherapy. If residual systemic disease contributes to relapse, the neomycin resistance gene will not be detected in the CML leukemic blasts at the time of relapse.

This study will compare the relative contributions of the peripheral blood and bone marrow to generate hematopoietic recovery after bone marrow transplantation and evaluate purging and selection of peripheral blood or bone marrow as a source of stem cells for transplant. The percentage of neomycin resistant CML cells which are leukemic will be determined by PCR analysis and detection of bcr-abl mRNA. (Protocol #9206-020)

Appendix D-31. Dr. Cynthia Dunbar of the National Institutes of Health, Bethesda, Maryland, can conduct gene transfer experiments on up to 48 patients with multiple myeloma, breast cancer, or chronic myelogenous leukemia. The retroviral vectors G1N and LNL6 will be used to transfer the neomycin resistance marker gene into autologous bone marrow and peripheral blood stem cells in the presence of growth factors to examine hematopoietic reconstitution after bone marrow transplantation. The efficiency of transduction of both short and long term autologous bone marrow reconstituting cells will be examined.

Autologous bone marrow and CD34+ peripheral blood stem cells will be enriched prior to transduction. Myeloma and CML patients will receive both autologous bone marrow and peripheral blood stem cell transplantation. These separate populations will be marked with both the G1N and LNL6 retroviral vectors. If short and long term marking experiments are successful, important information may be obtained regarding the biology of autologous reconstitution, the feasibility of retroviral gene transfer into hematopoietic cells, and the contribution of viable tumor cells within the autograft to disease relapse. (Protocol #9206-023/9206-024/9206-025)

Appendix D-32. Dr. Bernd Gansbacher of the Memorial Sloan-Kettering Cancer Center, New York, New York, can conduct gene therapy experiments on twelve patients over 18 years of age with metastatic melanoma who are HLA-A2 positive and who have failed conventional therapy. This is a phase I study to examine whether allogeneic HLA-A2 matched melanoma cells expressing recombinant human Interleukin-2 (IL-2) can be injected subcutaneously and used to create a potent tumor specific immune response without producing toxicity. By

allowing the tumor cells to present the MHC Class I molecule as well as the secreted IL-2, a clonal expansion of tumor specific effector cells is expected. These effector populations may access residual tumor at distant sites via the systemic circulation. (Protocol #9206-021)

Appendix D-33. Dr. Bernd Gansbacher of the Memorial Sloan-Kettering Cancer Center, New York, New York, can conduct gene therapy experiments on twelve patients over 18 years of age with renal cell carcinoma who are HLA-A2 positive and who have failed conventional therapy. This Phase I study will examine whether allogeneic HLA-A2 matched renal cell carcinoma cells expressing recombinant human Interleukin-2 (IL-2) can be injected subcutaneously and used to create a potent tumor specific immune response without producing toxicity. By allowing the tumor cells to present the MHC Class I molecule as well as the secreted IL-2, a clonal expansion of tumor specific effector cells is expected. These effector populations may access residual tumor at distant sites via the systemic circulation. (Protocol #9206-022)

Appendix D-34. Dr. Michael T. Lotze, University of Pittsburgh, Pittsburgh, Pennsylvania, can conduct experiments on twenty patients with metastatic, and/or unresectable, locally advanced melanoma, renal cell carcinoma, breast cancer, or colon cancer who have failed standard therapy. Patients will receive multiple subcutaneous injections of autologous tumor cells combined with an autologous fibroblast cell line that has been transduced *in vitro* with the gene coding for Interleukin-4 (IL-4) to augment the *in vivo* antitumor effect. Patients will be monitored for antitumor effect by PCR analysis and multiple biopsy of the injection site. (Protocol #9209-033)

Appendix D-35. Dr. Friedrich G. Schuening, Fred Hutchinson Cancer Research Center, Seattle, Washington, can conduct human gene transfer experiments on patients ≥ 18 years of age with breast cancer, Hodgkin's disease, or non-Hodgkin's lymphoma. A total of 10 patients per year will be enrolled in the studies over a period of four years. Patients will undergo autologous bone marrow transplantation with a selected population of Interleukin-3 (IL-3) or granulocyte colony-stimulating factor (G-CSF) stimulated CD34(+) peripheral blood repopulating cells (PBRC) that have been transduced with the gene coding for neomycin resistance (neo^R) using the retroviral vector, LN. Patients will be continuously monitored for neo^R to determine the relative contribution of autologous PBRCs to long-term hematopoietic reconstitution. Demonstration of long-term contribution of autologous PBRC to hematopoiesis will enable the use of PBRC alone for autologous transplants and suggest the use of PBRC as long-term carriers of therapeutically relevant genes. (Protocol #9209-027/9209-028)

Appendix D-36. Dr. Friedrich G. Schuening, Fred Hutchinson Cancer Research Center, Seattle, Washington, can conduct human gene transfer experiments on patients ≥ 18 years of age with breast cancer, Hodgkin's disease, or non-Hodgkin's lymphoma. A total of 5 patients per year will be enrolled in the study over a period of four years. Patients will undergo allogeneic bone marrow transplant with granulocyte colony-stimulating factor (G-CSF) stimulated CD34(+) PBRC harvested from an identical twin that have been transduced with neo^R using the retroviral vector, LN. Patients will be continuously monitored for neo^R to determine the relative contribution of G-CSF stimulated allogeneic PBRCs to long-term bone marrow engraftment. Demonstration of long-term contribution of allogeneic PBRC to hematopoiesis will enable the use of PBRC alone for allogeneic transplants and suggest the use of PBRC as long-term carriers of therapeutically relevant genes. (Protocol #9209-029)

Appendix D-37. Dr. Malcolm K. Brenner of St. Jude Children's Hospital, Memphis, Tennessee, and Dr. Bonnie J. Mills of Baxter Healthcare Corporation, Santa Ana, California, can conduct a multicenter uncontrolled human gene transfer experiment on 12 patients ≤ 21 years of age with Stage D Neuroblastoma in first or second marrow remission. Autologous bone marrow cells will be separated into two fractions, purged and unpurged. Each fraction will be transduced with the neo^R gene by either LNL6 or G1Na. Patients will be monitored by the polymerase chain reaction (PCR) for the presence of neo^R. The protocol is designed to evaluate the safety and efficacy of the Neuroblastoma Bone Marrow Purging System following high dose chemotherapy. (Protocol #9209-032)

Appendix D-38. Drs. Carolyn Keierleber and Ann Progulske-Fox of the University of Florida, Gainesville, Florida, can conduct experiments involving the introduction of a gene coding for tetracycline resistance into *Porphyromonas gingivalis* at a physical containment level of Biosafety Level-2 (BL-2).

Appendix D-39. Dr. Scott M. Freeman of Tulane University Medical Center, New Orleans, Louisiana, can conduct experiments on patients with epithelial ovarian carcinoma who have clinical evidence of recurrent, progressive, or residual disease who have no other therapy available to prolong survival. Patients will be injected intraperitoneally with the irradiated PA-1 ovarian carcinoma cell line which has been transduced with

the herpes simplex thymidine kinase (HSV-TK) gene. The patients will then receive ganciclovir therapy. Previous, data indicates that HSV-TK+ tumor cells exhibit a killing effect on HSV-TK- cells when exposed to ganciclovir therapy. Patients will be evaluated for safety and side effects of this treatment. (Protocol #9206-016)

Appendix D-40. Dr. Michael J. Welsh, Howard Hughes Medical Institute Research Laboratories, University of Iowa College of Medicine, Iowa City, Iowa, may conduct experiments on 3 cystic fibrosis (CF) patients ≥ 18 years of age with mild to moderate disease. This Phase I study will determine the: (1) *in vivo* safety and efficacy of the administration of the replication-deficient type 2 adenovirus vector, Ad2/CFTR-1, to the nasal epithelium; (2) efficacy in correcting the CF chloride transport defect *in vivo*; and (3) effect of adenovirus vector dosage on safety and efficacy. (Protocol #9212-036)

Appendix D-41. Dr. Ronald G. Crystal, National Institutes of Health, Bethesda, Maryland, may conduct experiments on 10 cystic fibrosis (CF) patients ≥ 21 years of age. Patients will receive an initial administration of the replication-deficient type 5 adenovirus vector, AdCFTR, to their left nares. If no toxicity is observed from intranasal administration, patients will receive a single administration of AdCFTR to the respiratory epithelium of their left large bronchi. Five groups of patients (2 patients per group) will be studied based on increased dosage administration of AdCFTR. This study will determine the: (1) *in vivo* safety and efficacy of the administration of AdCFTR into the respiratory epithelium; (2) efficacy of the correction of the biologic abnormalities of CF in the respiratory epithelium; (3) duration of the biologic correction; (4) efficacy of the correction of the abnormal electrical potential difference of the airway epithelial sheet; (5) clinical parameters relevant to the disease process; and (6) if humoral immunity develops against AdCFTR sufficient to prevent repeat administration. (Protocol #9212-034)

Appendix D-42. Dr. Kenneth Culver, Iowa Methodist Medical Center, Des Moines, Iowa, and Dr. John Van Gilder, University of Iowa, Iowa City, Iowa, may conduct experiments on 15 patients ≥ 18 years of age with recurrent malignant primary brain tumors or lung, melanoma, renal cell carcinoma, or breast carcinoma brain metastases who have failed standard therapy for their disease. Patient eligibility will be limited to those patients who have measurable residual tumor immediately following the post-operative procedure as demonstrated by imaging studies. The number of patients treated will be equally divided between the Iowa Methodist Medical Center and the University of Iowa. If a positive response is observed in any of the first 15 patients, the investigators may submit a request to treat an additional 15 patients.

Following surgical debulking, patients will receive a maximum of 3 intralesional injections of the G1TkSvNa vector- producing cell line (VPC) to induce regression of residual tumor cells by ganciclovir (GCV) therapy. Patients who demonstrate stable disease for a minimum of 6 months following this treatment will be eligible for additional VPC injections and subsequent GCV therapy. (Protocol #9303-037)

Appendix D-43. Drs. Malcolm Brenner, Robert Krance, Helen E. Heslop, Victor Santana, and James Ihle, St. Jude Children's Research Hospital, Memphis, Tennessee, may conduct experiments on 35 patients ≥ 1 year and ≤ 21 years of age at the time of initial diagnosis of acute myelogenous leukemia (AML). The investigators will use the two retroviral vectors, LNL6 and G1Na, to determine the efficacy of the bone marrow purging techniques: 4-hydroxyperoxicyclophosphamide and interleukin-2 (IL-2) activation of endogenous cytotoxic effector cells, in preventing relapse from the reinfusion of autologous bone marrow cells. (Protocol #9303-039)

Appendix D-44. Drs. Helen E. Heslop, Malcolm Brenner, and Cliona Rooney, St Jude Children's Research Hospital, Memphis, Tennessee, may conduct experiments of 35 patients ≤ 21 years of age who will be recipients of mismatched-related or phenotypically similar unrelated donor marrow grafts for leukemia. In this Phase I dose escalation study, spontaneous lymphoblastoid cell lines will be established that express the same range of Epstein-Barr Virus (EBV) encoded proteins as the recipient. These EBV-specific cell lines will be transduced with LNL6 or G1Na and readministered at the time of bone marrow transplant. This study will determine: (1) survival and expansion of these EBV-specific cell lines *in vivo*, (2) the ability of these adoptively transferred cells to confer protection against EBV infection, and (3) appropriate dosage and administration schedules. (Protocol #9303-038)

Appendix D-45. Drs. Robert W. Wilmott and Jeffrey Whitsett, Children's Hospital Medical Center, Cincinnati, Ohio, and Dr. Bruce Trapnell, Genetic Therapy, Inc., Gaithersburg, Maryland, may conduct experiments on 15 cystic fibrosis (CF) patients who have mild to moderate disease ≥ 21 years of age. The replication-deficient type 5 adenovirus vector, Av1CF2, will be administered to the nasal and lobar bronchial respiratory tract of patients. This study will demonstrate the: (1) expression of normal cystic fibrosis transmembrane conductance regulator