SUMMARY OF SECTION III of the NIH GUIDELINES for rDNA RESEARCH

Adapted from Section III of the 2016 *NIH Guidelines*. Please review the criteria listed in the full version of the NIH Guidelines to ensure that your study meets the criteria of the summarized versions listed below.

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*.

SECTION III-A: Experiments that Require Institutional Biosafety Committee Registration, RAC Review, and NIH Director Approval Before Initiation

III-A-1-a: 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.

SECTION III-B: Experiments That Require NIH/OBA and Institutional Biosafety Committee Registration Before Initiation

III-B-1: Cloning of Toxin Molecules with LD_{50} of <100ng/kg of body weight.
III-B-2: Experiments that have been Approved (under Section III-A-1-a) as Major Actions under the *NIH Guidelines*

SECTION III-C: Experiments that Require Institutional Biosafety Committee Registration and Institutional Review Board and RAC Approval Before Research Participant Enrollment

III-C-1: Deliberate transfer of rDNA, or DNA or RNA derived from rDNA into human subjects.

SECTION III-D: Experiments that Require Institutional Biosafety Committee Registration Before Initiation

III-D-1 Experiments Using Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents as Host-Vector Systems

III-D-1-a: Introduction of rDNA into Risk Group 2 agents; BSL-2/ABSL-2
Examples: Using Adenovirus, Adenovirus-luciferase or adeno-associated virus to transfect cells; Typically involves use of pathogens or defective pathogen vectors (with or without helper virus), such as Adenovirus, Adeno-Associated virus, Baculovirus, Herpes virus, Lentivirus, Retrovirus, Vaccinia and Vesicular Stomatitis Virus, *Shigella*, *Salmonella*, and *E. histolytica*.

III-D-1-b: Introduction of rDNA into Risk Group 3 agents; BSL-3/ABSL-3 (**unusual at UTHSC**)

III-D-1-c: Introduction of rDNA into Risk Group 4 agents; BSL-4/ABSL-4 (**Not Allowed at UTHSC**)

III-D-1-d: Introduction of rDNA into restricted agents at BSL-4/ABSL-4 not permitted except on a case-by-case basis following NIH/OBA review and USDA permit (**Not Allowed at UTHSC**)

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

III-D-2-a: DNA from Risk Group 2 or Risk Group 3 agents transferred into nonpathogenic prokaryotes or lower eukaryotes or exempt from the *NIH Guidelines* (see Section III-F). May be performed at BSL-1or BSL-2 depending on the risk assessment by the IBC.
Examples: *Yersinia* pseudotuberculosis genes encoding outer membrane adhesins are cloned into plasmid vectors for re-introduction into mutant strains of the same bacteria or *E. coli*.

III-D-2-b: DNA from Risk Group 4 and restricted agents transferred into nonpathogenic prokaryotes or lower eukaryotes not permitted except on a case-by-case basis following NIH/OBA review and FDA permit (**Not Allowed at UTHSC**)

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

III-D-3-a: Infectious or defective (defective eukaryotic viruses contain < 2/3 of the genome) Risk Group 2 viruses in the presence of helper virus in tissue culture may be conducted at BL-2.
Examples: Insertion of genes into defective lentiviral, retroviral, or adenoviral vectors (creation of recombinant vectors).

III-D-3-b: Infectious or defective Risk Group 3 viruses and prions in the presence of helper virus in tissue culture may be conducted at BL-3. (**unusual at UTHSC**)

III-D-3-c: Infectious or defective Risk Group 4 viruses in the presence of helper virus in tissue culture may be conducted at BL-4 (**Not Allowed at UTHSC**)

III-D-3-d: Infectious or defective restricted poxviruses in the presence of helper virus in tissue culture not permitted except on a case-by-case basis following NIH/OBA review and USDA permit (for animal

pathogens). (**unusual at UTHSC**)

III-D-3-e: Infectious or defective viruses in the presence of helper virus in tissue culture not covered in III-D above may be conducted at BL1. IBC reserves right to determine Risk Group Classification for novel agents.

III-D-4 Experiments involving whole animals

III-D-4-a: Recombinant/synthetic nucleic acid molecules, or DNA or RNA molecules derived therefrom, from any source except for greater than two-thirds of eukaryotic viral genome transferred to any non-human vertebrate or any invertebrate organism (ABSL-1). **Example: making transgenic mice**.

III-D-4-b: For experiments involving recombinant or synthetic nucleic acid molecules, or DNA or RNA derived therefrom, involving whole animals, including transgenic animals, and not covered by Section 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 Section III-D-4-a, the appropriate containment shall be determined by the Institutional Biosafety Committee

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

III-D-4-c-(1) Generation of transgenic rodents that require BL1 containment are described under Section III-E-3

III-D-4-c-(2) The purchase or transfer of transgenic rodents is exempt from the *NIH Guidelines* under Section III-F.

III-D-5 Experiments involving whole plants (**not typically done at UTHSC**)

III-D-5-a: Recombinant techniques with exotic infectious agents with recognized potential for serious detrimental impact on managed or natural ecosystems when recombinant DNA techniques are associated with whole plants. BL3-P

III-D-5-b: Plants with cloned genomes of readily transmissible exotic infectious agents that may reconstitute by genomic complementation.

III-D-5-c: Readily transmissible exotic infectious agents, such as the soybean rust fungus, maize streak or other viruses in the presence of their specific arthropod vectors. BSL-4P

III-D-5-d: Sequence encoding vertebrate toxins introduced into plants or associated organisms. BL-3P

III-D-5-e: Microbial pathogens of insects or small animals associated with plants if the rDNA-modified organism has a recognized detrimental impact on ecosystems.

III-D-6 Experiments involving more than 10 liters of culture. IBC determines containment level (See Appendix K)

III-D-7 Experiments involving influenza viruses

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 or synthetic virus. Experiments with Risk Group 2 influenza viruses containing genes from human H2N2 other than the HA gene can be worked on at BL2.

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). NIH OSP 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).

III-D-7-c: **1918 H1N1.** Experiments involving influenza viruses containing any gene or segment from 1918 H1N1 shall be conducted at BSL-3/ABSL3.

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 Requiring IBC Notice Simultaneous with initiation

III-E-1: Formation of rDNA molecules containing no more than 2/3 of the genome of any eukaryotic virus in tissue culture. BL-1 with no helper virus. IBC classifies retroviral vectors with packaging system capable of infecting human cells as BL-2.

Examples: Inserting DNA sequences that encode reporters that are measured (lacZ, luciferase, eGFP, dsRed2, etc), or that encode enzymes that are potentially therapeutic (nitric oxide synthases, superoxide dismutase, siRNA) against mRNAs that promote disease, etc into viral vectors that retain no more than 2/3 of the original viral genomic sequence. The cDNAs will be driven by the following promoters: CMV IE, RSV LTR, and cardiac Troponin T (cTnT).

III-E-2 Experiments Involving Whole Plants (**not typically done at UTHSC**)

III-E-2-a: rDNA-modified plants and rDNA-modified organisms not in section III-E-2-b. BL-1-P.

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

III-E-2-b-(1): Plants modified by recombinant or synthetic nucleic acid molecules that are noxious weeds or can interbreed with noxious weeds in the immediate geographic area

III-E-2-b-(2): Plants in which the introduced DNA represents the complete genome of a non-exotic infectious agent

III-E-2-b-(3): Plants associated with recombinant or synthetic nucleic acid molecule-modified non-exotic microorganisms that have a recognized potential for serious detrimental impact on managed or natural ecosystems

III-E-2-b-(4): Plants associated with recombinant or synthetic nucleic acid molecule-modified exotic microorganisms that have no recognized potential for serious detrimental impact on managed or natural ecosystems

III-E-2-b-(5): rDNA-modified arthropods or small animals associated with plants or with arthropods or small animals associated with them if the rDNA-modified microorganisms have no serious impact on ecosystems.

III-E-3: Generation of rodents with stable introduction of DNA into the animal's genome if BL-1. Otherwise see Section III D-4.

SECTION III-F: Exempt Experiments (require IBC registration before initiation)

III-F-1: No organisms or viruses.

III-F-2: DNA segments from a single nonchromosomal or viral DNA source.

III-F-3: DNA from a prokaryotic host when propagated only in that host or when transferred to another host by well established physiological means.

III-F-4: DNA from an eukaryotic host when propagated only in that host.

III-F-5: DNA segments from different species that exchange DNA by known physiological processes.

III-F-6: Those that do not present a significant risk to health or the environment as determined by NIH & RAC.

Appendix C: Exemptions under III-F-6

Appendix C-I rDNA (not virus sector) in tissue culture. (See C-IV for exceptions)

Appendix C-II E.coli K-12 host-vector systems. (See C-II-A for exceptions)

Appendix C-III Saccharomyces host-vector systems (See C-III-A for exceptions)

Appendix C-VI Purchase or transfer of transgenic rodents

III-F-7: Those genomic DNA molecules that have acquired a transposable element, provided the transposable element does not contain any recombinant and/or synthetic DNA

III-F-8: 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