Summary of Section III of the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)[1]

Section	Subsection	Research Description	Comments
Experiments that Require NIH Director Approval and IBC Approval Before Initiation (III-A)			
	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	Very rarely done, See Section IV-C-1-b-(1), Major Actions **Example:** Transferring a drug resistance trait that is used, had previously been used, may be used (outside the U.S.), or that is related to other drugs that are used to treat or control disease agents. These include: Transfer of Erythromycin resistance into *Borrelia burgdorferi*; Transfer of Pyrimethamine resistance into Toxoplasma gondii; Transfer of Chloramphenicol resistance into *Rickettsia conorii*; Transfer of Tetracycline resistance into *Porphyromonas gingivalis*.
Experiments That Require NIH OSP and IBC Registration Before Initiation (III-B)			
	III-B-1	Cloning of Toxin Molecules with LD50 of <100ng/kg of body weight	Very rarely done
	III-B-2	Experiments that have been Approved (under Section III-A-1-a) as Major Actions under the NIH Guidelines	Very rarely done
Experiments that Require IBC and Institutional Review Board Approvals and RAC Review before Research Participant Enrollment (III-C)			
III-C-1		Deliberate Transfer of r/sNA, or DNA or RNA Derived from r/sNA, into One or More Human Research Participants	Clinical studies which require IRB review in addition to IBC review
Experiments that Require IBC Approval Before Initiation (III-D)			
III-D-1 - Experiments using Risk Group (RG) 2, 3, or 4, or Restricted Agents as host-vector systems			
	III-D-1-a	Recombinant or synthetic (r/sNA) into RG2 agents	Require BSL-2/ABSL-2 **Example:** 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.

[1] NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines), April 2019. 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 in this document

	III-D-1-b	r/sNA into RG3 agents	Require BSL-3/ABSL-3
	III-D-1-c	r/sNA into RG4 agents	Require BSL4/ABSL-4
	III-D-1-d	r/sNA into restricted agents	Containment determined on a case-by-case basis following NIH OSP review
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 RG2 or RG3 agents transferred into nonpathogenic prokaryotes or lower eukaryotes or exempt from the NIH Guidelines (see Section III-F).	**Example:** 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.* May be performed at BSL-1 or BSL-2 depending on the risk assessment by the IBC.
	III-D-2-b	DNA from RG4 and restricted agents transferred into nonpathogenic prokaryotes or lower eukaryotes	Containment determined by NIH OSP following a case-by-case basis review. Very rarely done
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) RG2 viruses in the presence of helper virus in tissue culture	Usually conducted as BSL-2 Example: 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.	Conducted at BSL-3 Likely to be rare
	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	Conducted at BSL-4 Likely to be rare
	III-D-3-d	Infectious or defective restricted poxviruses in the presence of helper virus in tissue culture	Containment determined on a case-by-case basis following NIH OSP review Very rarely done
	III-D-3-e	Viruses not covered in III-D-3-a through III-D-3-d.	Usually conducted at BSL-1

III-D-4 - Experiments Involving Whole Animals

Experiments involving whole animals in which the animal's genome has been altered by stable introduction of recombinant or synthetic nucleic acid molecules, or nucleic acids derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant or synthetic nucleic acid molecule-modified microorganisms tested on whole animals.

Example: Creation of transgenic animals (mice, rats, zebrafish, drosophila, etc.), or knockout animals that leave genetic material in the animal as part of the silencing of the gene. Note: the purchase (or transfer to your lab) of previously created transgenic rodents is exempt from the regulations.

Example: Viral vector (e.g., adenoviral vector) into rodent			
	III-D-4-a	rDNA from any source except for > 2/3 of eukaryotic viral genome	**Example:** r/sNA or viral vectors (which does not lead to transmissible infection) into rodent Usually conducted as BSL-
	III-D-4-b	rDNA involving whole animals and not covered by III-D-1 or III-D-4-a	Appropriate containment decided by IBC
	III-D-4-c-(1)	Generating transgenic rodents that require BSL-1 containment	Covered under Section III-E-3
	III-D-4-c-(2)	Purchase or transfer of transgenic rodents	Exempt under Section III-F-6, Appendix C-VI
III-D-5 - Experiments involving whole plants – genetically engineering plants by r/sNA methods, using or propagating such plants, using plants with microorganisms or insects containing r/sNA **Example:** Creation of transgenic plants; Inserting a gene for herbicide tolerance in food or ornamental plants.			
	III-D-5-a	Exotic plant pathogens with recognized potential for serious detrimental impact on ecosystems	Usually conducted at BSL-2+P/BSL-3P
	III-D-5-b	Readily transmissible exotic agents in which the complete and functional genome may be reconstituted in planta	Usually conducted at BSL-2+P/BSL-3P
	III-D-5-c	Readily transmissible exotic agents in the presence of their arthropod vector	Conducted at BSL-4P
	III-D-5-d	Sequences encoding potent vertebrate toxins introduced into plants or associated organisms	Conducted at BSL-3P
	III-D-5-e	Microbial pathogens of insects or small animals associated with plants	Usually conducted at BSL-2+P/BSL-3P
III-D-6 - Experiments involving more than 10 liters of culture. IBC determines containment level Appendix K describes containment conditions Good Large-Scale Practice through BL3-Large Scale **Example:** Use of a 10-Liter fermenter or growing up five 2-Liter flasks of Recombinant or Synthetic Nucleic Acid Molecule culture (i.e. E. coli K-12) qualifies as a large scale experiment.			
III-D-7 - Experiments involving influenza viruses Conducted at the containment level corresponding to the RG of the virus that is the source of the majority of segments			
	III-D-7-a	Human H2N2 (1957-1968)	BSL-2 for H2 HA gene in cold-adapted, live attenuated vaccine strains and for H2N2 genes other than HA
	III-D-7-b	Highly pathogenic avian influenza H5N1	Usually conducted at BSL-3+
	III-D-7-c	1918 H1N1	Usually conducted at BSL-3+

	III-D-7-d	Antiviral susceptibility for genes from viruses in III-D-7-a through III-D-7-c	Higher containment may be required if any of the genes are resistant to both classes of current antiviral agents (adamantanes and neuraminidase inhibitors)
Experiments that require IBC notice simultaneous with initiation (III-E)			
III-E-1		Experiments Involving the Formation of r/sNA Containing no more than 2/3rds of the Genome of any Eukaryotic Virus	**Example:** 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. **Example:** Generation and maintenance of recombinant DNA in E coli non K-12
III-E-2		Experiments Involving Whole Plants	
	III-E-2-a through III-E-2-b	Generation of transgenic plants or testing of nucleic acid-modified microorganisms or insects on whole plants at BSL1P+ or BSL2P	BSL-1P or BSL-2P
III-E-3		Creation of transgenic rodents	BSL-1; experiments requiring BSL-2 or higher covered under section III-D-4
	III-E-3-a	Breeding of BSL-1 transgenic rodents	Exempt under Section III-F-6, Appendix C-VII
Exempt experiments (III-F)			
	III-F-1	Synthetic nucleic acids that cannot replicate nor integrate on a living cell (i.e., oligos)	**Example:** Recombinant or synthetic nucleic acids not in organisms or viruses.
	III-F-2	Nucleic acids that are not in organisms, cells or viruses	
	III-F-3	Exact nucleic acid sequence from a single source that exists contemporaneously in nature	**Example:** Synthetic short interfering RNA (siRNA) that targets an HIV viral protein required for transcription activation, even if this siRNA is injected into animals or used in cell culture.
	III-F-4	Prokaryotic host plasmids or viruses used only in that host or closely related strain	
	III-F-5	Eukaryotic host nucleic acids used only in that host or closely related strain	
	III-F-6	Nucleic acids entirely of DNA segments from different species that exchange DNA by known physiological processes	See Appendix A for list of natural exchangers
	III-F-7	Genomic DNA molecules that have acquired a transposable element	
	III-F-8	Do not present significant risk to health or environment	**Example:** Generation and maintenance of recombinant DNA in *E coli* K-12; Propagating and maintaining r/sNA containing less than one-half of any eukaryotic viral

			genome in tissue culture; experiments involving *Saccharomyces cerevisiae* as a host-vector system See Appendix C, Exemptions under Section III-F-8 for other classes of experiments which are exempt from the NIH Guidelines.