RESEARCH INVOLVING RECOMBINANT OR SYNTHETIC NUCLEIC ACID (R/SNA) MOLECULES

Yale is a NIH funded institution and must comply with the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines), April 2019 or latest edition. The NIH Guidelines are a condition of funding that must be followed by all Yale researchers regardless of their source of funding.

PIs are responsible for determining if they are conducting recombinant or synthetic nucleic acid (r/sNA) research and correctly classifying their experiments as defined by the NIH Guidelines. PIs must also select the appropriate combination of work practices, protective equipment including PPE, engineering controls, and a facility where the work can be done safely. If a PI has questions regarding this classification or appropriate work practices, they should contact EHS for additional support.

R/sNA research is split into non-exempt and exempt categories. However, as the Yale IBC reviews a wide range of biohazardous and biological materials research, both exempt and non-exempt r/sNA research must be registered and authorized by the IBC before research may begin.

Definition of r/sNA Molecules

NIH Guidelines, Section I-B, defines recombinant and synthetic nucleic acids as:

(i) molecules that a) are constructed by joining nucleic acid molecules and b) that can replicate in a living cell, i.e., recombinant nucleic acids.

(ii) nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, i.e., synthetic nucleic acids, or

(iii) molecules that result from the replication of those described in (i) or (ii) above.

The following table provides a quick overview of common r/sNA research experiments, the classification from the NIH Guidelines and the likely biocontainment level (which depends on other factors associated with your research). The table below can help PIs in classifying their own research materials by comparing to the examples and notes below.

Common r/sNA Experiments

Example r/sNA Experiment	Classification NIH Section	Containment Level
Use of a defective adenoviral vector in absence of packaging cell line	III-D-1-a	BSL2
Use of a defective adenoviral vector in animals	III-D-1-a	ABSL2
Use of a defective lentiviral vector (HIV backbone) with packaging cell line	*III-D-3-b	BSL2 (enhanced)
Amphotropic retroviral vector with packaging cells or helper systems (e.g., helper viruses, packaging cell lines, transient transfection systems, replicon systems).	Appendix B-V, III-D-3-a	BSL2
Insertion of Risk Group 2 defective vectors or r/sNA modified Risk Group 2 pathogens into vertebrate or invertebrate animals	III-D-4-b	BSL2
Inserting DNA into Risk Group 2, 3 or 4 pathogens	III-D-1	BSL2 or higher
Inserting nucleic acids from Risk Group 2, 3 or 4 pathogens into prokaryotic or lower eukaryotic cells	III-D-2	BSL2 or higher

Example r/sNA Experiment	Classification NIH Section	Containment Level
Cell culture experiments utilizing standard cloning vectors that have less than 50% of a Risk Group 2 pathogen	III-F, Appendix C-I	Exempt, BSL1
Cloning GFP in non-conjugative *E. coli* with standard cloning vectors (that contain less than 50% of a Risk Group 2 pathogen)	III-F, Appendix C-II	Exempt, BSL1
Expressing r/sNA into *E. coli* B cells (e.g. strain BL21) with standard cloning vectors (that contain less than 50% of a Risk Group 2 pathogen)	III-E	BSL1
Growing > 10 Liters of *Saccharomyces cerevisiae* expressing a gene requiring BSL1 containment	III-D-6	BSL1-LS
Purchase of transgenic rodents or use of transgenic rodents created by another group	III-F, Appendix C-VI	Exempt, BSL1
Creation of transgenic rodents or where the transgene is not a biohazard (e.g. virus, oncogene or toxin)	III-E-3	BSL1
Transgenic drosophila experiments where transgene is not a biohazard	III-D-4-a	BSL1
Non-*E. coli* K12 r/sNA experiments (e.g. *E. coli* B cells)	III-E	BSL1
Baculovirus r/sNA research	III-E	BSL1
r/sNA Research with other Animals (anything in the Kingdom Animalia, e.g. *Caenorhabditis elegans*, flies, mosquitoes, Zebrafish, snails etc)	III-D-4-a	BSL1

*Lentiviral vector work could be classified in III-D-3-a, III-E depending on the system utilized. III-D-3-b is a default setting to include the use of BSL2-enhanced and stringent work practices to ensure that the risk of insertional mutagenesis associated with exposures are considered by the researchers. Replication defective retroviruses are classified in Appendix B-V, III-D-3-a. BSL2 is designated for amphotropic retroviral vectors and BSL1 is the starting point for ecotropic retroviral vectors. BSL2 is advised as retroviruses can integrate into genome and has the potential also to combine with endogenous retroviruses inside the human body to become replication competent. Retroviruses are referred to as "ecotropic" if they can infect only murine cells, or 'amphotropic' if they can infect and transduce both murine and human cells.

The final biocontainment level or the combination of work practices, protective clothing and equipment and where the work will be done, is a factor of the starting Risk Group of the biohazard utilized and its inherent risk to those handling the materials in and outside the laboratory. It also considers the proposed research procedures (e.g., cell sorting, necropsy, sonicating, centrifuging) as part of selecting the biocontainment level and corresponding additional controls that may be needed. Risk assessment also encompasses a review of the vectors and plasmids utilized, the inserted r/sNA molecules and the host cells, microorganisms or organisms involved.

All NIH Guideline Section Classifications from A to F (including Appendix C)

NIH Guideline Section Classifications

NIH Guideline Section	Description	Example
III-A-1	Major Action – antibiotic resistance genes in pathogens (not acquired naturally and could compromise ability to treat the disease)	Inserting Vancomycin gene into *Staphylococcus aureus*
III-B-1	Cloning toxins with an LD50 < 100 ng/kg in *E. coli* K12	Using *E. coli* K12 to express tetanus toxin
III-C-1	Human gene transfer experiments Introduction of r/sNA into human subjects	CAR-T, mRNA, nanoparticles with r/sNA, *ex vivo* transduction of cells delivered to human subjects, direct injection of defective vectors into human subjects
III-D-1	Introduction of r/sNA into a pathogen – use of a r/sNA pathogen in a host-vector system	Defective adenoviral vector Introduction of r/sNA into a pathogen (OVA in L. monocytogenes)
III-D-2	Introduction of r/sNA from pathogens into non-pathogenic prokaryotes and non-pathogenic lower eukaryotes	Defective lentiviral vector in *E. coli* Genes from *Vaccinia virus* in *E. coli*
III-D-3	Use of infectious or defective DNA or RNA viruses with helper systems (e.g., helper viruses, packaging cell lines, transient transfection systems, replicon systems). in cell culture	Defective retroviral, lentiviral vectors
III-D-4	Animal r/sNA experiments	Any r/sNA molecules or cells in animals. r/sNA vectors in animals. r/sNA pathogens in animals.
III-D-5	High risk plant r/sNA research	Exotic, high risk plant pathogens, pests, etc.
III-D-6	Large Scale r/sNA experiments	More than 10 liters of culture in one vessel as part of a r/sNA experiment
III-D-7	r/sNA experiments with high-risk Influenza viruses	Human H2N2 (1957-1968) HPAIV H5N1, or 1918 H1N1
III-D-8	Experiments involving gene drive modified organisms generated by recombinant or synthetic nucleic acid molecules shall be conducted at a minimum of Biosafety Level (BL) 2, BL2-N (Animals) or BL2-P (plant) containment.	Use of a gene drive in mosquitoes within a contained facility that leads to the all mosquitoes harboring the desired gene.
III-E	Experiments that are not described elsewhere in the NIH Guidelines	*E. coli* B cells AAV (> 2/3 AAV genome), Baculovirus
III-F-1	Cell culture experiments with <2/3 pathogen	Cell culture experiments where there is < 2/3 of

NIH Guideline Section	Description	Example
III-E-2	Low risk plant r/sNA experiments	Lower risk r/sNA experiments involving plants, includes: Not a noxious weed and cannot interbreed with noxious weeds in local area. No recognized serious detrimental impact to the environment (*Agrobacterium* spp., *Rhizobium* spp.) Plants that are noxious weeds or can interbreed with noxious weeds in immediate local area. Plants with introduced r/sNA that contains full genome of a nonexotic infectious agent. Non-exotic microorganisms already present in the local area that can have a serious impact on the environment. r/sNA modified Exotic microorganisms that present NO risk to the environment. r/sNA modified arthropods or small animals associated with plants that have NO recognized potential for serious damage to the environment.
III-E-3	Generation of transgenic rodents that require BSL1 containment	Creation of transgenic rodents that require BSL1 containment by the PI using them (the PI that creates the transgenic rodent in this category must register this work. The PI could create the gene or r/sNA sequence for the creation, but the core laboratory or laboratory that inserts the gene into the embryo is the laboratory that registers the work.
III-F-1	Use of only oligonucleotides that lack an origin of replication	r/sNA materials that cannot replicate in a living cell (oligonucleotides that do not have an origin of replication, cannot integrate into DNA, does not produce a toxin with LD50 < 100 ng/kg body weight)
III-F-2	PCR	Those not in organisms, cells, or viruses, unmodified (not put into a delivery vehicle that can get it across a cell membrane), e.g. PCR
III-F-3	r/sNA experiments using the exact sequence that exists already in nature	
III-F-4	r/sNA from a prokaryotic host propagated only in that host, or when transferred by an existing physiological means	
III-F-5	r/sNA from a eukaryotic host propagated only in that host	
III-F-6	r/sNA from different species that exchange DNA by a known physiological process, if on the list of known exchangers published by the NIH.	See Appendix A of the NIH Guidelines for the 4 lists. Exchangers must between microorganisms on the same sublist.

NIH Guideline Section	Description	Example
III-F-7	r/sNA experiments involving genomic DNA molecules that have acquired a transposon or other transposable element that does not contain r/sNA.	
III-F-8	Those listed by the NIH in Appendices C-1 to C-8 that do not present a significant risk to man or the environment	See Appendix C for examples (directly below)
III-F-8 App C-I	Cell culture < 50% Risk Group 2 pathogen (r/sNA from Risk Group and 3 and 4 not exempt)	Cell culture r/sNA with <50% of a pathogen genome. (Exceptions: r/sNA with RNA or DNA from Risk Group 3, 4 or restricted pathogens USDA permitted materials, genes from toxins that have an LD50 < 100 ug/kg body weight, large-scale or human gene transfer experiments)
III-F-8 App C-II	*E. coli* K12 (not Risk Group 3 and 4 and experiments not described in other sections)	Use of *E. coli* K-12 host-vector systems, provided that: (Exceptions: r/sNA with RNA or DNA from Risk Group 3, 4 or restricted pathogens USDA permitted materials, genes from toxins that have an LD50 < 100 ug/kg body weight, large-scale or human gene transfer experiments)
III-F-8 App C-III	*Saccharomyces cerevisiae* (not Risk Group 3 and 4 and experiments not described in other sections)	Experiments involving *Saccharomyces cerevisiae* and *Saccharomyces uvarum* host-vector systems: (Exceptions: r/sNA with RNA or DNA from Risk Group 3, 4 or restricted pathogens USDA permitted materials, genes from toxins that have an LD50 < 100 ug/kg body weight, large-scale or human gene transfer experiments)
III-F-8 App C-IV	*Kluyveromyces lactis* Host-Vector Systems	Experiments involving *Kluyveromyces lactis* host-vector systems (Exceptions: r/sNA with RNA or DNA from Risk Group 3, 4 or restricted pathogens USDA permitted materials, genes from toxins that have an LD50 < 100 ug/kg body weight, large-scale or human gene transfer experiments)
III-F-8 App C-V	*Bacillus subtilis* (not Risk Group 3 and 4 and experiments not described in other sections)	*Bacillus subtilis* or *Bacillus licheniformis* Host-Vector Systems: (Exceptions: r/sNA with RNA or DNA from Risk Group 3, 4 or restricted pathogens USDA permitted materials, genes from toxins that have an LD50 < 100 ug/kg body weight, large-scale or human gene transfer experiments)
III-F-8 App C-VI	r/sNA molecules derived entirely from extrachromosomal elements of the Gram Positive organisms listed in App C-VI	It is only applicable to the limited microbes listed in this Appendix

NIH Guideline Section	Description	Example
III-F-8 App C-VII	Purchase or transfer of transgenic rodents for experiments that require BSL1 containment	Only applicable to rodents and must not exceed BSL1 containment
III-F-8 App C-VIII	Generation of BSL1 transgenic rodents via breeding	Generation of transgenic rodents via Breeding that can be housed at BSL1, if: (1) Both parental rodents can be housed under BSL1 containment; and (2) neither parental transgenic rodent contains the following genetic modifications: (i) incorporation of more than one-half of the genome of an exogenous eukaryotic virus from a single family of viruses; or (ii) incorporation of a transgene that is under the control of a gammaretroviral long terminal repeat (LTR); and (3) the transgenic rodent that results from this breeding is not expected to contain more than one-half of an exogenous viral genome from a single family of viruses.
Appendix F	Cloning Toxins with LD50 > 100 ng/kg and < 100 ug/kg in *E. coli* K12	Abrin, *Clostridium perfringens* epsilon toxin, *Staphylococcus aureus* alpha toxin, *Staphylococcus aureus* beta toxin, ricin, *Pseudomonas aeruginosa* exotoxin A, *Bordetella pertussis* toxin, the lethal factor of *Bacillus anthracis*, the *Pasteurella pestis* murine toxins, the oxygen-labile hemolysins such as streptolysin O, and certain neurotoxins present in snake venoms and other venoms