NIH Guideline Resources – NIH Guidelines Sections With Examples

Important Notes on Research with Recombinant or Synthetic Nucleic Acid (r/sNA) Molecules

PIs must register their r/sNA research materials whether they create, purchase, or obtain them from a colleague. A colleague's registration does not cover your research unless you are added as an assistant to that PI, perform the work in their laboratory, and that PI has assumed all responsibility for the research that you will perform on their protocol in their laboratory. This relationship would have to be updated and approved by the Yale Biological Safety Committee. For example, buying a fluorescent zebrafish for your child at home is not regulated, but purchasing one or creating one for research is and will require registration and approval by the Yale Biological Safety Committee and IACUC. The same holds true for obtaining replication defective retroviral vectors, lentiviral vectors, or recombinant cells from colleagues. PIs must register this r/sNA research prior to initiation.

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).

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) virus	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
Gene Drive Modified Organisms research developed with recombinant or synthetic nucleic acids molecules research	III-D-8	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.

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, its inherent risk to those handling the materials and other 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.

Higher Risk Lentiviral Vector Experiments

Be careful if purchasing or creating a lentiviral vector where the envelope gene is placed in cis with lentivirus sequences in the transfer vector as this defeats a safety mechanism of the defective vector. These genes should be placed on the helper systems (e.g., helper viruses, packaging cell lines, transient transfection systems, replicon systems) plasmids in trans. This research must be justified to the YALE BIOLOGICAL SAFETY COMMITTEE and will result in an elevation in risk and biocontainment. Be very careful if using lentiviral vectors and amphotropic retroviral vectors with a library of CRISPR/Cas9 sequences targeted against the human genome. Exposures to these vectors could result in the silencing of tumor suppressing genes (e.g. within the lung from aerosol exposures).

Copy of the April 2024 (or current edition) NIH Guidelines

Full text of the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf

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

NIH Guideline Section Classifications for recombinant or synthetic nucleic acid (r/sNA) molecules

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) viruses or helper systems (e.g., helper viruses, packaging cell lines, transient transfection systems, replicon systems)plasmids 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 to push a desired trait in mosquitoes in a contained environment to all mosquitoes within the population.

NIH Guideline Section	Description	Example
III-E	Experiments that are not described elsewhere in the NIH Guidelines	*E. coli* B cells AAV (> 2/3 AAV genome), Baculovirus
III-E-1	Cell culture experiments with <2/3 pathogen genome	Cell culture experiments where there is < 2/3 of any eukaryotic virus in the absence of helper systems (e.g., helper viruses, packaging cell lines, transient transfection systems, replicon systems) viruses or helper systems (e.g., helper viruses, packaging cell lines, transient transfection systems, replicon systems)plasmids
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

NIH Guideline Section	Description	Example
		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-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	
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)

NIH Guideline Section	Description	Example
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
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

NIH Guideline Section	Description	Example
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

Exempt Experiments

NIH Guidelines Section	Description
III-F-1	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	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.
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.
NIH Section III-F-8	Appendix C of the NIH Guidelines lists the exemptions under Section III-F-8, which are those that do not present a significant risk to health or the environment.

Supplemental Information on the NIH Guidelines Sections

r/sNA Research Requiring Authorization by the National Institutes of Health (NIH) **(Notify the Institutional Biosafety Committee (YALE BIOLOGICAL SAFETY COMMITTEE) for a separate application if Yes)**	
NIH Section **III-A-1**	Transfer of a drug resistance trait to microorganisms (e.g. pathogens) that are not acquired naturally, that could compromise the ability to treat the disease or control the agent in the environment (example: creating vancomycin resistant *Staphylococcus aureus*). This category does not include placing ampicillin resistance in *E. coli* K12. Note: NIH Director and YALE BIOLOGICAL SAFETY COMMITTEE registration and authorization is required prior to initiation for this work. NIH authorization is not required if the drug resistance is acquired naturally. Please include a reference to verify the natural acquisition of drug resistance if applicable.)
NIH Section **III-B-1**	Deliberate formation of recombinant or synthetic nucleic acid molecules 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). The NIH Office of Science Policy (OSP) and Yale Biological Safety Committee approval is required prior to initiation.
Human Subjects Experiments that Require YALE BIOLOGICAL SAFETY COMMITTEE and IRB Approval Prior to Initiation **(FDA Authorization is also required for this research category)**	
NIH Section **III-C-1**	Experiments Involving the Deliberate Transfer of Recombinant or Synthetic Nucleic Acid Molecules, or DNA or RNA Derived from Recombinant or Synthetic Nucleic Acid Molecules, into One or More Human Research Participants. (Clinical trials with replication defective vectors such as adeno-associated virus, lentivirus, retrovirus, adenovirus).

	Includes CAR T-cell therapies, introduction of mRNA into human subjects, and includes the use introduction of recombinant autologous or allogeneic cells that have been genetically modified *ex vivo*.

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

Human gene transfer is the deliberate transfer into human research participants of either:

1. Recombinant nucleic acid molecules, or DNA or RNA derived from recombinant nucleic acid molecules, or

2. Synthetic nucleic acid molecules, or DNA or RNA derived from synthetic nucleic acid molecules, that meet any one of the following criteria:

a. Contain more than 100 nucleotides; or

b. Possess biological properties that enable integration into the genome (e.g., *cis* elements involved in integration); or

c. Have the potential to replicate in a cell; or

d. Can be translated or transcribed.

Research cannot be initiated until YALE BIOLOGICAL SAFETY COMMITTEE and all other applicable institutional and regulatory authorization(s) and approvals have been obtained.

The deliberate transfer of recombinant or synthetic nucleic acids into one human research participant, conducted under a Food and Drug Administration (FDA) regulated individual patient expanded access Investigational New Drug (IND) or protocol, including for emergency use, is not research subject to the *NIH Guidelines* and thus does not need to be submitted to an YALE BIOLOGICAL SAFETY COMMITTEE for review and approval.

Human Gene Transfer Research Using Gene Editing Technologies

No gene editing of the germ line human embryos or germ cells for clinical application is allowed. Gene editing of human embryos and germ cells for scientific purpose may be allowed but must be evaluated and approved on a case-by-case basis by the appropriate federal and local scientific review committees.

r/sNA Research Requiring YALE BIOLOGICAL SAFETY COMMITTEE Authorization Prior to Initiation **(In addition to the r/sNA experiments listed above)**	
NIH III-D-1	Introduction of r/sNA into Risk Group 2 or higher Pathogens (as either host or vector)

Examples:

NIH Section III-D-1

Use of a Pathogen as a Host-Vector System

NIH Section III-D-1-a

Introduction of r/sNA into a Risk Group 2 Pathogen

Use of a replication defective adenovirus vector (83% of the adenovirus genome) to deliver r/sNA to cells.

Introduction of GFP into Plasmodium falciparum or Herpes Simplex Virus-1 (Risk Group 2 pathogens)

(Note: this same NIH Section Classification of III-D-1-a and BSL2/ABSL2 can be used for the use of these defective Risk Group 2 pathogens or pathogens harboring r/sNA in animals).

Introduction of r/sNA into pathogenic bacteria, e.g. GFP in *Salmonella typhimurium*, the ovalbumin gene (OVA) into *Listeria monocytogenes*, are classified under NIH Section III-D-1 (III-D-1-a for the introduction of r/sNA into Risk Group 2 pathogens) and BSL2 is the starting containment level. Purchasing a pathogen that contains r/sNA (e.g. buying *Listeria monocytogenes* with the OVA gene in it, LM-OVA, would still require registration and approval prior to use).

NIH Section III-D-1 (BSL2/ABSL2 or higher) or III-D-4-b (BSL2/ABSL2 or higher)

Hazardous experiments with animal species are III-D-4-b, BSL2/ABSL2 or higher depending on the Risk Group of the research materials utilized.

NIH Section III-D-1-b

Introduction of r/sNA into a Risk Group 3 Pathogen

Placing GFP into Mycobacterium tuberculosis (Risk Group 3 pathogen), BSL3.

(If also used in animals, III-D-4-b, BSL3/ABSL3)

r/sNA Research Requiring YALE BIOLOGICAL SAFETY COMMITTEE Authorization Prior to Initiation **(In addition to the r/sNA experiments listed above)**	
III-D-2	Cloning DNA or RNA from Risk Group 2 or higher Pathogens (>50% genome of a Risk Group 2 Pathogen, any percentage of Risk Group 3 and 4 Pathogens)

Examples:

NIH Section III-D-2

Cloning r/sNA from Pathogens into non-pathogen prokaryotes or lower eukaryotes host-vector systems

NIH Section III-D-2-a

r/sNA from *Mycobacterium tuberculosis* into *E. coli* K12.

Use of a 2nd generation defective lentivirus vector (which contains r/sNA from HIV) into *E. coli* K12.

BSL2 is the starting point and depends on the r/sNA utilized.

(These protocols in animals will fall under III-D-4, III-D-4-a or III-D-4-b depending on the percentage of the genomes and risk of the materials). Default to III-D-4-b if the r/sNA contains > 2/3 of a pathogen genome or replication competent r/sNA pathogens are used in animal experiments.

Check with the the Yale Biological Safety Committee prior to introducing r/SNA from Risk Group 4 pathogens (e.g. Lassa Fever, Ebola or Marburg viruses) into these host-vector systems to verify the safety and containment level.

NIH Section **III-D-3**	Use of infectious or defective pathogen vectors with helper systems (e.g., helper viruses, packaging cell lines, transient transfection systems, replicon systems) virus or packaging celllines in tissue culture.

Examples:

NIH Section III-D-3

Infectious DNA or RNA Viruses or Defective DNA or RNA viruses in the presence of helper systems (e.g., helper viruses, packaging cell lines, transient transfection systems, replicon systems) virus or helper systems (e.g., helper viruses, packaging cell lines, transient transfection systems, replicon systems) plasmids in tissue culture

NIH Section III-D-3-a

Infectious or defective Risk Group 2 viruses in the presence of helper systems (e.g., helper

viruses, packaging cell lines, transient transfection systems, replicon systems) virus or plasmids,

BSL2(e.g. Use of an amphotropic defective retroviral vector and helper systems (e.g., helper

viruses, packaging cell lines, transient transfection systems, replicon systems) virus or plasmids)

r/sNA Research Requiring YALE BIOLOGICAL SAFETY COMMITTEE Authorization Prior to Initiation

(In addition to the r/sNA experiments listed above)

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. Ecotropic and amphotropic are terms used only with retroviruses.

Pseudotyping a vector with the VSV-G gene can expand the host range of cells substantially, allowing the vector to infect a wide range of cell types. Pantropic is the range used to describe these vectors and is not associated with a specific type of vector.

NIH Section III-D-3-b

Infectious or defective Risk Group 3 viruses in the presence of helper systems (e.g., helper

viruses, packaging cell lines, transient transfection systems, replicon systems) virus or plasmids,

BSL3Note: Defective lentiviral vector systems contain HIV, which is a Risk Group 3 pathogen. A classification of III-D-3-b, using BSL2-enhanced work practice is generally utilized (BSL3 work practices in a BSL2 laboratory), especially if the defective lentivirus is pseudotyped with the VSV-G gene, that makes the vector pantropic.

These experiments in animals can be conducted at III-D-4-b, BSL2/ABSL2.

NIH Section **III-D-4**	Addresses r/sNA research involving animals. There are additional questions in this section, but the key take home message is that this covers ALL r/sNA work involving any type of animal, vertebrate or invertebrate, any species in the Kingdom Animalia (including *Caenorhabditis elegans*, other nematodes, arthropods and insects, including *Drosophila melanogaster*, zebrafish, etc.). It also includes the introduction of recombinant cells into animals (recombinant human or animal cells that have been made by other laboratories *ex vivo* or purchased are included in this section). r/sNA experiments involving stable introduction of r/sNA into a vertebrate animal's genome or the introduction of viable r/sNA modified microorganisms or cells into an animal (rodents, zebrafish).

Examples:

r/sNA Experiments with Animals

NIH Section III-D-1 (as described above)

NIH Section III-D-4-a, BSL1/ABSL1

This involves any r/sNA experiments involving any species in the Kingdom Animalia. This includes r/sNA research with mice, rats, guinea pigs, zebrafish, *Drosophila melanogaster*, other insects and arthropods, mosquitoes, flies, snails, *Caenorhabditis elegans*, sponges and any other species in the animal kingdom.

r/sNA Research Requiring YALE BIOLOGICAL SAFETY COMMITTEE Authorization Prior to Initiation

(In addition to the r/sNA experiments listed above)

NIH Section III-D-1 (BSL2/ABSL2 or higher) or III-D-4-b (BSL2/ABSL2 or higher)

Hazardous experiments with animal species are III-D-4-b, BSL2/ABSL2 or higher depending on the Risk Group of the research materials utilized.

NIH Section 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 YALE BIOLOGICAL SAFETY COMMITTEE.

NIH Section III-D-4-c.

NIH Section III-E-3, BSL1/ABSL1

Experiments involving the generation of transgenic rodents that require BSL1 containment

NIH Section III-D-5	r/sNA experiments involving exotic plants, plant pathogens, or weeds that have potential for serious detriment to crops and ecosystems

III-D-5

r/sNA experiments in plants (high risk potential)

r/s Plant pathogens that represent a serious risk to managed or natural ecosystems.

r/s plant pathogen genomes that could be infectious under experimental conditions with same serious risk to the environment.

r/s exotic plant pathogens used with the arthropod vectors that could cause serious damage to major U.S. crops.

r/s plant experiments involving the introduction of potent toxins to vertebrates into plants or associated organisms.

r/s experiments involving microbial pathogens of insects or small animals associated with plants that could have serious or detrimental impact to the environment.

NIH Section III-D-6	Large Scale r/sNA research with at least 10 L in a single vessel or container, such as a fermenter

r/sNA Research Requiring YALE BIOLOGICAL SAFETY COMMITTEE Authorization Prior to Initiation **(In addition to the r/sNA experiments listed above)**	
YALE BIOLOGICAL SAFETY COMMITTEE is attempting to identify if there is a significant volume of r/sNA materials generated as part of your experiment. A spill of this volume of material can easily leak into drains if present in the laboratory in the event of a spill. This release would be reportable to regulatory agencies, represent a loss of control, and make decontamination difficult. Please obtain authorization for large scale work prior to initiation.	
NIH Section **III-D-7**	High risk Influenza virus r/sNA experiments, involving the following strains of Influenza virus: Human H2N2 (1957-1968), HPAIV H5N1 and 1918 H1N1
This category is only for the 3 high-risk influenza viruses listed above. These are Risk Group 3 pathogens and can be higher depending on the experimental protocol. Standard Risk Group 2 influenza viruses are not classified in this section.	
NIH Section **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.
Suppression drives force deleterious traits into a population, leading those populations to crash or be much diminished. If the mosquito is eliminated, so too will all the diseases it can transmit. Population modification or replacement, leaves the insect in place in the environment but blocks disease transmission by modifying the insect vector to prevent pathogen transmission.	
NIH Section **III-E**	Recombinant or synthetic nucleic acid experiments that are not classified under Sections III-A, III-B, III-C, III-D, III-F? The categories of r/sNA research described in Section III-E are:

Examples:

E. coli B cells

Research in *E. coli* B cells and other non *E. coli* K12 cells are not exempt, are classified in Section III-E, and BSL1 is the general starting point.

Baculovirus

An insect virus not pathogenic to humans. This is classified at Section III-E (experiments not classified elsewhere in the NIH Guidelines) and is conducted at BSL1 containment.

Adeno-Associated virus (AAV)

AAV is not a human pathogen.

Classification of r/sNA research with AAV depends on the percentage of AAV in the vector.

If you are purchasing a prepared AAV vector for r/sNA research, it would likely have less than 10% of the AAV genome present and use in cell culture would be Exempt, III-F-8, Appendix C-I (and the BSL2 for human cells, BSL1 for murine cells).

r/sNA Research Requiring YALE BIOLOGICAL SAFETY COMMITTEE Authorization Prior to Initiation (In addition to the r/sNA experiments listed above)	
NIH Section III-E-1	Recombinant or synthetic nucleic acid molecules containing no more than two-thirds of the genome of any eukaryotic virus propagated and maintained in cells in tissue culture.
Example: If you are making your own AAV vectors and using helper systems (e.g., helper viruses, packaging cell lines, transient transfection systems, replicon systems) viruses or cells that contain r/sNA fromadenovirus (as AAV is dependent on adenovirus for replication), the classification is related to the percentage of the genome present. If the AAV vector has more than 50% of the AAV genome, then III-E would be the NIH Section for classification. BSL2 if human cells are used. (Placing this in animals would not be exempt however and classed at III-D-4-a, ABSL1).	
NIH Section 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.
NIH Section III-E-3	**Generation of transgenic rodents that require BSL1 containment** Section III-E-3. Experiments Involving Transgenic Rodents requiring BSL1 containment. This section covers experiments involving the generation of rodents in which the animal's genome has been altered by stable introduction of recombinant or synthetic

r/sNA Research Requiring YALE BIOLOGICAL SAFETY COMMITTEE Authorization Prior to Initiation (In addition to the r/sNA experiments listed above)	
	nucleic acid molecules, or nucleic acids derived therefrom, into the germ-line (transgenic rodents). 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.

Lower risk r/sNA Toxin Experiments that require authorization by the YALE BIOLOGICAL SAFETY COMMITTEE	
NIH Appendix F-II-a	Cloning of toxins in *E. coli* K12 that have an LD50 of > 100 ng/kg but < 1 ug/kg body weight
NIH Appendix F-II-b	Cloning of toxins in *E. coli* K12 that have an LD50 of > 1 ug/kg but < 100 ug/kg body weight