

FREQUENTLY ASKED QUESTIONS

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)

1. Why has the NIH modified the *NIH Guidelines* to include synthetic nucleic acid molecules?

The impetus for amending the *NIH Guidelines* was two-fold:

- (1) recognition that appropriate biosafety containment of an agent is critical regardless of the technology used to generate that agent (i.e., recombinant or synthetic techniques), and
- (2) a recommendation by the National Science Advisory Board for Biosecurity (NSABB) that the United States Government work with the scientific community to ensure that current biosafety guidelines are appropriate, adequate, and easily understood with respect to working with synthetic nucleic acids.

2. What has changed with respect to the scope of the *NIH Guidelines*?

The scope of the *NIH Guidelines* has been modified to cover explicitly certain types of basic and clinical research with nucleic acid molecules created solely by synthetic means. Certain classes of basic and clinical research with synthetic nucleic acids will be exempt.

The new language in Section I-A of the *NIH Guidelines* states “The purpose of the *NIH Guidelines* is to specify the practices for constructing and handling:

- (i) recombinant nucleic acid molecules,
- (ii) synthetic nucleic acid molecules, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, and
- (iii) cells, organisms, and viruses containing such molecules.”

Throughout the *NIH Guidelines*, the term “recombinant DNA molecules” has been replaced as appropriate with “recombinant or synthetic nucleic acid molecules” which encompasses research with both recombinant and/or synthetic nucleic acids. As a result, the amended *NIH Guidelines* apply (unless otherwise exempted by other sections of the *NIH Guidelines*, e.g. III-F) to research with recombinant or synthetically derived nucleic acids, including those that are chemically or otherwise modified analogs of nucleotides (e.g., morpholinos), or both.

3. How are recombinant and synthetic nucleic acids defined under the amended *NIH Guidelines*?

In the amended *NIH Guidelines*, recombinant and synthetic nucleic acid molecules are defined as:

- (i) molecules that a) are constructed by joining nucleic acid molecules and b) 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.

4. What institutions are subject to the amended *NIH Guidelines*?

Institutions that receive NIH funding for any research involving recombinant or synthetic nucleic acids, unless such research is specifically exempted by the *NIH Guidelines*, must comply. Previously, institutions were required to follow the *NIH Guidelines* only if they received NIH funding for recombinant DNA research. If an institution is receiving NIH funding for only research with synthetic nucleic acids, and that research is covered under the amended *NIH Guidelines*, any research with synthetic nucleic acids or recombinant DNA conducted at the institution, regardless of the source of funding, will need to comply with all of the requirements of the *NIH Guidelines*. For further information on institutional requirements under the *NIH Guidelines* see:

http://oba.od.nih.gov/oba/ibc/FAQs/IBC_Frequently_Asked_Questions7.24.09.pdf/.

Other Federal agencies, including the Departments of Energy, Veterans Affairs, and Agriculture, may have policies in place stating that recombinant or synthetic nucleic acid research conducted by or funded by these agencies must comply with the *NIH Guidelines*, and investigators receiving funding from other Government agencies need to check with those agencies regarding the applicability of the amended *NIH Guidelines*. While the *NIH Guidelines* may not govern all Government funded and privately funded research, it may be used as a tool for the entire research community to understand the potential biosafety implications of this type of research.

5. When do the requirements under the amended *NIH Guidelines* go into effect?

The changes are effective March 5, 2013, which is six months from the date of publication. All ongoing and proposed experiments with synthetic nucleic acids newly subject to the amended *NIH Guidelines* need to be registered by the Principal Investigator with the Institutional Biosafety Committee (IBC) by the effective date listed above. The six month time frame was deemed sufficient to allow institutions to develop new procedures, as well as

outreach and training for investigators whose research now will be subject to the amended *NIH Guidelines*.

6. What basic research with synthetic nucleic acids is covered under the amended *NIH Guidelines*?

The amended *NIH Guidelines* apply to research with synthetic nucleic acids that presents biosafety risks equivalent to recombinant DNA research that is subject to the *NIH Guidelines*. For example, research with a genetically modified virus or a vector derived solely by synthetic techniques is subject to the amended *NIH Guidelines*.

7. What impact is this change projected to have on institutions?

Presently, most research with synthetic nucleic acids also involves the use of recombinant techniques and is already covered by the *NIH Guidelines*. Thus, the NIH anticipates that in the near-term there will not be a significant increase in the amount of research subject to the amended *NIH Guidelines* due to these amendments.

8. What basic research with synthetic nucleic acids is exempt from the amended *NIH Guidelines*?

In keeping with the exemptions for recombinant DNA molecules described in Section III-F of the previous version of the *NIH Guidelines*, certain synthetic nucleic acid molecules are exempt from the amended *NIH Guidelines* because:

- (1) their introduction into a biological system is not expected to present a biosafety risk that requires review by an IBC, or
- (2) the introduction of these nucleic acid molecules into biological systems would be akin to processes of nucleic acid transfer that already occur in nature, so that the appropriate biosafety practices would be the same as those used for the natural organism.

Even though these molecules are exempt from the amended *NIH Guidelines*, other federal and state standards of biosafety may still apply to research with such molecules (e.g., the Centers for Disease Control and Prevention/NIH publication *Biosafety in Microbiological and Biomedical Laboratories*). The exemptions that were in the previous version of the *NIH Guidelines* under Section III-F also will apply to research with synthetic nucleic acids. This includes an exemption for any research with synthetic nucleic acids that are not contained in cells, organisms, or viruses.

In addition, in review of the *NIH Guidelines*, it was recognized that most biosafety risks arise from recombinant molecules because they are designed to replicate or are derived from molecules that can replicate. Synthetic nucleic acid molecules may not be designed to replicate and therefore may not pose the same biosafety risks. Also, a biosafety risk may arise due to the ability of nucleic acid molecules to integrate into the genome or produce a toxin. Therefore, an

exemption was made for research with certain low-biosafety-risk synthetic nucleic acid molecules. Specifically, synthetic nucleic acids molecules that meet the following criteria will be exempt:

Those synthetic nucleic acids that:

- (1) can neither replicate nor generate nucleic acids that can replicate in any living cell (e.g., oligonucleotides or other synthetic nucleic acids that do not contain an origin of replication or contain elements known to interact with either DNA or RNA polymerase), and
- (2) are not designed to integrate into DNA, and
- (3) do not produce a toxin that is lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight.

An example of an exempt molecule would be research with a 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.

9. Does the synthesis of a naturally occurring organism fall under the amended *NIH Guidelines*?

The *NIH Guidelines* always have exempted research with recombinant molecules that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, although one or more segments may be a synthetic equivalent. This language exempted nucleic acid sequences that are essentially copies of those found in nature. The language has been modified in the amended *NIH Guidelines* by limiting this exemption to those nucleic acid sequences that exist contemporaneously in nature. The new exemption now applies to those molecules that consist solely of the exact recombinant or synthetic nucleic acid sequence from a single source that exists outside of a laboratory setting.

Research with nucleic acid sequences for organisms that do not currently exist in nature outside of the laboratory setting would not be exempt (e.g., an identical copy of the 1918 H1N1 influenza virus).

10. Is the chemical synthesis of nucleic acids subject to the *NIH Guidelines*?

No. The amended *NIH Guidelines* do not cover the chemical synthesis of nucleic acids. While the scope of the amended *NIH Guidelines* refers to “constructing” nucleic acids, the amended *NIH Guidelines* exempts research with nucleic acids that are not contained in cells, organisms, or viruses. Therefore, the chemical synthesis of nucleic acids is exempt. The amended *NIH Guidelines* only apply once synthetic nucleic acids are placed in a biological system.

11. Will recombinant DNA protocols still need to be reviewed by an Institutional Biosafety Committee?

Yes. The existing requirements to review recombinant DNA research that were previously covered under the *NIH Guidelines* have not changed.

12. Does research need to encompass both recombinant and synthetic nucleic acids to be subject to the amended *NIH Guidelines*

No. The amended *NIH Guidelines* apply to research involving either recombinant or synthetic nucleic acid molecules (or both in combination) unless the research is specifically exempted under one or more conditions listed in Section III-F.

13. What clinical research with synthetic nucleic acids is covered under the amended *NIH Guidelines*?

Section III-C-1 of the amended *NIH Guidelines* covers human gene transfer experiments, i.e., research that involves the deliberate transfer into human research participants of either:

- Recombinant nucleic acid molecules, or DNA or RNA derived from recombinant nucleic acid molecules, or
- Synthetic nucleic acid molecules, or DNA or RNA derived from synthetic nucleic acid molecules, that meet any one of the following criteria:
 - Contains more than 100 nucleotides; or
 - Possesses biological properties that enable integration into the genome (e.g., *cis* elements involved in integration); or
 - Have the potential to replicate in a cell; or
 - Can be translated or transcribed.

The above criteria are designed to capture synthetic nucleic acid agents that share common characteristics with current gene transfer agents that are delivered by vectors. The key characteristics of gene transfer agents are their ability to be translated or transcribed, potential for replication, even if engineered to be replication incompetent; and their ability to integrate and persist. In contrast, synthetic oligonucleotides, such as short interfering RNAs or micro-RNAs, that are directly administered or delivered in chemical vehicles, such as nanoparticles, are very small molecules and do not share these same characteristics. This definition of human gene transfer is designed to exclude these constructs from the amended *NIH Guidelines* and instead to only capture agents that would be the equivalent of recombinant gene transfer agents, (e.g., short-hairpin RNA that is delivered in a plasmid, bacterial, or other viral vector).

14. How do the changes affect what gene transfer protocols need to be registered?

OBA does not anticipate a significant increase in the number or types of gene transfer protocols that will need to be registered. At this time, recombinant techniques are predominately used to create vectors, such as a plasmid. It is likely that over the next few years or longer recombinant techniques will continue to be used to create large nucleotides that are designed to integrate, replicate, or be translated or transcribed. However, as synthetic techniques evolve, this may change and it may be more efficient to produce synthetic vectors for gene transfer.

15. How does a risk assessment for research with synthetic nucleic acid molecules differ from a risk assessment for research with recombinant nucleic acid molecules?

The risk assessment framework of the *NIH Guidelines* uses the risk group of the parent organism as a starting point for determining the necessary containment level. For example, genetic modifications of a Risk Group 3 organism (defined as agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available) would generally be carried out at Biosafety Level 3 (BL3) containment but the containment level might be raised or lowered depending on the specific construct and the experimental manipulations.

In most instances, this risk assessment framework can be effectively applied to assess the biosafety risks of experiments with synthetic nucleic acids. However, synthetic biology research has the potential to create complex, novel organisms for which identification of a parent organism may be more difficult or may not be as relevant to the risk assessment as it is with more traditional recombinant organisms. The risk assessment may also be complicated by the limitations in predicting gene function from sequence(s) or the synergistic effects from combining sequences from different sources in a novel context. In such cases, the risk assessment should include at least two levels of analysis. The first involves a consideration of the Risk Groups of the source(s) of the sequences and the second involves an assessment of the functions that may be encoded by these sequences (e.g., virulence or transmissibility). It may be prudent to first consider the highest risk group classification of all agents that are the source of sequences included in the construct. Other factors to be considered include the percentage of the genome contributed by each parent agent and the predicted function or intended purpose of each contributing sequence. The initial assumption should be that all sequences will function as they did in the original host context.

The risk assessment should also consider that the combination of certain sequences in a new biological context may result in an organism for which the risk profile could be higher than that of the contributing organisms or sequences. The synergistic function of these sequences may be one of the key attributes to consider in deciding whether a higher containment level is warranted, at least until further assessments can be carried out. A new biosafety risk may occur with an organism formed through the combination of sequences from a number of organisms or due to the synergistic effect of combining transgenes that results in a new phenotype.

16. Will the composition and expertise of IBCs need to change to address the changes to the amended *NIH Guidelines*?

No. The required composition of an IBC has not changed under the amended *NIH Guidelines*. In general, when constituting an IBC, institutions should consider their research portfolios and constitute the IBC so that it has the necessary expertise to review research that is subject to the amended *NIH Guidelines*.

17. Where can I get more information about the *NIH Guidelines*?

Further information is available on the NIH OBA website at:

http://oba.od.nih.gov/rdna/nih_guidelines_oba.html

If you have specific questions about the *NIH Guidelines*, please contact the NIH Office of Biotechnology Activities by e-mail at oba@od.nih.gov, by telephone at 301-496-9838, by fax to 301-496-9839, or by mail to:

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