

Stakeholder Workshop
“Future Directions & Research Priorities for the
USDA Biotechnology Risk Assessment Grants Program”
Washington, DC
June 9-10, 2003

Research Needs & Priorities for **Microorganisms**¹

Summary

Assessing and managing environmental risks from transgenic microorganisms formed the focus of our discussion. Risk is defined as a function of hazard and exposure. Research activities aimed at assessing potential hazards from the use of transgenic microorganisms and assessing levels and routes of exposure were deemed high priorities. The group acknowledged that our ability to measure differences resulting from the use of transgenic organisms and to interpret the ecological meaning of observed change(s) was fundamental to risk assessment. Hence, priority should be given to improving the capacity to detect differences in behavior and ecology between parent and transgenic organisms that also allow us to better interpret changes at the ecosystem level. In addition to defining and prioritizing research needs for current technologies, the group recommends that the BRARG program take a proactive role in anticipating regulatory needs for technologies in the pipeline and providing funding for associated risk assessment.

The top priorities as expressed by the Federal regulatory representatives are:

1. Improved laboratory methods involving relevant organisms that can be used to assess potential impact on non-target organisms (particularly for aquatic species).
2. Ability to predict and monitor distribution post release (including marker and detection systems).
3. What are the safety issues and precautions necessary with DNA vaccines?
4. Containment methods development.
5. Understanding the behavior of the organism in the environment. (including survival, transport, host range, genetic transfer, vectors, virulence, etc).
6. Use of genetic resources to distinguish pathogens from closely related taxa.

Our discussions centered on three general areas: 1) ecology of transgenic microorganisms, 2) exploitation of new genomic resources, and 3) containment. The research needs identified within these three areas are prioritized by section below (**H** = high priority, **HM** = high to medium priority, **M** = medium priority, **ML** = medium to low priority, and **L** = low priority).

I. Ecology of transgenic microorganisms

A key priority research area for this program is assessing the stability of introduced genetic material and evaluating the likelihood that it may be transferred to other organisms upon release of the transgenic into the environment. Among the questions that need to be addressed are:

- **(H)** Is the gene(s) more or less stable in the recombinant than it is in the parental background? Gene stability will depend on the transgenic host and the location in the genome where the gene(s) has been inserted; hence evaluation on a case by case basis is needed.
- **(H)** What is the potential for genetic mobility or recombination? The presence of transposons, insertion sequences and other mobile genetic elements in the recombinant will influence this; hence the presence, location, and activity of such elements and their potential influence should be known.

- (HM) What is the role of natural selection in the maintenance or stability of introduced genetic material? When, how, and why might inserted genetic material ‘disappear’ from the organism?
 - (M) What methods can best be used to detect gene mutations and/or gene evolution over time?
 - (ML) If liberated, how long does extracellular DNA persist in the environment? Is this a potential ‘problem’?

Another priority with regard to the ecology of transgenic microorganisms is assessing the capacity for transgenic microorganisms to survive and spread once they are introduced into the environment. Host range, ecological fitness relative to the wild type, ability to compete with other organisms and impacts on non-targets are all components of this issue. Questions that need to be answered are:

- (H) Does genetic engineering change the host range or ecological fitness of the organism?
 - (H) What are the genetic determinants of fitness for the wild type and are these altered in the transgenic? An example would be to assess the saprophytic competence of the transgenic in relation to its host dependency.
 - (H) Make better use of what is known about the ecology of non-genetically modified organisms to guide specific research questions with transgenic microorganisms.
- (HM) Are there changes in ecosystem function?
 - (HM) What magnitude of change is ecologically relevant?
 - (HM) Tools are needed to evaluate the ecological significance of any observed differences.

The above issues are components of exposure assessment. Other needs include:

- (H) Improved laboratory methods involving relevant organisms that can be used to assess potential impact on non-target organisms.
- (H) Ability to predict and monitor distribution post release. To address this issue, more sensitive methods/tools are needed, especially when there is the potential for background interference (e.g., green fluorescent protein versus fluorescent pseudomonads).
- (H) Evaluate the environmental persistence, replicative host range and zoonotic emergence and re-emergence of viruses.
- (ML) Establish if marker genes used for monitoring will affect the fitness of the organism or pose a potential risk in and of themselves.
- (ML) Develop means to evaluate *functional* changes in community ecology relevant at both local and ecosystem scales.
- (L) Develop scale-relevant testing systems.

(L) Lastly, the need for use of appropriate controls was discussed. This was deemed a function of good experimental design for any given study. The following were specific suggestions:

- Compare transgenic with parent and non-genetically modified organisms as options.
- Examine performance and risk in relation to other technologies used to address the same issue (e.g., pesticide use).
- Include revertants, gene knockouts, and/or gene amplification as relevant.
- Test in multiple environments.
- Perform a risk/benefit analysis. What is the risk of not adopting the technology?

II. Fully Exploit New Genetic Resources

This area was discussed in relation to identifying and characterizing transgenic microorganisms or products. Specifically, the group agreed that criteria for or standardized ways to identify organisms for taxonomic assignment are critically needed in order to differentiate pathogens from closely related microbes under field conditions. A key priority here is developing methods to accomplish this. To this end, the rapidly developing genomic resources becoming available through a myriad of sequencing projects need to be fully exploited. The following needs were recognized:

- **(H)** Innovative methods for identifying and detecting risk factors (e.g., exotoxin variation). An example of this is to identify the active moiety of a given toxin and then use it to trace the toxin in tissue or the environment as relevant.
- **(HM)** Identify components of the genome that are relevant to environmental risk assessment by use of bioinformatics and related tools for data mining.
- **(HM)** Use comparative genomics between a transgenic and its parent to identify regions of the genome that are of particular concern.
- **(HM)** Identify sequences that could flag higher risk potential, particularly when the taxon has pathogenic relatives.
- **(ML)** Cross-reference new taxonomic information to alternative or prior nomenclature.

III. (H) Containment

Transgenic microorganisms cannot be contained by “fences”, nor -as history has shown- do islands work. Hence, a focus on research into genetically-based containment (e.g., terminator genes) was deemed a high priority. Here the focus should be on reducing, rather than eliminating risk. Post-release attenuation of activity and reduced ability of the organism to spread, through a lack of saprophytic competence for example, were proposed as means to accomplish this. Containment issues for prokaryotes differ from those for eukaryotes, hence approaches here need to take into account the differences in the biology and ecology of these groups.

In cases of limited releases that go awry, new approaches to mitigate potential hazard(s) and exposure need to be developed.

Concluding remarks

In general, the group acknowledged that we are stuck on the treadmill of “case by case” assessment of both hazard and exposure. We need to determine at what stage we can move beyond this to developing “guidelines” that cover similar technologies. New tools, such as real-time PCR, GIS, and genomics, need to be used to derive more immediate information on larger scales so that such guidelines can be developed.

Lastly, there is an urgent need to address biosecurity issues in relation to transgenic microorganisms; however, the group did not have time to address these specific issues in sufficient detail.

¹ Some of the research needs and priorities listed in this document may be outside the scope of the USDA Biotechnology Risk Assessment Grants Program. This document was prepared by one or more of the individuals listed below. USDA program staff did not edit the content of this document. The USDA Biotechnology Risk Assessment Grants Program supports risk assessment and risk management research projects regarding the safety of introducing into the environment genetically modified animals, plants, and microorganisms. More information is available at: www.reeusda.gov/crgam/biotechrisk/biotech.htm. Questions regarding the suitability of research proposals should be discussed with the Program Director (dhamernik@csrees.usda.gov).

A list of people that attended this workshop is available at: http://www.isb.vt.edu/brarg/brarg_wshop/brarg_meeting.htm. The following individuals contributed to the discussion of this topic at the workshop and/or preparation of this document after the workshop:

Representatives from U.S. Regulatory Agencies:

Patricia Foley (USDA-APHIS)
Bill Schneider (EPA)
Mark Segal (EPA)

Discussion Leader:

Bryce Falk (University of California, Davis)

Reporters:

Jim Fuxa (Louisiana State University)
Janice Thies (Cornell University)

Science Facilitators:

Byrony Bonning (Iowa State University)
Dennis Gonsalves (USDA-ARS)
Linda Thomashow (USDA-ARS)