The biological science component of the Forest Health Initiative (FHI) supports core activities needed to promote the health and/or restoration of threatened forest tree species. The first target species, American chestnut, was almost lost from its former range by introduction of an exotic fungal pathogen that causes chestnut blight. Over the past fifty years, many public and private organizations, motivated by concerned citizens throughout the U.S., have tried to restore the American chestnut to its former status in the Eastern hardwood forest. These activities, usually involving selection and breeding or attempts to attenuate the pathogen, have not yet achieved success. FHI is poised to build on these past efforts, introduce new biotechnology tools and knowledge not previously available, and serve as an integrated platform to accelerate species restoration.

American chestnut restoration presents enormous challenges. Conventional backcross breeding programs introduce blight resistance from related species, followed by crossing of hybrid offspring to recover resistant and nearly pure American chestnut trees. These programs require many years of effort for each breeding cycle. Similarly, reliable blight resistance screening requires many years for trees to reach appropriate size for inoculation and genetic screening.

How is FHI contributing? Transgenic technology is rapid in that it requires no breeding, and is precise in that the immediate product is a nearly pure American chestnut genome. However, there are still knowledge gaps regarding the most appropriate transgenes to introduce that will improve resistance to chestnut blight. Advanced marker-informed breeding and selection may prove successful, but it is not known whether and how genome rearrangements between donor species and American chestnut may affect efficiency of backcross breeding. Additional knowledge gaps surround accurate rating of heritable blight resistance in very young seedlings. To fill these gaps, FHI will support the discovery of genes and their organization from blight resistant species and hybrids through genome sequencing and DNA marker development, thereby creating platforms for accelerated breeding strategies and for evaluating candidate transgenes. FHI will also support the development of early, accurate blight resistance screening protocols in vegetatively propagated material.

The activities of this biological research component of FHI will be reinforced by parallel activities focused on social, environmental and policy aspects, in order to engage the broad communities demanding options for restoration of American chestnut.
• FHI provides a complete genetic "parts list" for the American chestnut genome and for hybrids between American chestnut and other species.

• FHI provides a platform to integrate several biotechnology activities including genomics, mapping, propagation and early clonal testing for blight resistance.

• Shared resources foster coordination and efficiency.

• FHI enables examination of breeding lines to determine the origins of blight resistance.

• The precision afforded by a genome sequence will make future breeding faster and more precise, since the trees carrying blight resistance genes can be identified.

• FHI enables identification, introduction and evaluation of transgenes to accelerate recovery of blight resistant American chestnut.

• Engages the Social/Environmental and Policy/Regulatory arms of FHI.
FHI Biotechnology Research Workflow by Year

**Genomics**
- Genome Sequence

**Germplasm**
- Collection/Coordination
- Population Genotyping

**Clonal Testing**
- Early Screen
- Transgenic Technology
- Veg Prop Technology

**Year 1**
- CC Genome
- Cloned Embryos
- Improved Markers
- Pilot Test
- Ramp-up to Testing Scale

**Year 2**
- ID All Genes, Incl. QTL
- Cloned Embryos
- Integrated Mapping
- Validate
- New Candidate Transgenes
- Greenhouse/Field Tests

**Year 3**
- Web Portal
- Cloned Embryos
- Marker-Aided Breeding
- Apply Screen
- Greenhouse/Field Tests
- Greenhouse/Field Tests