MAXYGEN

725 San Aleso Avenue, Suite 2
Sunnyvale, CA 94085-1400
www.maxygen.com

Modifying Tropism of AAV with Molecular Breeding

– March 2019 –

Background

Maxygen is a leader in the application of synthetic biology to directed evolution. The Company's molecular breeding technology can be applied to the enhancement of protein function in many areas of biotechnology. Associated expertise in molecular and cellular biology has also led Maxygen to acquire experience in a range of areas from enzyme optimization to gene editing and viral vectors. Particular focus has been placed on adeno-associated virus (AAV) as the preeminent gene-delivery vector for a variety of therapeutic applications. We are undertaking efforts to optimize the viral expression elements, capsid assembly and tropism, and replicase activity. We are also creating improved plasmids to provide the necessary helper functions for AAV production.

AAV Capsids Lacking Liver Tropism

Goal. Identify variants of the AAV capsid protein that exhibit low to no binding to liver cells while retaining high infectivity of other tissues or specific tissues.

Approach. Libraries of genes encoding variant AAV capsid proteins will be created by molecular breeding, which creates chimeric genes from existing functional capsid genes. Typically, these libraries contain a high proportion (>50%) of genes encoding functional variants. Variants can be evaluated either as individuals or as a pool by taking precautions to ensure a linkage between the capsid gene and the expressed protein that forms the AAV particles. Assays that evaluate the infectivity of the particles on various cells types can be used to identify capsid proteins that have an enhanced or decrease binding.

Workplan. The following is a simplified description of a possible approach.

1. Choose capsid genes from one or more AAV serotypes
2. Perform molecular breeding to create a library of chimeric genes encoding variant capsid proteins
3. Produce AAV particles by transfection of individual or pooled variant capsid genes
4. Incubate AAV particles with human hepatocytes in culture; recover particles from media that do not transduce hepatocytes
5. Incubate AAV particles with non-liver human cells in culture (e.g., HEK293); recover genes from transduced cells that are derived from particles carrying functional variant capsid proteins
6. Repeat cell culture selections on different cell types as needed
7. Inject recovered AAV particles into mice carrying humanized liver; determine the number of AAV genomes that transduce liver tissue compared to the number that transduce non-liver human cells in culture
8. Repeat various rounds of the positive and negative selection steps until one or more AAV capsid variant is identified with low or no transduction activity on liver tissue