

Innovative Techniques and Reagents for Improved Genetic Engineering

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Donald L. Court, Ph.D.,

Senior Investigator & Head, Molecular Control and Genetics Section, NIH/NCI/CCR, Gene Regulation and Chromosome Biology Laboratory

Ms. Nina C. Constantino,

Senior Research Assistant, NIH/NCI/CCR, Gene Regulation and Chromosome Biology Laboratory

Neal G. Copeland, Ph.D.,

Chief (Former), NIH/NCI/CCR, Mouse Cancer Genetics Program

Nancy A. Jenkins, Ph.D.,

Senior Investigator (Former), NIH/NCI/CCR, Mouse Cancer Genetics Program

Hilary M. Ellis, Ph.D.,

E-Chiang Lee, Ph.D.,

Soren Warming, Ph.D.,

Daiguan Yu, Ph.D.,

Postdoctoral Fellow (Former), NIH/NCI/CCR, Mouse Cancer Genetics Program

Simanti Datta, Ph.D.,

Postdoctoral Fellow (Former), NIH/NCI/CCR, Gene Regulation and Chromosome Biology Laboratory

Contact:

Dr. Thomas M. Stackhouse
Associate Director
Technology Transfer Center,
National Cancer Institute, NIH
1003 West 7th Street,
Fairview Center Suite 500
Frederick, MD 21701
301-846-5071
Fax: 301-846-6820
stackhot@mail.nih.gov

The development of restriction enzyme technology in the

1970s was a breakthrough in molecular biology research. For the first time, scientists were able to cut DNA at specific sites, and insert sequences with matching ends. However, the technology was limited to insertion at particular sites in the host vector, and the size of the inserted DNA quickly became a limiting factor.

Through the research of Dr. Donald Court and colleagues at the National Cancer Institute's Center for Cancer Research, a set of recombination-mediated genetic engineering, or "recombineering," reagents was developed. Three specialized bacterial strains and seven plasmids were developed, based upon a genetic system in *E. coli* that was harnessed into a powerful platform technology allowing for highly efficient and rapid genomic manipulation in comparison to previous techniques. Additionally, much larger DNA sequences (up to 100kb) can be inserted. Besides improving standard molecular biology research, this technique is used to generate Bacterial Artificial Chromosomes (BACs) and conditional knockout mice.

The research community has enthusiastically received this technique, and 795 non-profit researchers have received the materials thus far. The technology transfer efforts initially focused on the negotiation of individual Material Transfer Agreements with each recipient. Growing interest created the need for a simple and streamlined process, leading to the deposit of the materials in the NCI's Preclinical Repository in 2007 and making the NIH Simple Letter Agreement available online. This has greatly expedited the transfer of the materials. Additionally, the inventors have three issued patents and several applications pending, and the technology has been non-exclusively licensed to 18 commercial entities.

Dr. Court and his colleagues continue to develop the technology, making improvements to the initial bacterial cell lines

resulting in a "second generation" set that, together with a selection plasmid construct, added the functionality of positive/negative selection and are specifically designed for BAC generation. His laboratory continues to use the technology in research on gene regulation and initiation of transcription and translation, and it has been the subject of a number of publications by both the inventors and outside investigators. Other projects utilizing recombineering are diverse and have included stem cell research, genetic studies in model organisms, creating research tools such as transgenic mice and specialized imaging vectors, and high-throughput screening.

The investigators are credited for not only discovering and developing this revolutionary technology, but also for seeing a need for widespread distribution within the research community and seeking out the technical support and technology transfer mechanisms needed to provide these materials as broadly as possible. They also anticipated recipients wanting to access unpublished information regarding protocols and experimental design techniques in order to use these materials, and have made this technical know-how available through their Recombineering Website.