



About the Author: Dr.

Sen Wu is currently a post-doctoral fellow working at Professor Mario Capecchi's laboratory in Howard Hughes Medical Institute, University of Utah. Dr. Wu was graduated from the College of Veterinary Medicine, Beijing Agricultural University in 1993. He received his PhD degree from University of Utah in 2006 with an Interdepartmental Program in Neuroscience provided by the Department of Human Genetics, University of Utah. Between 1993 to 1998, he worked as a Clinical Veterinarian for Laboratory Animals at National Vaccine and Serum Institute, Beijing, PR China.

Manipulating the Entire Mouse Genome: Gene Targeting and Beyond

Sen Wu

Gene targeting technology has been used widely in biomedical research for ~20 years. Three scientists, Mario Capecchi, Oliver Smithies, and Martin Evans, have been awarded the 2007 Nobel Prize in Physiology or Medicine for their work developing gene targeting or “knockout (KO) mouse technology”.

International efforts from large-scale genome projects in the past decade or so have obtained sequences for human, mouse and other mammalian genomes. However, the biggest challenge now is to functionally annotate these genomes. Among many reasons, the powerful genetic tools available to manipulate the mouse genome have made mice an ideal model tool for functional genomics. In fact, the usefulness of knockout mice has prompted three major international knockout programs in the US, Canada, and Europe to disrupt every gene (~25,000) in mouse ES cells¹. These international efforts only concentrate on gene coding regions, which represent ~2.5% of the total mouse genome. The remaining 97.5% noncoding sequences can also have important regulatory functions.

It costs ~\$10,000—100,000 to generate a mouse KO in the US. So knocking out the 25,000 mouse genes would cost \$250 million, and knocking out ~300,000 conserved non-coding DNA sequences would need billions of dollars. Therefore, even these international efforts can plan to produce 900 mouse lines by 2010.

It became clear that a faster, simpler, and much cheaper method is absolutely required to address the needs for functional genomics in the post-genomic era.

We have recently transformed the *piggyBac* transposon into a gene-trap system that can produce multipurpose loss-of-function and conditional rescue (OFF→ON) alleles. We have demonstrated that alleles produced by *piggyBac* transposition are of very high quality and can compare to alleles generated by conventional gene targeting. This is largely due to properties of *piggyBac*. First, *piggyBac* uses a perfect cut-and-paste transposition mechanism, and leaves no DNA scar upon



jumping. Second, it tends to insert in the 5' intron of a gene, allowing gene trapping to generate a null allele in most cases.

Since production of *piggyBac* alleles is just breeding, it is extremely simple, fast and cost-effective. Basically, only a few founders are needed to start breeding with wild type females, and each newborn harbors on average one new transposition. It is therefore easy to start this project and see results immediately, in contrast to the relatively more demanding setup of the more conventional KO technology.

Based on our pilot breeding, we have estimated that the generation of mice containing at least one *piggyBac* insertion in most mouse genes would require breeding of <100,000 (assuming a total of 25,000 genes and ~85% coverage) mice, which can be finished in <3 years. The cost of this will be estimated at \$20 million in the US. The maintenance fee for all the lines produced can be further cut down by freezing down sperm for any line.

In addition, we have found that Cre-*loxP* site-specific recombination system can mediate trans-allelic recombination in vivo, generating large deletions and duplications simply by breeding, at an unexpected high frequency than previously imagined². Since our *piggyBac* gene-trap vectors have *loxP* sites, we can easily choose two such *loxP*-containing alleles to generate large deletions at 1000 kb distance. To cover the entire 2.5 gb mouse genome, we would only need 1000–2000 mouse deletional lines.

Our system also makes it possible to perform large-scale screens in mammalian models, such as screening for tumor enhancers and suppressors, cardiovascular diseases, diabetes, longevity, neural phenotypes and so on. Mice are still a huge valuable mine that has not been systematically screened.

More importantly, this technique can be readily used in other vertebrate model animals. For example, rats, chicken, and fish. The utilization of this new technique to generate rat models for vast collection of human diseases can be appealing to drug developers. Rats are widely used for development and preclinical trial for most drugs, yet there is currently no knockout technology to generate useful genetic models for rats. Since our new technique only requires pronuclear injection of rats, which is routine for many labs, it can be used essentially the same as in mice. We envision that this *piggyBac* and Cre-*loxP*-based technology would greatly facilitate drug discovery and development.

References

1. The International Mouse Knockout Consortium. A mouse for all reasons. *Cell* 128, 9-13 (2007).
2. Wu, S., Ying, G., Wu, Q. & Capecchi, M.R. Toward simpler and faster genome-wide mutagenesis in mice. *Nat Genet* (2007).

