B-MO'S

TIP OF THE MONTH

JULY 2016

KNOCKING OUT ESSENTIAL GENES

Use of CNTRL™ Cell Lines

B-MoGen Biotechnologies Inc

Knocking Out Essential Genes
Use of CNTRL™ Cell Lines

Studying in living cells the function of essential genes, or genes which confer a strong selective disadvantage is critical.

The CNTRL™ technology, or Conditional Transgene Rescue Line, at B-MoGen enables a more efficient method compared to today's standard of deleting genes in order to observe the effects on the cell.

When the method of deleting genes is used on genes that are essential, then recovering a knockout (KO) clone becomes impossible. These genes, when lost, confer a strong selective disadvantage, or loss of fitness to the cell. It has recently been estimated that about 1,600 genes constitute a core set of essential genes and genes which incur a very strong fitness defect when deleted in human cells.[1] Added to this list are many genes that are lethal or required for fitness, in special circumstances, such as when another gene is mutated. These are called synthetic lethal genes. In order to study genes that are essential, required for fitness, or are synthetic lethal we need facile methods to conditionally KO gene function. This has been done in the past by making conditional KO's using Cre/loxP technology, in which homologous recombination is used to place loxP sites on either side of an essential exon or exons.[3] However this method is very time and labor intensive. Others have used conditional expression of a short hairpin RNA (shRNA) vector.[4] Yet this approach usually does not result in 100% loss of function for the gene.

At B-MoGen our CNTRL™ technology offers a highly facile and effective method to generate cells with KO's in essential genes. We make KO's for essential genes using targeted nucleases either at the same time or after we have introduced a transposon vector containing a tetracycline-inducible rescue cDNA of the gene to be targeted, as well as a puromycin gene. **SEE FIGURE 1A.** Selection in puromycin for the transposon, and doxycycline to induce the rescue cDNA, allows recovery of clones in which all copies of the endogenous gene are KO's. Removal of doxycycline from the media results in loss of gene expression and determination of the role of the gene in the cell, even if it is an essential gene **SEE FIGURE 1B.**

Scientists affiliated with B-MoGen have described the CNTRL™ method in literature[2] and believe that the technology will be of high value to the research community, especially when isolating clones with KO's in genes that are essential. It's value is also apparent for the general study of effects of varying gene levels on the cell.

FIGURE 1A.

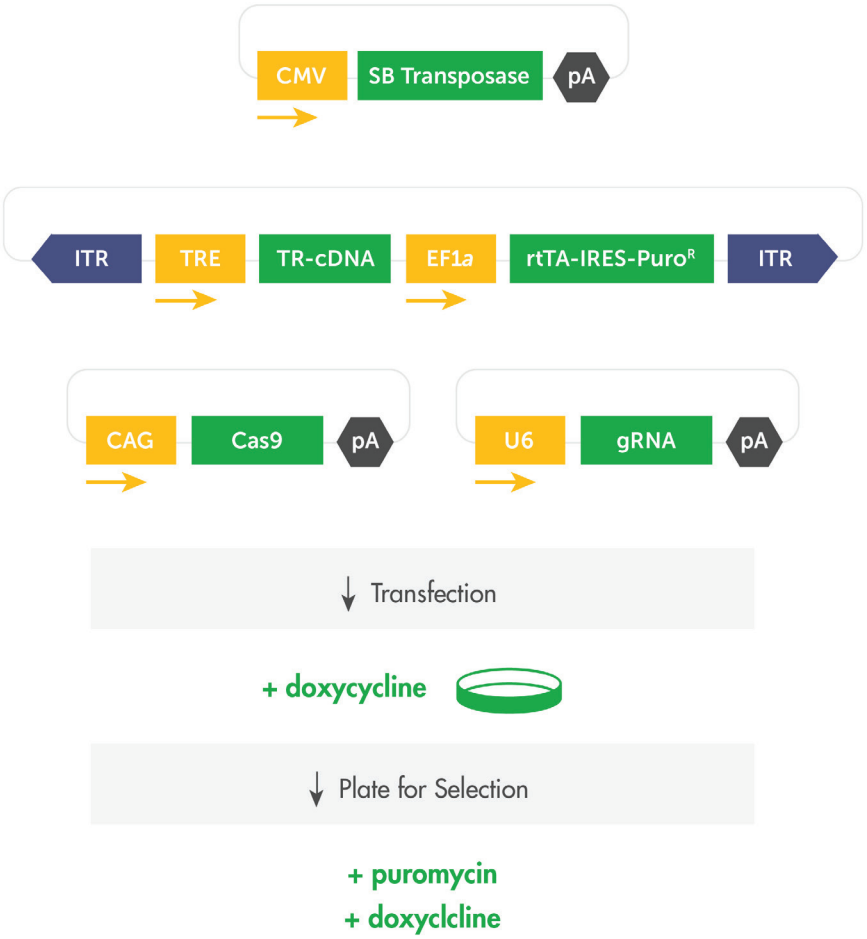
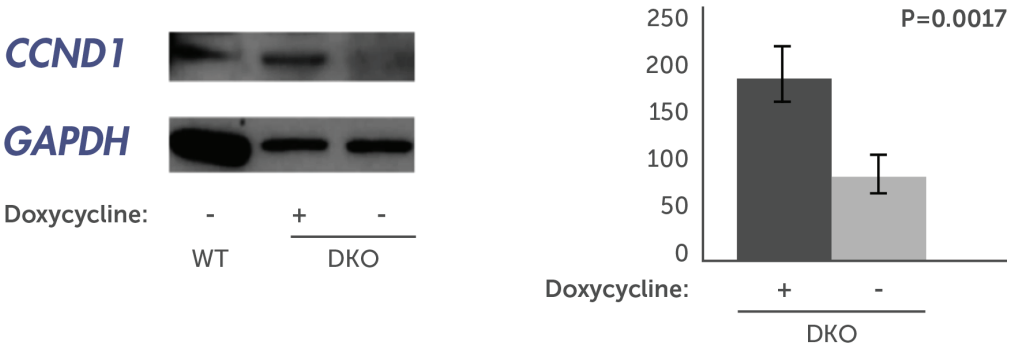


FIGURE 1B.



CNTRL™ TECHNOLOGY.

A. Co-transfection of PiggyBac (PB) transposase and transposon vectors, along with vectors for targeting an endogenous gene using CRISPR/Cas9, allos recovery of puromycin-resistant, doxycycline-induced rescue cDNA (TR-cDNA).

B. The left panel shows western blot anlysis of cyclin D1 (CCND1) and GAPDH protein expression in wild type (WT) or *CCDN1* double knockout (DKO) cells in the presence or absence of doxycycline. The right panel shows the average soft agar colony number in the same *CCND1* DKO cells with and without doxycyclin induction of the *CCDN1* rescue cDNA.

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