B-MO'S

TIP OF THE MONTH

AUG 2016

B-MOGEN'S CO-TRANSPOSITION KIT

A Simple Way to Enrich for Knockout Cell Lines

B-MoGen Biotechnologies Inc

B-MoGen's Co-Transposition Kit
A Simple Way to Enrich for Knockout Cell Lines

Targeted nucleases are revolutionizing genome engineering.[1] Targeted nucleases have made it much easier to create "knockouts", in which loss of function mutations are introduced into specific genes in a variety of cultured cell lines. TAL endonculeases (TALENs), CRISPR/Cas9, or other systems make it possible to introduce double strand breaks into specific loci in living cells. These double strand breaks can be repaired by non-homologous end joining (NHEJ), an error prone process that creates frameshifting insertion or deletion mutations (in/dels) that impair gene function. Alternatively, the double strand break can be resolved by homology dependent repair (HDR) using an exogenously supplied DNA substrate and in this way a specific alteration can be introduced at the cut site. Additional applications of targeted nucleases include the generation of large deletions, chromosomal translocations, or other genetic alterations. However, the biggest bottleneck in generating genetically altered cells with targeted nucleases is the recovery and screening of many individual clones for study.

At B-MoGen we are focused on methods for generation of many knockout clones at one time, on methods to recover only cells that have undergone specific genetic alterations, and on simple methods to enrich for knockout cell clones.

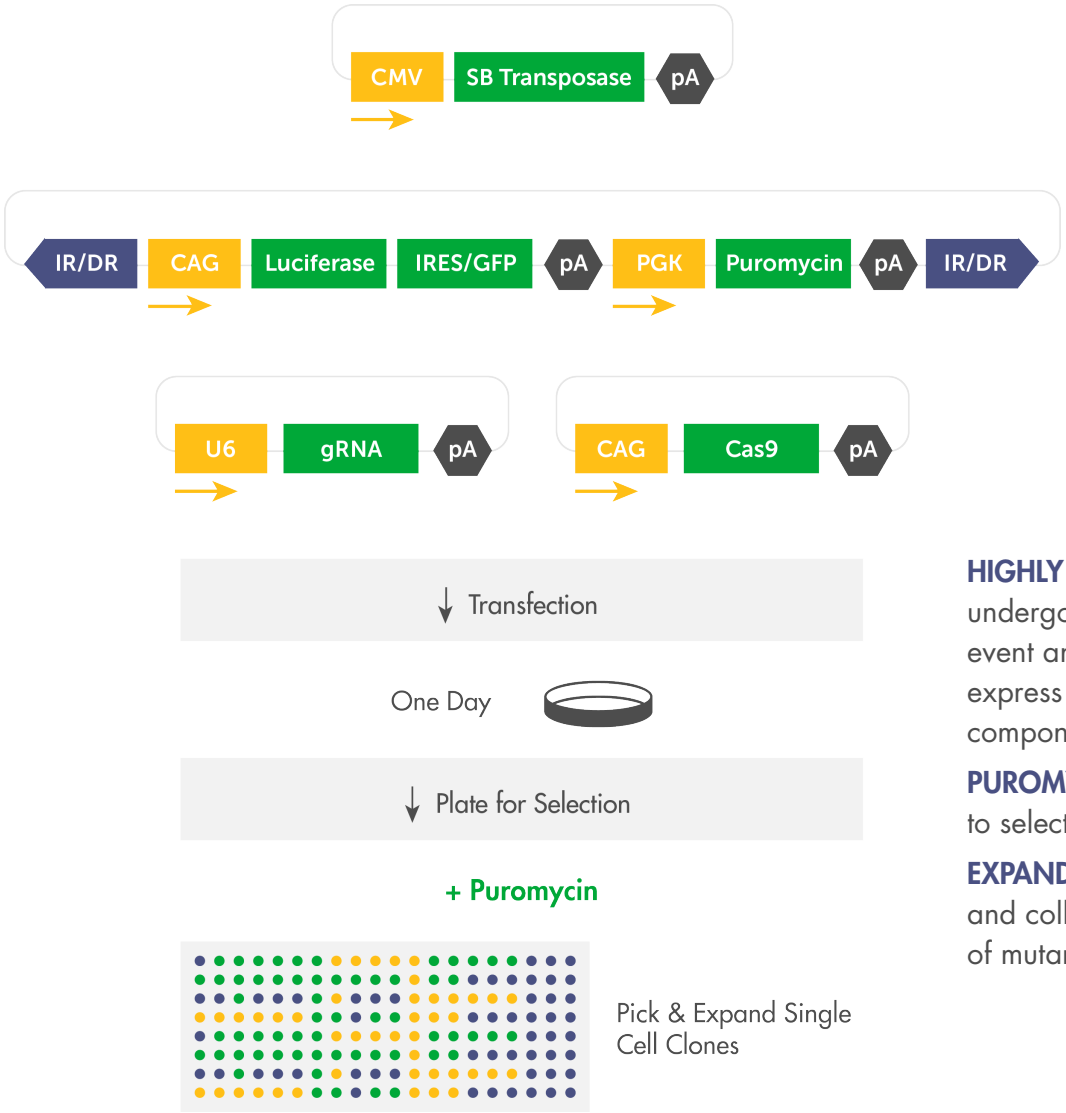
We offer an easy to use kit for this last purpose that should find very wide utility for many research laboratories. Called the "Co-Transposition Kit", it provides a simple way to generate single cell clones that are enriched for those bearing targeted nuclease-mediated knockout or other mutations. SEE FIGURE 1.

The Co-Transposition Kit is based on co-delivery of the vectors for CRISPR/Cas9 gene targeting (i.e. guide RNA and Cas9 vectors) plus transposase and puromycin resistance transposon vectors. SEE FIGURE 2. The transposase and transposon allow for selection of cells that have taken up and expressed transgenes.

Our published work demonstrates that selection for puromycin-resistant clones dramatically enriches for those that have undergone heterozygous or homozygous loss of function mutations.[2] Using co-transpostion, as many as 90% and 30% of clones carried heterozygous or homozygous mutations respectively were recovered. It has even been possible to isolate triple knockout clones with biallelic mutations present in three different genes.[2]

The Co-Transposition Kit also enriches for clones that have undergone HDR after co-introduction of either large double stranded targeting vectors or single stand oligonucleotides, thus making "knocking" projects much more efficient also. The B-MoGen Co-Transposition Kit and detailed protocols are now available to researchers to assist them in all their genome engineering projects using targeted nucleases.

FIGURE 1.



HIGHLY TRANSFECTED CELLS will undergo transposon integration event and are also likely to express targeted nuclease system components.

PUROMYCIN SELECTION is used to select for individual clones.

EXPAND CLONES, viably freeze and collect DNA for identification of mutants.

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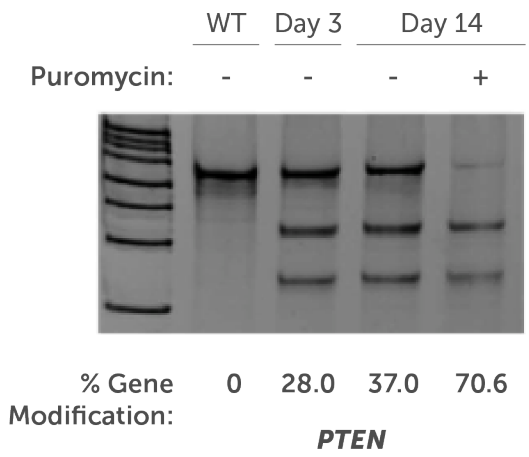
1. THE CO-TRANSPOSITION METHOD FOR ENRICHMENT OF NUCLEASE MODIFIED CELL CLONES.

Co-transfection of plasmids expressing the Sleeping Beauty (SB) transposase and a transposon containing a puromycin resistance cassette allows for recovery of single drug resistant clones. These clones tend to have also expressed the components of the co-transfected nuclease system, in this case a guide RNA (gRNA) and the Cas9 nuclease.

FIGURE 2.

A.

B.



C.

	Day 3	Day 14		Fold Increase +/day3
Puromycin:	-	-	+	
PTEN	28.0%	37.0%	70.6%	1.9
NF1	7.8%	7.0%	40.0%	5.1
NF2	13.9%	9.8%	35.6%	3.6
TP53	17.7%	18.5%	13.1%	-1.3
HPRT	15.9%	14.1%	41.4%	2.9
ERAS	22.9%	13.7%	34.0%	2.5
FOXR2	16.2%	15.2%	3.03%	2.0
CCND1	15.0%	18.6%	55.3%	3.7
MYC	1.1%	<1.0%	28.0%	25.4
CTNNB1	<1.0%	<1.0%	8.0%	>8
ARH-GAP36	3.3%	1.8%	18.9%	5.7
KRAS	6.0%	5.0%	31.0%	5.2

2. CO-TRANSPOSITION ENRICHES FOR NUCLEASE MODIFIED CELL CLONES.

(A) Experimental scheme. Cells were transfected with transposase and transposon vectors and targeted nucleases for 12 different genes. CEL-1 assays were performed at day 3 before puromycin selection and at 14 days with or without puromycin selection. (B) Example CEL-1 assays for the PTEN gene. (C) Tabulation of results for percent gene modification 3 days after puromycin selection or 14 days with or without puromycin selection. The fold increase in percent gene modification are shown. Results were initially described in Moriarity et al. (2014).[2]

REFERENCES

1. Carroll, D. (2015). "Genome editing by targeted chromosomal mutagenesis." **Methods Mol Biol** 1239: 1-13.
2. Moriarity, B. S., E. P. Rahrmann, D. A. Beckmann, C. B. Conboy, A. L. Watson, D. F. Carlson, E. R. Olson, K. A. Hyland, S. C. Fahrenkrug, R. S. McIvor and D. A. Largaespada (2014). "Simple and efficient methods for enrichment and isolation of endonuclease modified cells." **PLoS One** 9(5): e96114.