Citations

One Out of Two Ain't Bad

Envy can stir in researchers working with mammalian systems when they hear descriptions of genetic screens in yeast. Compared with their diploid cousins, yeast mutants are simpler to generate, not to mention that the resulting phenotypes are easier to interpret, thanks to the presence of just one copy of each allele. Although a mostly haploid leukemia cell line (save for chromosome 8) has been described for use in loss-of-function genetic screens, it is only with the publication of an article in *Cell Stem Cell* from Elling et al. that a fully haploid mammalian somatic cell line has become available. To prepare the haploid cells, the authors collected embryonic stem cells (ESCs) from early-stage murine parthenogenetic embryos. By flow cytometry sorting to capture cells with reduced DNA content, Elling et al. isolated two cell lines with haploid chromosome sets. The lines continue to proliferate despite ongoing culture (>50 passages) and genomic analysis confirms no gross chromosomal deletions or duplications, though some cells do become diploid over time. The cells' bona fides as ESCs are clear as well: expression profiles of relevant markers mirror those of a characterized diploid ESC line, and they can differentiate into cells of all three germ layers. The cells' particular appeal, of course, is their compatibility with genetic screens. To demonstrate this, Elling et al. transduced freshly sorted haploid ESCs with a retroviral vector-borne gene trap. The vector is designed for efficient insertional inactivation

of even poorly expressed genes and has an 'on/off switch' based on Cre-mediated reversal (and thus inactivation) of the trap, a splice acceptor sequence that prematurely truncates the gene product by fusing the transcript's 5' end to the marker. Using this technique, the authors generated a knock out of the RNase Drosha, which is involved in miRNA processing, and for which no true knockout cell line has been developed. Using this technique with other genes could make haploid ESCs an alternative to RNAi knockdown, which can be incomplete, transient, or suffer from off-target effects. Haploid ESCs are equally suitable to forward genetic screens, as shown by an experiment in which the retroviral vector-mutagenized haploid ESCs were exposed to a lethal dose of ricin to identify those with mutations enabling survival. In this way haploid ESCs—or cells differentiated from them—offer rapid, yeast-style forward genetic screens in metazoan systems, bringing the malleability of a 1n chromosome set to a mammalian model organism well poised to engender progress in studies of development, pathogenesis, and physiology.

Elling et al. 2011. Forward and reverse genetics through derivation of haploid mouse embryonic stem cells. *Cell Stem Cell*. 9(6):563-74.

EASE Does It

Transforming DNA into the *Arabidopsis* germ line presents no real difficulties, but integrating exogenous DNA into specific genome locations in this model organism is no easy feat. Typically, transformed DNA integrates randomly, making gene knockout or replacement a frustrating proposition. In a technical advance published in *The Plant Journal*, Even-Faitelson et al. offer a simple strategy for targeted modification of the *Arabidopsis* genome. The procedure begins with the typical transformation method: exogenous DNA is engineered into *Agrobacterium tumefaciens* DNA, and delivered into the flower by dipping the budding plant into a solution containing the bacteria. It is transformation of the egg cell that transmits the exogenous DNA to progeny, so Even-Faitelson et al. directed their attention to an egg cell-based strategy. They hypothesized that egg cell-specific expression of proteins involved in targeted gene mutagenesis or gene targeting would make these strategies easily implementable in *Arabidopsis*. To this end, the authors prepared constructs containing a 77-base pair enhancer sequence called the egg apparatus-specific enhancer (EASE), which stimulates expression of adjoining

Seedlings were stained with X-Gluc staining solution, to detect NHEJ repair of the mutated GUS gene. A blue seedling represents a mutated seedling. Source: *The Plant Journal*

genes in the egg cell or the very early zygote. For gene targeting, the effector protein is RAD54, a chromatin remodeling factor that promotes the homologous recombination of exogenous DNA with its genomic target. For targeted gene mutagenesis, EASE-mediated expression of a zinc finger nuclease is employed to trigger gene mutagenesis by the error-prone non-homologous joining of termini created by the engineered endonuclease. In tests using reporter vectors to detect successful gene manipulation events, the EASE-regulated

expression method yielded rates of gene targeting and targeted gene mutagenesis of 4×10^{-3} and 3×10^{-3} , respectively. The same strategy should also be useful for appropriate spatiotemporal expression of other genome-modifying proteins such as transcription activator-like effector nucleases (TALENs), which offer particular flexibility in customizing target sequence recognition. The advent of the EASE system should help make precise genome modification in *Arabidopsis* as routine as mutating specific loci in yeast or mice.

Even-Faitelson et al. 2011. Localized egg-cell expression of effector proteins for targeted modification of the *Arabidopsis* genome. *Plant J*. 68(5):929-37.

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