Genetic modification is critically enabling for studies addressing specification and maintenance of cell fate; however, methods for engineering modifications are inefficient. We demonstrate a rapid and efficient recombination system in which an inducible, floxed cre allele replaces itself with an incoming transgene. We target this inducible cassette exchange (ICE) allele to the (HPRT) locus and demonstrate recombination in murine embryonic stem cells (mESCs) and primary cells from derivative ICE mice. Using lentivectors, we demonstrate recombination at a randomly integrated ICE locus in human ESCs. To illustrate the utility of this system, we insert the myogenic regulator, Myf5, into the ICE locus in each platform. This enables efficient directed differentiation of mouse and human ESCs into skeletal muscle and conditional myogenic transdifferentiation of primary cells cultured in vitro. This versatile tool is thus well suited to gain-of-function studies probing gene function in the specification and in and reprogramming of cell fate.

**ICE recombination in murine embryonic stem cells**

**Fig. 1.** Schematic representation of the lentiviral constructs carrying the components of the ICE system, and the proviral ICE locus before and after recombination.

**Fig. 2.** Flow cytometry of iC2C12 cells, which carry an inducible cre-ires-GFP proviral locus before recombination, and derivative cell lines carrying DsRed2 or luciferase, after recombination (left panel). Fluorescence microscopy of iC2C12-DsRed2 during selection (central). Dose response and time course of luciferase gene expression in response to doxycycline (500 ng/mL).

**Fig. 3.** Inducible cassette exchange recombination in A2lox.cre mouse embryonic stem cells (mESCs). The incoming plasmid is shown above the inducible cassette exchange (ICE) target locus, which is integrated at a unique site in the genome (50 of HPRT in the mESCs). At the ICE target locus, cre is flanked by heterologous loxP sites and is downstream of a TRE. Arrows indicate recombination between homologous loxP sites. The heterologous loxP sites on the incoming plasmid are in the opposite orientation relative to their cognates on the chromosome, therefore recombination catalyzes the integration of the entire plasmid. Cre is exchanged for the incoming gene of interest, rendering that gene doxycycline-inducible in the derivative recombinants. Selection is enabled by correction of the Dreo gene which acquires a PGK promoter, Kozak translational consensus and ATG (left) FACS analysis of a derivative inducible GFP cell line and control (E14) exposed to doxycycline (right).

**CONCLUSION**

- ICE recombination system is versatile—easily set up in any cellular model system.
- Cre recombination in ICE locus is at levels of two to three orders of magnitude above conventional homologous recombination.
- Pure population of derivative inducible gene-expressing recombinants can be obtained within a days.
- All cre-mediated integrations into a given cell type are targeted to the same locus.
- Variations in expression associated with integration site and copy are eliminated. These features allow elements of an allelic series to be compared to one another directly.
- The conditional doxycycline-inducible nature of the target locus is well suited to gain-of-function experiments.