Generation of stable cell lines using DNA transposases

DNA transposons are mobile genetic elements that integrate transgenes into chromosomal DNA via a cut-and-paste mechanism. They are mobilized by transient expression of a transposase, which excises the donor sequence and inserts it into target sites through strand transfer reactions—without causing double-strand breaks at the insertion site.

Different transposase systems have been developed to support stable transgene integration in mammalian cells. These systems vary in integration efficiency, sequence preference, and genomic stability.

The Sleeping Beauty (SB) system (Ivics et al., 1997), including hyperactive versions such as hySB100X, integrates at near-random sites but has relatively low transposition efficiency and a tendency for local hopping on the X chromosome.

The PiggyBac (PB) system (Ding et al., 2005), by contrast, is ten- to hundred-fold more efficient, with no evidence of local hopping. It shows a preference for intragenic regions such as transcription start sites, making it well-suited for insertional mutagenesis.

Risk assessment

– Work with human-derived material or transgenic cell lines (BSL-2)

▷ Wear gloves, safety glasses, lab coat

□ Collect and dispose waste after inactivation as REGULATED MEDICAL WASTE

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Procedures

Transfection of host cells

(1.) Seed cells to reach 70% confluence (50 000 cells per well in a 6-well plate) at the time of transfection.

(2.) Prepare a mix of the transposase and transfer plasmids at a sub-stoichiometric ratio.

Plasmid		**Per well**	
Transposase plasmid	pPBase-RFP	0.05 pmol	0.32 µg
Transfer plasmid	Custom	0.20 pmol	1.00 µg

Note: The number of integrated copies depends on transposase activity, not transfection efficiency.

(3.) Transfect the cells using a non-viral method such as Lipofectamine® 0012.

(4.) After 24 h, replace the medium.

(5.) Begin selection or screening for stably transfected cells according to 0019.

Hint: Selection can begin as early as 24 h post-transfection, but may benefit from a 48 h recovery window in sensitive lines.

List of references

S. Ding, X. Wu, G. Li, M. Han, Y. Zhuang, and T. Xu, *Cell* **122**(3), 473—483 (2005).

Z. Ivics, P. Hackett, R. Plasterk, and Z. Izsvák, *Cell* **91**(4), 501—510 (1997).

Change log

2023-11-12 Benjamin C. Buchmuller Adaptation as SOP.