

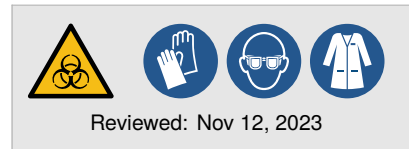
Generation of stable cell lines using DNA transposases

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.

This is a bench card. Full protocol available online.







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	pPBBase-RFP	0.05 pmol	0.32 µg
Transfer plasmid	Custom	0.20 pmol	1.00 µg

- (3.) Transfect the cells using a non-viral method such as Lipofectamine®  SOP0012.
- (4.) After 24 h, replace the medium. 
- (5.) Begin selection or screening for stably transfected cells according to  SOP0019. 

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).

 Notes (available online)

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