Sleeping Beauty Transposon System User Manual

DS: SB001

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SYSTEM OVERVIEW

The *Sleeping Beauty* transposon system is a DNA transfer method that employs a transposase enzyme to cut and paste donor DNA flanked by *Sleeping Beauty*-specific inverted repeats/direct repeats (IR/DRs) into chromosomal DNA (1). This system allows for highly efficient transfer of desired genetic material into host cell genomes of vertebrate species. The system can be used to deliver genes to a variety of cell types including adherent and suspension cultured cell lines, primary cells and cells in situ in a laboratory animal (2,3,4). *Sleeping Beauty* transposons integrate in a random distribution at TA sites without a preference to integrate in or near endogenous genes, making them less likely to cause off-target effects when compared to other transposons and viral gene delivery methods (4,5,6).

B-MoGen and Neuromics offer a variety of SBT™ transposon donor vectors and *Sleeping Beauty* transposase expression vectors for combined use to stably integrate transgenes into the genome of your desired cells. Sample protocols for SBT MCS cloning, co-transfection of SBT and *Sleeping Beauty* transposase vectors, and selection of SBT integrated cells follow under the PROTOCOLS section.

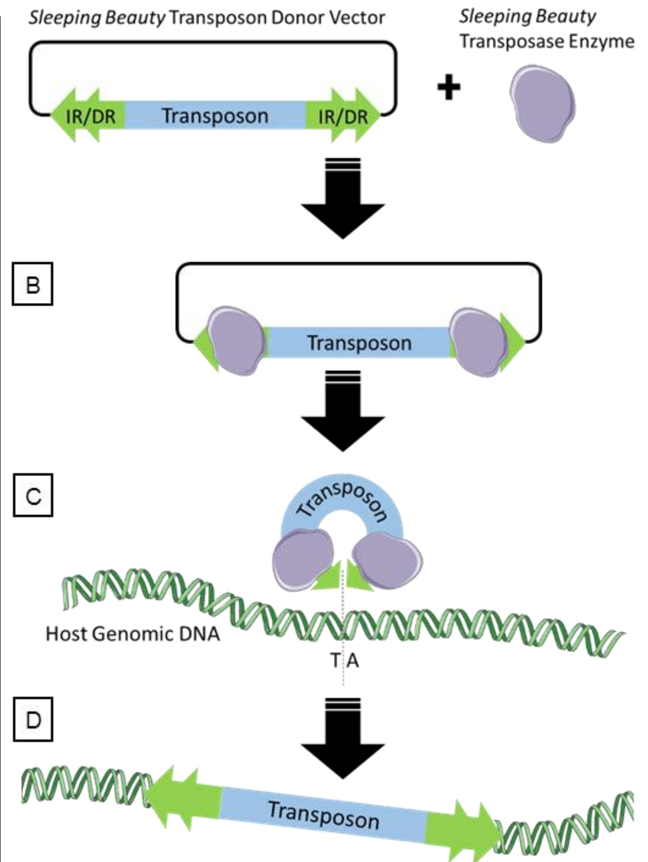

Figure Key: (A) A depiction of Sleeping Beauty transposon plasmid and Sleeping Beauty transposase enzyme active in cellular nuclei. (B) Transposase enzyme binds to Sleeping Beauty-specific IR/DR sites. (C) Transposase enzyme excises transposon sequence from transposon vector. (D) Transposase enzyme stably integrates transposon sequence at TA site in host cell genome.

Product Specifications

Shipped with each SBT and *Sleeping Beauty* transposase vector purchased is a *Sleeping Beauty* Transposon System User Manual (DS: SB001) and a vector data sheet per product ordered. Vector maps and characteristics are found in vector data sheets, usage guides and protocols are found in the *Sleeping Beauty* Transposon System User Manual.

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Shipping and Storage

SBT and *Sleeping Beauty* transposase vectors are shipped at ambient temperature in Tris-EDTA buffer (TE) at pH 7.5~8. Plasmid vector products are best stored in TE at room temp or 4°C for short-term storage, up to one month, and at -20 to -80°C for long-term storage, months to years. Avoid repeat thawing and freezing of product. Spin vials for 1 minute in a micro-centrifuge prior to opening.

PROTOCOLS

Co-transfection

The SBT vector and the *Sleeping Beauty* transposase expression vector must be co-transfected in order to effectively integrate the desired SBT vector transposon into the host cell genome. The specific co-transfection method to be used is the user's choice. We suggest choosing a co-transfection method that is amiable to the target cell type and familiar to the user. A 1:5 to 1:10 mass ratio of *Sleeping Beauty* transposase:transposon vector is suggested for optimal transposon integration (3,7). Different cell types will vary in optimal transposase:transposon co-transfection ratio.

SAMPLE CO-TRANSFECTION METHODS

Cell Line or Cell Type	Co-transfection Method	Cell concentration	Mass Transposon Vector	Mass Transposase Vector
HCT116 colorectal carcinoma	Electroporation	1.0×10^6 cells	1000ng	100ng
HCT116 colorectal carcinoma	Lipofectamine	6.25×10^5 per well (6- well plate)	500ng to 2 ug	50ng to 200ng
Hela Cells (3)	FuGene Reagent	2.5×10^{5n} per well (6-well plate)	500ng	50ng
OG2 Mouse Embryonic Fibroblasts (3)	Electroporation	5×10^5 cells	500ng	50ng
HFF-1 human fibroblast (3)	Nucleofection	4×10^5 cells per well (6-well plate)	2ug	200ng
Other Stem and Primary Cells	pn-Fect™ or p-Fect™	Contact Technical Services*		

Service Contact: Rose Ludescher, Manager Customer Satisfaction rose@neuromics.com or US toll free 866-350-1500 or 952-374-6161.

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MCS Cloning Guide

Multiple Cloning Site (MCS) ready SBT™ vectors are ideal for easily creating custom transgene transposon vectors. The MCS in our vectors allow researchers to choose from a variety of unique restriction enzyme sites for insertion of donor DNA, validating insertion of donor DNA, and excising donor DNA. Because different restriction enzymes create different DNA strand overhangs it is crucial to match the appropriate restriction enzyme with the overhangs of the desired DNA insert. Restriction enzyme cut site sequences, overhangs, and digestion protocols are available thru restriction enzyme provider.

Restriction Enzyme Cut Sites Available in B-MoGen/Neuromics MCS
EcoRV, EcoRI, XhoI, SciI

SAMPLE MCS PROTOCOL

1. Digest 2ug of MCS ready SBT vector according to specific restriction enzyme digest protocol.
2. Prepare DNA insert by PCR amplification.
3. Gel purify digested SBT-MCS vector and desired PCR insert.
4. Ligate insert into digested MCS ready SBT vector. (*Depending upon overhangs from DNA insert and restriction enzyme chosen, different ligation parameters may be necessary. We recommend a T4 DNA ligase reaction.)*
5. Transform ligation reaction into competent E. Coli cells on bacterial media agar plates with ampicillin resistance. Incubate bacterial plates at 37° overnight.
6. Pick desired number of colonies from bacterial plates and grow small bacterial cultures (2-6ml) shaking at 37°C overnight.
7. Mini-prep plasmid DNA from small growth cultures.
8. Validate insertion of desired DNA in mini-prep plasmids via restriction enzyme digest and/or plasmid sequencing using appropriate sequencing primer. *(Reference vector data sheet for restriction enzyme and sequencing primer sites)*

Selection of Modified Cells

The final step in creating a stable transgenic cell line with the *Sleeping* Beauty transposon system is isolating transposon carrying cells from the population of co-transfected cells. SBT vectors offer a variety of reporter genes for selection that fall into two categories; fluorescence reporters and selectable drug markers. Begin cell selection 4 days post co-transfection with transposon and transposase vectors.

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Selection Using a Fluorescence Reporter

The most common method of isolating fluorescence-reporter-transposon positive cells from a population is manual single cell cloning. We recommend plating co-transfected cells in serial dilutions of 1:2 volumes across a 96-well tissue culture plate. 3-4 days post plating in 96-wells plate screen wells for single colonies and validate colonies for fluorescence activity using fluorescence imaging. Select and expand positive clones for use in downstream experiments. Alternatively, one can utilize fluorescence activated cell sorting (FACS) to automatically isolate fluorescence positive cells from a population.

Selection Using a Drug Resistance Marker

Eliminating transposon negative cells from a population of co-transfected cells is easily achieved by culturing treated cells in medium containing appropriate drug. Below are starting culture specifications according to the specific selectable drug markers available in SBT vectors. These values may change depending on cell type. We recommend testing the sensitivity of target cells with varying concentration of selection drug prior to co-transfection and selection.

Selection Drug	Working Concentration in Culture Medium	Length of Selection Process
Puromycin	1-10 ug/ml	5 days
Neomycin (G418)	400-1000 ug/ml	8+ days
Zeocin (Sh ble)	50-400 ug/ml	8+ days
Hygromycin	50-500 ug/ml	8+ days

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APPENDIX

Technical Support

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