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Minimal *Piggybac* Functionality in Mammalian Cells

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Extended Abstract

We recently developed a modified *piggyBac* (transposon) delivery system in which most of both terminal domains have been relocated from the delivery cassette into the helper (non-integrating) part of the same plasmid to minimize the size of the delivered transposon (Solodushko et al., 2014). This minimal *piggyBac* system is potentially safer and less mutagenic than other transposons and retroviruses, and has the advantage of requiring only a *single*-plasmid for delivery. We have demonstrated that this vector stably and efficiently transposes mammalian cells. Herein we test various characteristics of the plasmid including the size of insert it can accommodate, the effect of DNA conformation on transposition efficiency, and the impact of methylation on transgene expression.

We constructed five minimal *piggyBac* vectors each of which included minimal terminal repeats flanking a DNA insert and a CMV-RFP-polyA sequence, but which contained increasing sizes of DNA fragments within the delivery cassette. HEK293 cells were transfected with each plasmid and RFP positive cells sorted 48 hours later. The sorted cells were seeded and RFP expression monitored over 28 days. The percentage of RFP positive cells decreased initially, but stabilized 21 days after transfection indicating the cells that had successfully integrated the transposon. Vectors with smaller delivered fragments demonstrated higher integration efficiency; the vector with a 1.6kB delivery fragment was detected in 25% of cells 21 days after reseeding, whereas the 15kB delivery fragment was present in only

5%; all vectors significantly outperformed the non-transposable RFP expressing control. The integration efficiency of these minimal piggyBac vectors depended on both the size of the transposon and the size of the delivery plasmid. We then determined the effect of DNA conformation and methylation on the functionality of these vectors. Open vectors demonstrated the same integration efficiency as their super-coiled counterparts. DNA methylation decreased the integration of minimal *piggyBac* sequences, and also silenced the expression of previously integrated minimal *piggyBac* sequences in some cell types. Inhibiting methylation of genomic DNA at the time of transfection increased the integration rate of minimal piggyBac vectors.

The minimal *piggyBac* vectors can efficiently, and stably, deliver large DNA sequences to host cells. Transposition efficiency decreases as the size of either the insert or the entire plasmid increases. The integration of these minimal vectors is inhibited by methylation of host chromatin. DNA methylation within the delivered fragment also reduces gene expression from successfully integrated sequences. Unlike standard piggyBac vectors, however, the integration efficiency of these minimal piggyback vectors is not adversely affected by DNA conformation and thus can be delivered in either super-coiled or linear form allowing them to be combined with other gene delivery technologies.

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Solodushko, V., Bitko, V., & Fouty, B. (2014). Minimal Piggybac Vectors For Chromatin Integration. *Gene Therapy,* 21, 1-9.