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RESEARCH ARTICLE

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Diphtheria toxin-mediated transposon-driven poly (A)-trapping efficiently disrupts transcriptionally silent genes in embryonic stem cells

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Summary

Random gene trapping is the application of insertional mutagenesis techniques that are conventionally used to inactivate protein-coding genes in mouse embryonic stem (ES) cells. Transcriptionally silent genes are not effectively targeted by conventional random gene trapping techniques, thus we herein developed an unbiased poly (A) trap (UPATrap) method using a *Tol2* transposon, which preferentially integrated into active genes rather than silent genes in ES cells. To achieve efficient trapping at transcriptionally silent genes using random insertional mutagenesis in ES cells, we generated a new diphtheria toxin (DT)-mediated trapping vector, DTrap that removed cells, through the expression of DT that was induced by the promoter activity of the trapped genes, and selected trapped clones using the neomycin-resistance gene of the vector. We found that a double-DT, the dDT vector, dominantly induced the disruption of silent genes, but not active genes, and showed more stable integration in ES cells than the UPATrap vector. The dDT vector disrupted differentiated cell lineage genes, which were silent in ES cells, and labeled trapped clone cells by the expression of EGFP upon differentiation. Thus, the dDT vector provides a systematic approach to disrupt silent genes and examine the cellular functions of trapped genes in the differentiation of target cells and development.