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## The Sea Urchin *sns5* Chromatin Insulator Improves the Likelihood of Lentiviral Vectors in Erythroid Milieu By Organizing an Independent Chromatin Domain at the Integration Site

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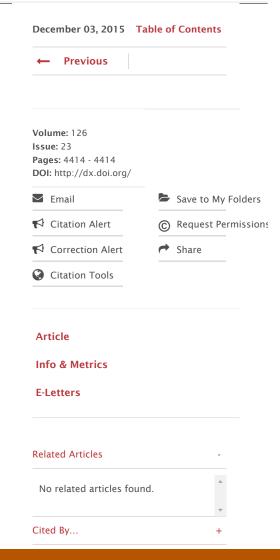
## Abstract

Retroviral vectors are currently the most suitable vehicles for therapeutic gene transfer in hematopoietic stem cells. However, these vectors are known to integrate rather randomly throughout the genome, suffering the so called chromosomal position effects (PE). Such a critical occurrence most probably depends upon the ability of heterochromatin to spread in the inserted vector sequences. Moreover, the use of transgenes imply genotoxicity effects, since the *cis*-regulatory sequences harbored by the vector can disturb the proper transcription of the resident genes neighboring the integration site, potentially leading to malignant transformation.

Due to their enhancer blocker activity, the incorporation of chromatin insulators in flanking position to the transferred unit can reduce the mentioned dangerous effects. Moreover, by acting as barriers to the spread of heterochromatin, chromatin insulators can also mitigate vector silencing.

We have previously shown that the sea urchin *sns5* chromatin insulator activity is conserved in mouse and human erythroid milieu: it blocks the βglobin-LCR-HS2 enhancer/globin promoter interaction when placed between them. In addition, when placed in flanking location of a γ-retrovirus vector, *sns5* impedes PE variegation and improves vector-specific expression following integration in the erythroid genome. Importantly, by binding both erythroid-specific and ubiquitous factors, *sns5* favors the accumulation inside the provirus locus of epigenetic marks commonly associated to an euchromatic state (Acuto S. et al., BCMD 2005; D'Apolito D. et al., 2009; Di Caro D. et al., *J Mol Biol* 2004; Cavalieri V. et al., NAR 2009).

In this study we extend these findings, demonstrating that sns5 works as



chromatin insulator also when placed in flanking position of a GFP transgene contained in a lentivirus vector (LV-GFP). A large panel of mouse erythroleukemic clones (MELC) was generated after transduction with uninsulated and sns5-insulated LV-GFP. Individual clones were screened for single vector integrants (by Q-PCR), and for GFP-expression (by cytofluorimetry). Our results shown that the inclusion of the sns5 element in a forward orientation increased the fraction of vector expressing cells (89% for the insulated vector vs 42% for the uninsulated ones). The clonal variegation of expression, assessed as frequency of clones that showed a percentage of GFP-negative cells in the progeny, decreased in clones transduced with the insulated vectors (7.4% vs 13,9%).

It has been suggested that chromatin insulators could shape the architecture of topologically independent chromosome domains. High resolution mapping of chromosomal domains in drosophila and higher eukaryotes highlighted that chromatin insulators play a critical role in shaping the architectural genome organization both in a local chromosome environment and in long range chromosomal interaction. Intriguingly, by using the Chromosome Conformation Capture (3C) technology, we demonstrated that the sns5 flanked LV-GFP integrated at a single copy in the erythroid cell genome is organized into an independent chromatin loop at the integration site. Worth to mention, no looping was detected in the absence of sns5, indicating that the two flanking copies of sns5 are specifically involved in the reorganization of the chromatin structure at the provirus locus.

In conclusion our results not only confirm the conserved and striking boundary function of sns5, but also provide a new clue concerning the molecular mechanism that allows this function to occur. On these basis, our findings reassure the use of sns5 to improve both efficacy and safety of lentiviral vectors for gene therapy.

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