Discovery of Genetic Elements that Protect Against Transgene Silencing

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Abstract:

Transgene silencing remains a major obstacle in biotechnology, limiting the long-term effectiveness of gene therapies and recombinant protein production by causing a gradual loss of transgene expression. Although advances in genome editing and vector design have improved transgene delivery, achieving stable and sustained expression in mammalian cells remains challenging. DNA barrier elements, such as cHS4 and A2UCOE, can help prevent heterochromatin spreading and maintain a permissive chromatin environment, thereby supporting long-term transgene activity. However, their effectiveness often varies depending on the cell type, chromosomal integration site, and promoter used, highlighting the need for the discovery of novel and more robust barrier elements.

To address this challenge, we developed the RGBarrier assay system, a high-throughput platform designed to evaluate the anti-silencing activity of putative DNA barrier elements within consistent chromosomal environments. The system combines targeted integration into repressive genomic loci with recombinase-mediated cassette exchange, enabling direct comparison of multiple barrier elements at identical genomic locations.

Using this platform, we identified a repressive chromosomal locus, H3K9me3_3271-A43, suitable for transgene silencing studies and demonstrated the utility of RGBarrier as a scalable screening tool. Furthermore, we identified the putative barrier element pBarrier 2102, which maintained stable transgene expression for up to 14 weeks, suggesting effective protection against heterochromatin-mediated silencing. Collectively, these findings advance our understanding of chromatin barrier mechanisms and provide valuable tools for improving transgene stability in synthetic biology, biomanufacturing, and gene therapy applications.