Creating New β-Globin-Expressing Lentiviral Vectors by High-Resolution Mapping of Locus Control Region Enhancer Sequences

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Relevance

Our lab has developed a lentiviral vector (LV) containing an anti-sickling version of the β-globin gene (β-AS3) driven by human genomic elements that confer high-level and erythroid specific expression of β-AS3 globin.

Figure 1: Proviral Map of CCLc-AS3-FB

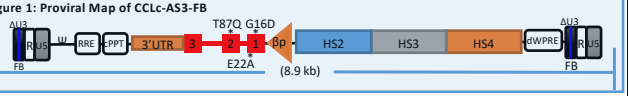


Figure 2: Map of β-AS3 Interactions in HBS Fibers

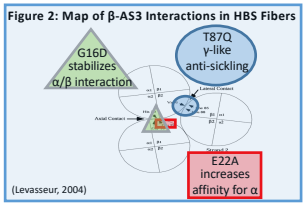


Figure 3: Polymerization Delay Time Measurements

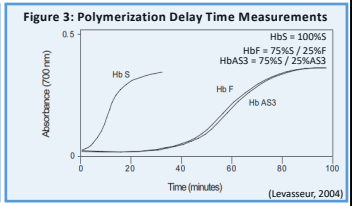
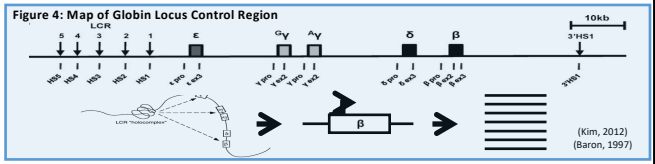
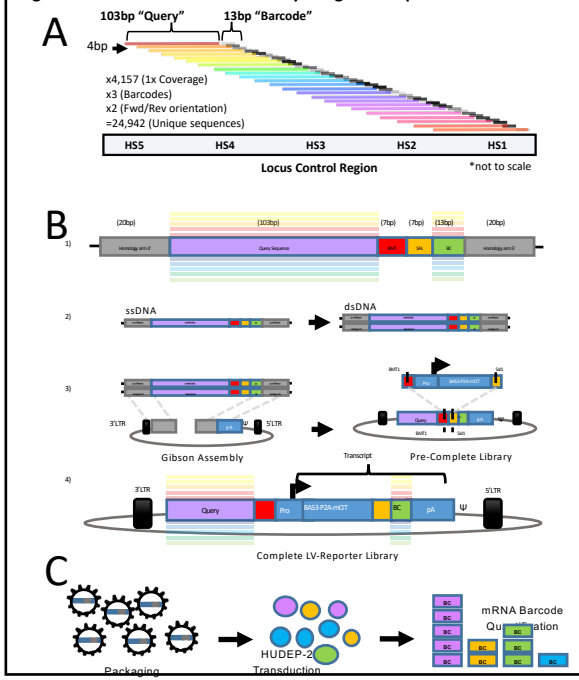


Figure 4: Map of Globin Locus Control Region



Methods

Figure 5: Overview of LV-MPRA library design and experimental workflow



Results

Figure 6: LV-MPRA Guided Therapeutic Vector design and characterization

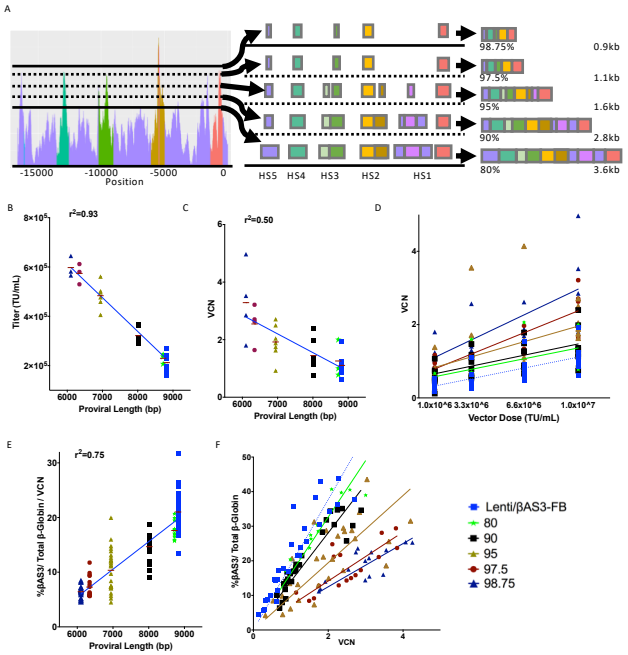
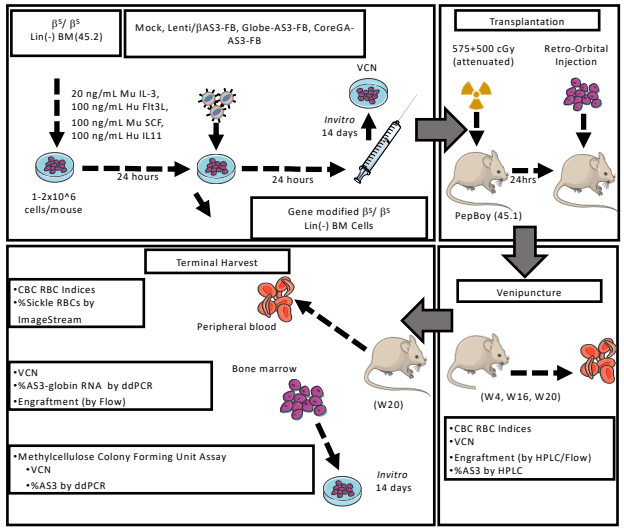
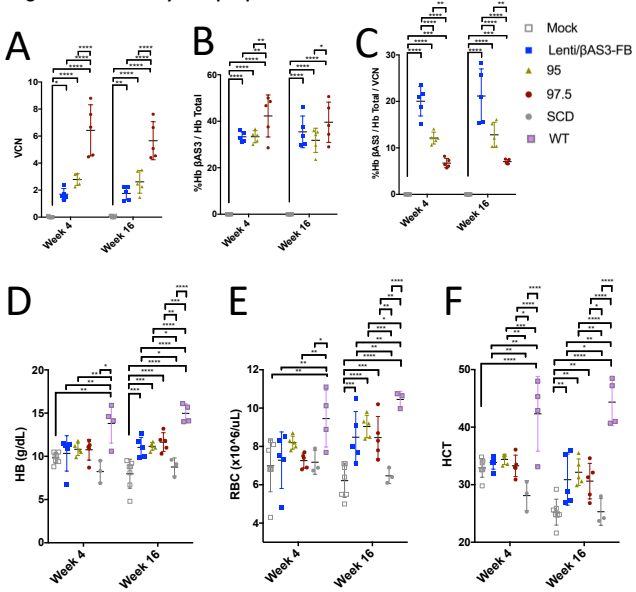


Figure 7: Outline of *in vivo* experiments in a sickle cell disease (SCD) mouse model



Results

Figure 8. In vivo analysis of peripheral blood from "Townes" mouse model of SCD



Conclusion

We have harnessed the power of massively parallel automated DNA synthesis and NGS to simultaneously analyze thousands of synthetic DNA fragments in parallel to identify "sequence intrinsic" enhancers of the LCR at near base pair resolution. These maps were used to generate novel LVs that ameliorated hematologic parameters defining the pathological phenotype of SCD in the mouse model of the disease. Case reports describing patients afflicted with both SCD and hereditary persistence of fetal hemoglobin often describe the clinical course as benign when HbF levels are 10% or higher (likely due to pancellular distribution of HBF). Thus, the *in vivo* percentages of βAS3-globin seen for 95 and 97.5 are at levels of expression expected to be therapeutic. These new LV designs should have advantages for clinical-scale production providing the highest level of gene transfer for the lowest amount of vector.

References

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