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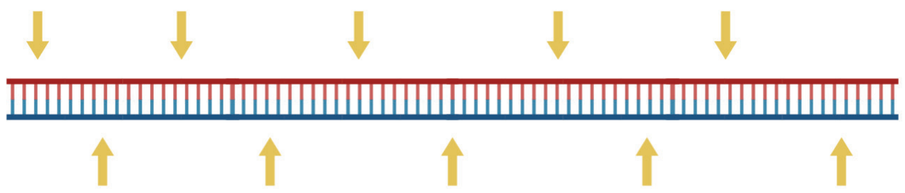
Developing a pooled screening platform for evaluating CRISPR knock-in loci.

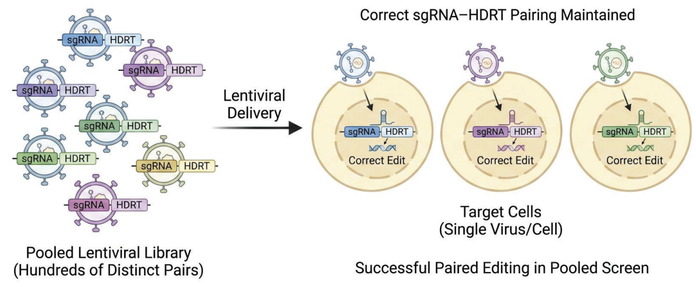
CRISPR-Cas9 knock-in screening usually tests sgRNA and repair template pairs one at a time, which is slow. This project encodes both on a single lentivirus, using a retron to convert the repair template from RNA into DNA, so many pairs can be screened at once to quickly find the best gene insertion sites for cell and gene therapies.

The Goal: Identifying the Most Efficient Cut Site for Knock-Ins

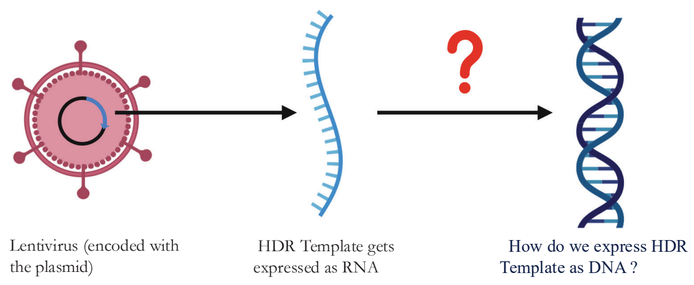
Many Different Cut-Sites on a Locus:



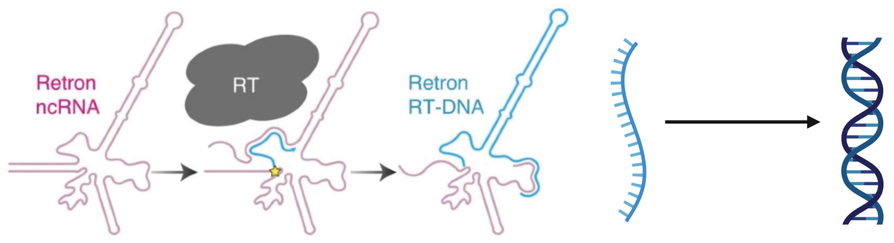
Our Approach: Pooled Screening with Lentivirus



The Problem: HDRT will be Expressed as RNA and not DNA

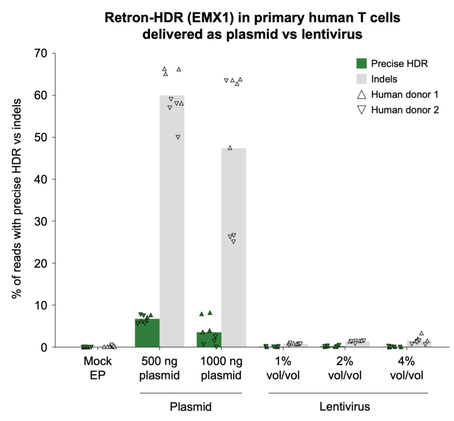


The Solution: Retrons

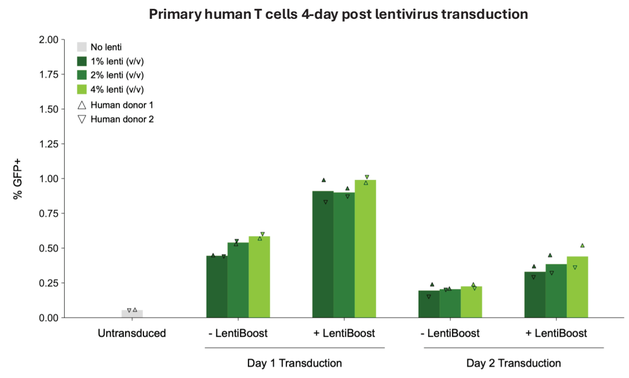


Lopez, S.C., Crawford, K.D., Lear, S.K. et al.

Results: Retron-HDR did not work in T cells via **lentiviral** delivery



Results: The Lentivirus was the issue not the Retron



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