A Genome Editing Lead Finding Platform for Therapeutics

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Background

- Editas Medicine is a leading genome editing company that is translating new genome editing technologies into therapeutics. To this end, we have developed a flexible lead finding platform to identify and characterize highly active and specific genome editing agents. Hundreds of RNA – protein complexes (RNPs) are assayed directly in primary cells at any locus. Genome editing rates are measured with next-gen sequencing using targeted PCR amplification. This process is equally applicable to all RNA guided nucleases including Cas9 and Cpf1 orthologues and variants.

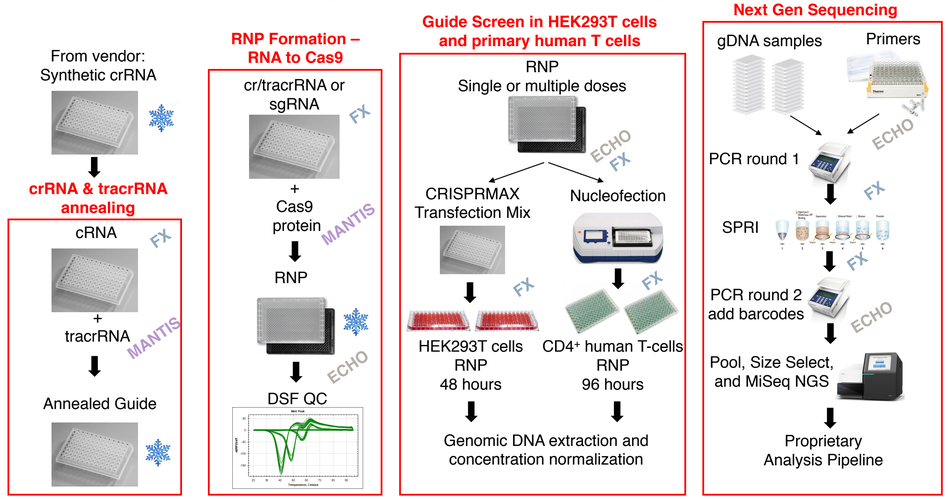
- Targeted amplification and sequencing, while a broadly used tool in the editing field, has critical limitations due to being anchored by two PCR primers. This includes a size bias making large insertions and deletions poorly detected and unexpected translocation events undetectable. To eliminate these challenges we have developed a uni-directional targeted sequencing methodology, **UDiTaS**, that is rapid, quantitative, removes bias associated with variable length PCR amplification, and is capable of measuring large deletions and translocations as well as more typical indels. We show that **UDiTaS** can detect a 1kb deletion generated by a dual editing event that has been confirmed by Sanger sequencing and droplet digital PCR. **UDiTaS** has successfully detected a known translocation in K562 cells, the BCR-ABL fusion gene (chr22/9, not shown). A multiplexed version is forthcoming for simultaneous on- & off-target assessment.

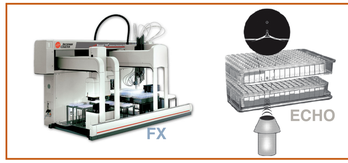
Conclusions

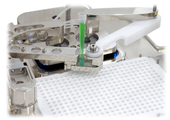
- Screening and optimization platform developed for unbiased investigation of RNA-Protein complex (RNPs) in cell lines and primary cell models

- **UDiTaS** is a uni-directional targeted sequencing method useful for simultaneous measurement of small genome edits and the junctions of larger chromosomal rearrangements

Modular Screening Process for Screening and Sequencing



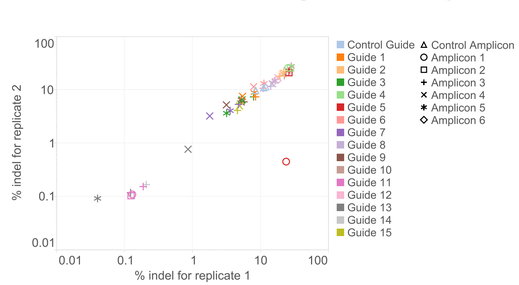




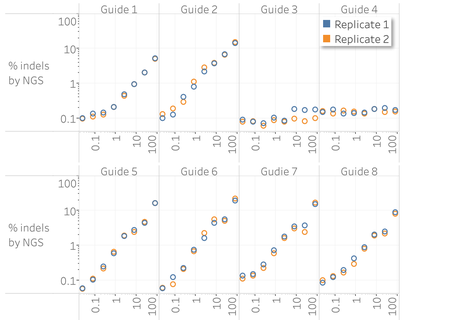
Key liquid handling equipment that enable rapid RNP screening process.

A Lab Information Management System (LIMS) tracks process steps throughout

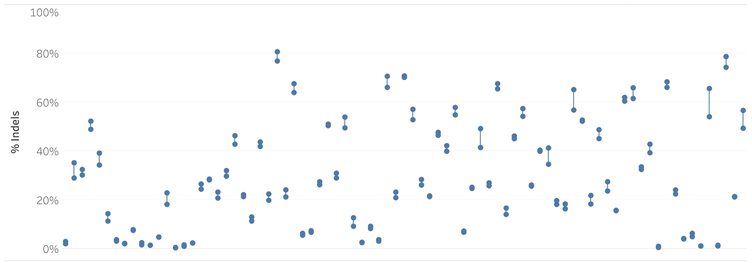
Example Editing Data from Screens



Comparison of bio-replicates from a HEK293T transfection experiment. 15 Guides each assayed with 3 different amplicons.



8-point dose response data with RNPs (x axis in **nM**) with 8 different guide RNAs/Cas9 nucleofected into **Primary Human Erythroblasts** and measuring %indels with NGS (y axis) after 96 hours



81 S py Guides screened in duplicate, ordered by cut site position →

Screening example:

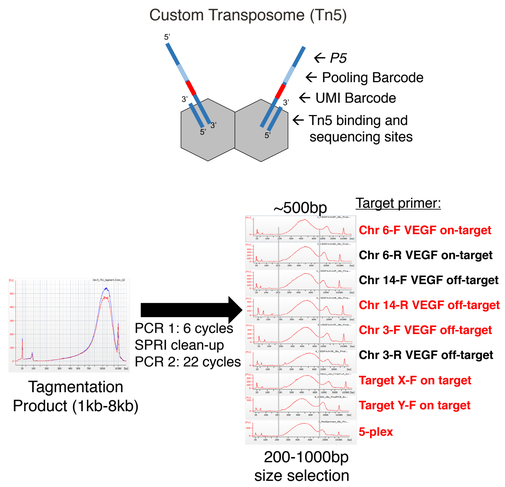
81 S py guides assayed in duplicates as RNPs.

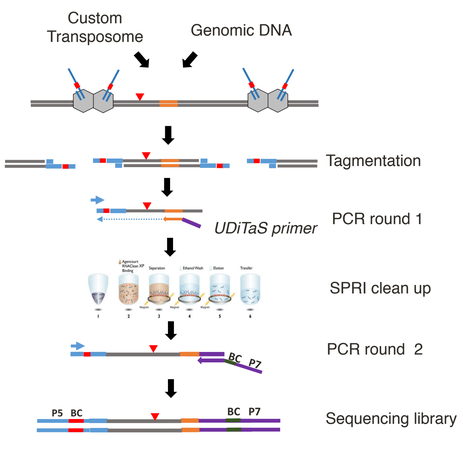
Primary T-cell.

NGS readout for indels.

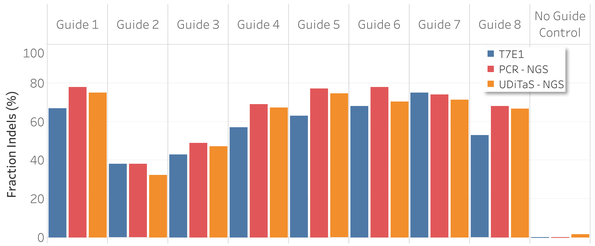
UDiTaS process

Uni-Directional **Ta**rgeted **S**equencing



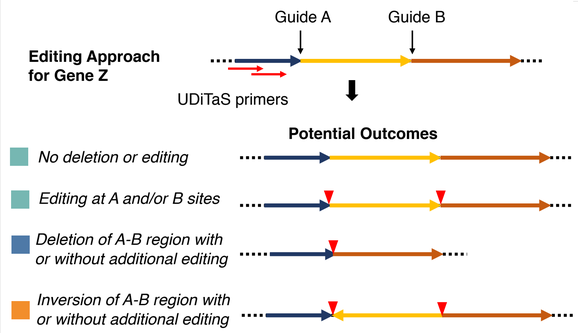


Example Editing and Large Deletion Detection with UDiTaS



Comparison of editing events at Gene X with eight different guides in K562 cells.

T7E1, PCR amplification with NGS, and UDiTaS preparation with NGS give similar results.



A HEK293T clonal line was generated that contains 3 alleles of Gene Z: two deletions and one inversion between the cut sites. The clone genomic DNA was mixed with HEK293T wt genomic DNA at various percentages to produce a range of alleles between ~1% and 100%. The expected allele percentage versus the measured is plotted. The grey line is y=x.