Multiplexed Transposase-Based NGS Tools for QC Assays in Genome Editing Applications

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Introduction

- Genome editing with technologies such as CRISPR/Cas9 can be accompanied by off-target mutagenesis that are critical to measure and characterize. Double-stranded DNA breaks at sites with near-matching target sequence occurs via nonhomologous end-joining, producing unwanted off-target mutations.
- Methods efficiently screen genome editing events in an unbiased manner, such as UDiTaS (Giannoukos et al., 2018) and GUIDE-Seq (Tsai et al., 2015) can help detect and quantify the on- and off-target effects of CRISPR/Cas9 and other genome engineering enzymes.
- We describe the application of a customized Tn5 transposase (Tagify™ i5 UMI TR) to characterize and quantify transgene induced editing in thehuman genome, and the application of the UDiTaS technology to assays of genome editing of the clinically relevant human gene CEP290.

Toolkit for Assays of Genome Editing: UDiTaS and Tagify Reagents

- UDiTaS **(Fig 1)** uses i5 UMI-barcoded transposition to tag positions in genomic DNA with unique sample-specific barcodes, allowing for highly multiplexed **(Fig 2)** targeted amplification around tagged transposition sites.

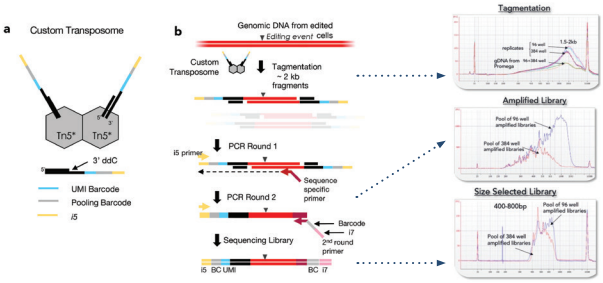
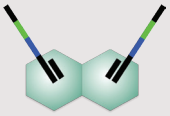


Fig 1: UDiTaS method. Custom i5-UMI barcoded Tn5 transposase (Tagify i5 UMI TR, seqWell) tags gDNA from CRISPR-edited cells; locus-specific primers are used to amplify regions flanking barcoded transposition sites.

Tagify™ i5 UMI Tagging Reagent



- i5 Tagging Reagent with inline Unique Molecular Identifier (UMI) barcode
- Each Tagging Reagent has a different unique i5 barcode plus UMI
- Full-length Illumina-compatible P5+i5+UMI+Read1 priming sequence
- **Applications: Targeted sequencing assays, CRISPR QC, Cell/Gene Therapy QC**

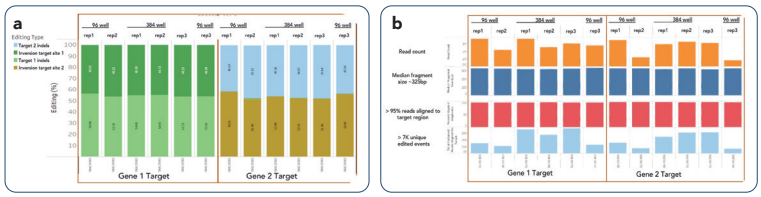


Figure 2: Scaling of UDiTaS method to 96 and 384 sample configurations. (a) Application of 96 and 384 plex UDiTas to detection of multiplexed RNP editing. (b) 384-plex reactions capture reproducibly higher numbers of unique editing events generated during multiplexed editing.

Application of UDiTaS to NGS-based QC of Human Gene CEP290 Editing

- Leber Congenital Amaurosis Type 10 (LCA10) represents a severe retinal dystrophy, caused by mutations in the CEP290 gene on chromosome 12.
- In LCA10, an intronic mutation in intron 26 generates a cryptic splice donor site "IVS26".
- CEP290 is present in the connecting cilium and is important for ciliogenesis, ciliary trafficking, and outer

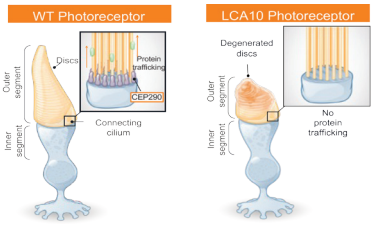


Figure 3: Model of dysfunction mediated by a CEP290 intronic mutation present in LCA10.

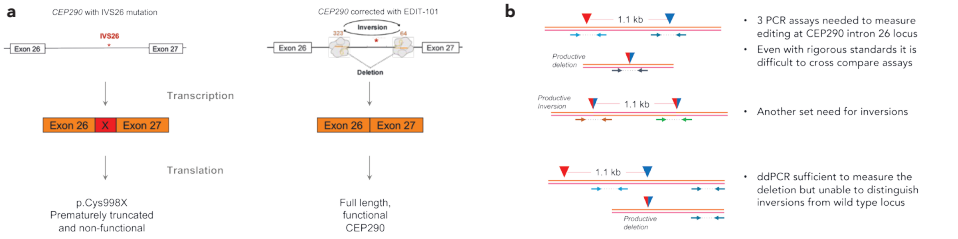


Figure 4: Overview of (a) targeted genome editing via EDIT-101 to remove cryptic splice site in CEP290, and (b) how genome editing of CEP290 creates a number of potential translocation events that are difficult to resolve with conventional PCR assays alone.

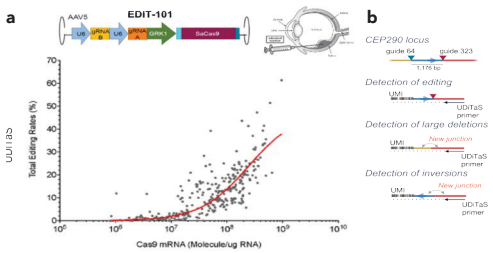


Fig 5: Overview of UDiTaS QC of targeted genome editing via EDIT-101 to remove cryptic splice site in CEP290, and how genome editing of CEP290 creates a number of potential translocation events that are difficult to resolve with conventional PCR assays alone. (a) Example showing highly multiplexed application of UDiTaS. The rate of editing in cells detected with UDiTaS correlates with the transgene expression level induced b EDIT-101 in HuCEP290 knock-in mice. (b) UDiTas is agnostic to the translocation type (e.g. deletion/ inversion) induced by different genome editing events.

Summary and Conclusions

- The UDiTaS method represents a streamlined, highly multiplexed, transposase-based workflow for putative target site or unbiased insertion and translocation site profiling in a variety of genome editing applications. Tagify™ UMI tagging reagents help enable UDiTaS and related methods for counting of targeted editing and translocation sites in different genome editing applications
- For more information regarding Tagify i5 UMI reagents utilized in these studies, or other Tagify custom-loaded transposase reagents, please contact info@seqwell.com