UDiTaS™: A streamlined genome editing detection method for on- and off-target edits, large deletions, and translocations

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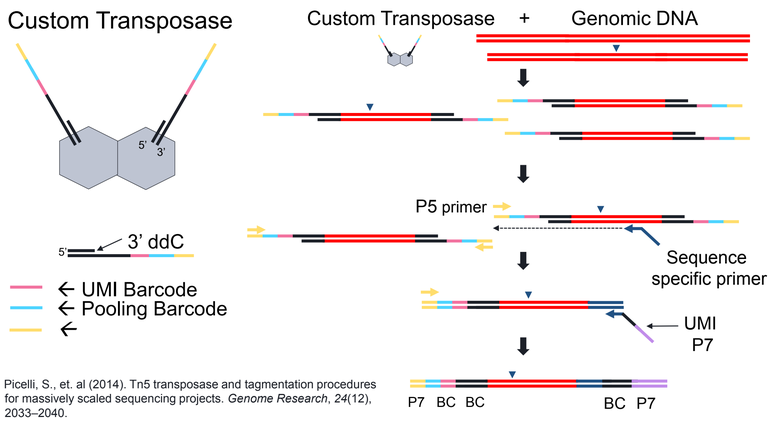
Introduction

Genome editing technologies, including the CRISPR/Cas9 and Cpf1 systems, allow for precise and corrective DNA modifications to treat the underlying cause of genetic diseases. Development of accurate measurement technologies with nucleotide resolution are key to successfully translate these therapies to the clinic as quantitation of genome edits will be important in the overall safety assessment. We will want to track these changes to make sure there is no deleterious consequences as we move therapeutic candidates through appropriate pre-clinical safety assessments.

We have developed a **U**ni-**Di**rectional **Ta**rgeted **S**equencing methodology, **UDiTaS™**, for simultaneous measurement of on-target editing, off-target editing, large insertions, deletions, translocations, and other structural changes. The method improves on previous uni-directional amplification techniques (eg: AMP-Seq and HTGTS), implementing a robust and rapid tagmentation step with a custom designed Tn5 transposase which includes barcodes for better quantitation.

Larger genomic rearrangements such as translocations, inversions, and duplications are generally rare when compared to the highly active non-homologous end joining (NHEJ) result of small insertions and deletions at the cut site. With UDiTaS, editing events not possible to measure using standard technologies are now measurable across a >3 log range. Controlled spiking experiments with intra- and inter- chromosomal alterations allow for determination of reliable lower limits of detection and accurate measurement of editing events. UDiTaS has successfully detected anticipated translocations, a designed 1 kb deletion, small insertions and deletions, and, in addition, can find *de novo* translocations originating at endonuclease cut sites. Identification and quantitation of these events will support subsequent monitoring for safety purposes.

The UDiTaS™ Method

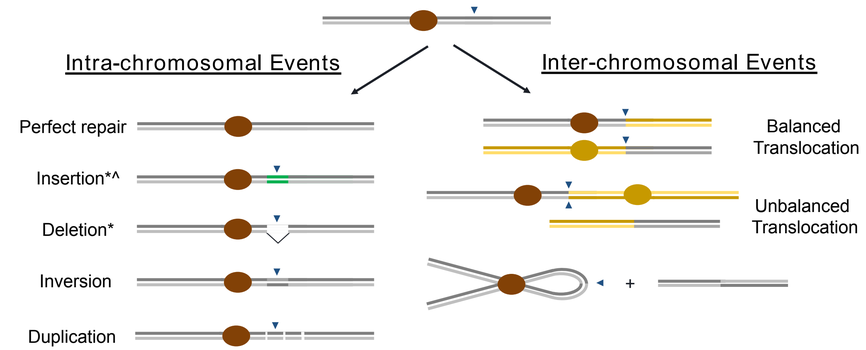


Picelli, S., et. al (2014). Tn5 transposase and tagmentation procedures for massively scaled sequencing projects. *Genome Research*, *24*(12), 2033–2040.

UDiTaS™ takes advantage of the highly efficient transposase mediated DNA fragmentation ('tagmentation') approach developed for preparation of sequencing libraries. We have produced a Tn5 transposase which is loaded with a custom, asymmetric DNA oligo nucleotide as diagrammed in the figure.

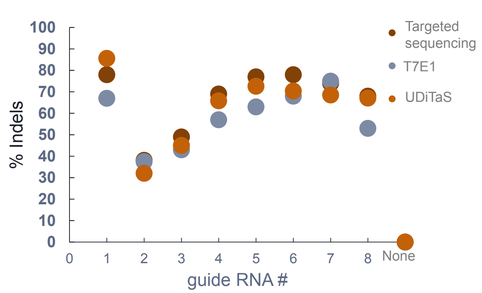
For each editing event at least two assays would be generated that probe the potential cut site from either side. Because the reaction is unidirectional (contains only 1 sequence specific primer) the contents distal to the cut site are irrelevant and a inter-chromosomal event is as readily detected as a small indel.

Genomic Modifications Come in Various Categories

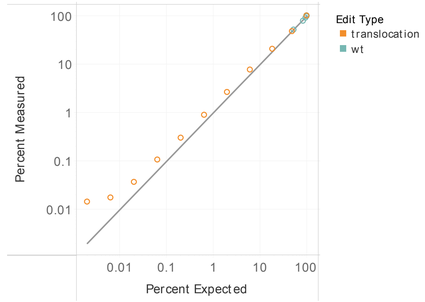


* Small Insertions and Deletions (<100) are "Indels" and are categorically distinct from large insertions or deletions.
^ Inserted DNA can come from 'bone fide' sources (e.g. other chromosomes, delivered DNA or can be non-templated.

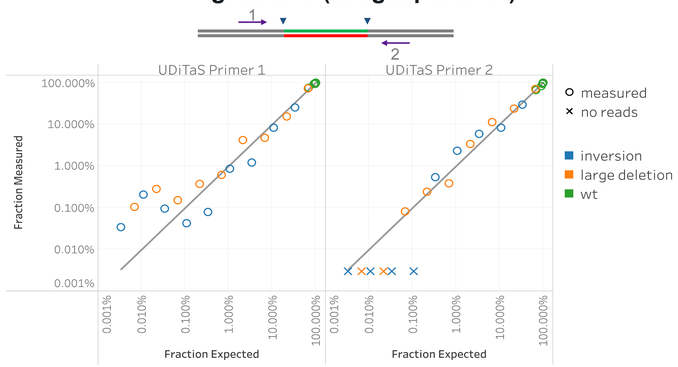
UDiTaS™ measures small Indels similar to T7E1 and targeted sequencing



Sensitivity of UDiTaS™ determined by plasmid dilution experiments



Detection of large intra-chromosomal modifications in HEK 293 cell clones with ~ 1kb deletion or inversion down to ~ 1 event in 1000 with 3,500 genomes (50ng input DNA)



Cutting 2 different chromosomes simultaneously: UDiTaS™ measures large inter-chromosomal translocations (balanced or unbalanced)

Result		Assay 1a (%)	Assay 2b (%)
1a 1b	no edit	3.89	-
1a 1b	with indel	91.38	-
1a 1a		0.42	-
1b 1b		-	-
2a 2b	no edit	-	14.87
2a 2b	with indel	-	82.35
2a 2a		-	-
2b 2b		-	0.001
1a 2a		1.62	-
1a 2b		2.63	2.04
1b 2b		-	0.66
2a 1b		-	-

Conclusion

UDiTaS™ is a novel and robust sequencing method within that has tremendous value for the translation of genome editing therapies. Insights gained from simultaneous measurement of small and large editing events, nucleotide resolution, with accurate quantification will augment therapeutic candidate selection and safety assessment.