In Silico, Biochemical and Cell-based Integrative Genomics Identifies Precise CRISPR/Cas9 Targets for Human Therapeutics

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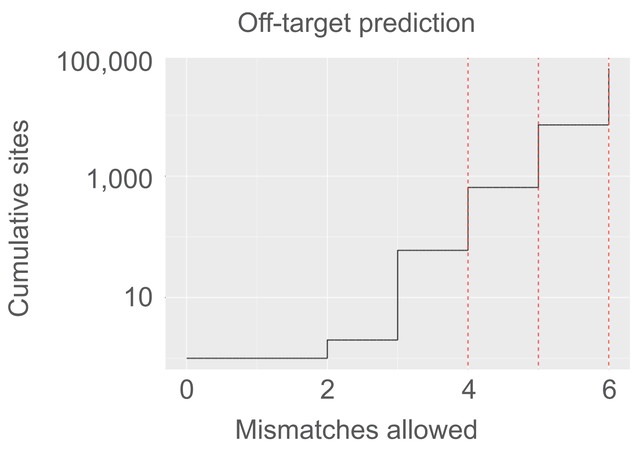
ABSTRACT

For CRISPR/Cas9 to be an effective and safe therapeutic drug in the treatment of diseases that would benefit from the reduction of pathogenic proteins, it can be beneficial to induce a high rate of knockout through indel frameshifts at the on-target locus and minimize any off-target editing. We present an integrative genomics approach that combines *in silico*, biochemical and cell-based genome-wide off-target discovery of 12 CRISPR/Cas9 guides designed to eliminate the expression of a secreted protein. *In silico* prediction that allows 3 mismatches in the genome and 4 mismatches in coding DNA sequence identified loci, the biochemical discovery assay *SITE-Seq*® discovered loci, and a cell-based oligo capture assay identified loci. Validation of these off-target discovery profiles was conducted through targeted off-target sequencing on empirical loci discovered and the top 30 *in silico* predicted coordinates. Our targeted off-target sequencing revealed that *in silico*-based methods had the lowest contribution to validated off-target discovery. The biochemical discovery assay *SITE-Seq*® was the most sensitive and identified validated indels that were missed in the cell-based oligo capture assay. Therefore, an integrative genomics approach that applies *in silico* CRISPR guide design followed by empirical biochemical off-target discovery and validation through targeted off-target sequencing is useful to identify precise human CRISPR/Cas9 therapeutic candidates.

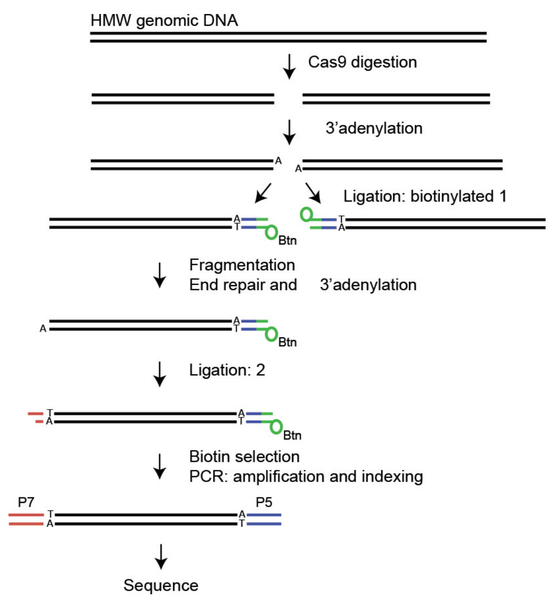
IN SILICO OFF-TARGET DISCOVERY



In Silico Site Prediction Based on Mismatches

The cumulative number of sites predicted is represented on the Y-axis. There is log-linear relationship in the number of potential off-target loci as the number of mismatches allowed increases (X-axis).

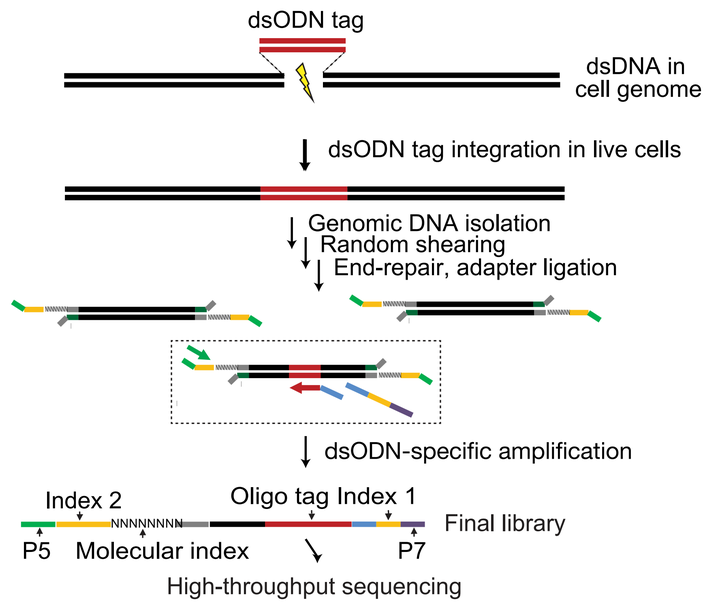
BIOCHEMICAL[1] OFF-TARGET DISCOVERY



SITE-Seq® NGS library construction

This is a species genome-wide off-target discovery assay because it is executed on deproteinated and purified genomic DNA.

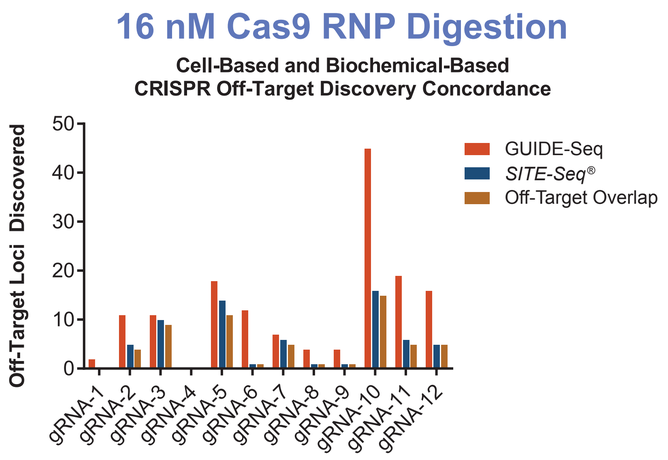
CELL-BASED[2] OFF-TARGET DISCOVERY



GUIDE-Seq NGS Library Construction

This is a tissue specific genome-wide off-target discovery assay because it is executed in cells and relies on endogenous repair machinery.

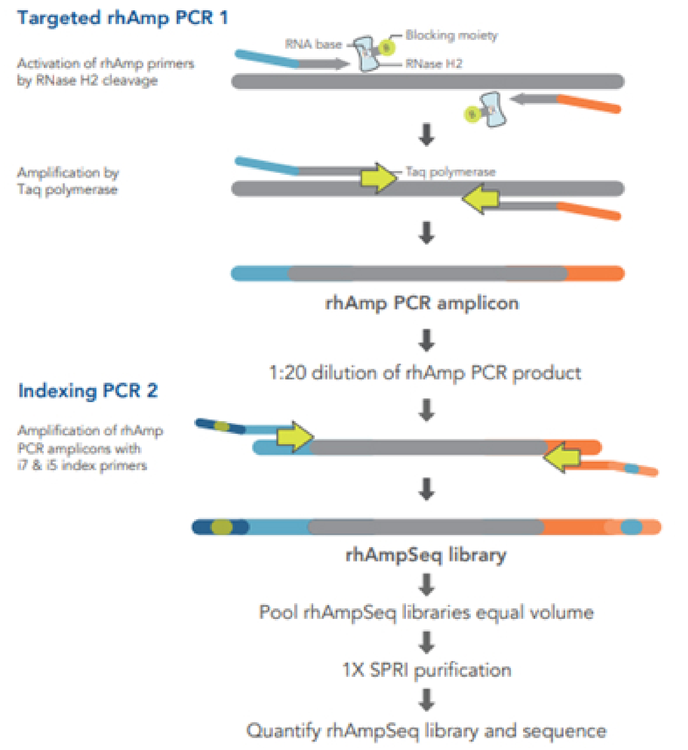
BIOCHEMICAL VERSUS CELL-BASED AND *IN SILICO* OFF-TARGET PROFILESS



Comparison of Off-Target Profiles

The number of potential off-target sites discovered by *SITE-Seq*® increases with Cas9 RNP concentration and is a super-set of the potential off-target loci discovered by GUIDE-Seq.

OFF-TARGET INDEL VALIDATION WITH TARGETED OFF-TARGET SEQUENCING



ID	Top 30 *In silico*	GUIDE-Seq	64 nM RNP *SITE-Seq*®
gRNA-1	0/30	0/2	0/1
gRNA-2	0/30	1/11	1/14
gRNA-3	0/30	1/11	1/27
gRNA-4	0/30	0/0	0/4
gRNA-5	1/30	1/18	2/37
gRNA-6	0/30	0/12	0/3
gRNA-7	0/30	0/7	0/15
gRNA-8	0/30	0/4	0/9
gRNA-9	0/30	1/4	1/4
gRNA-10	0/30	2/45	6/43
gRNA-11	0/30	0/19	1/12
gRNA-12	0/30	0/16	0/31

rhAMPSeq®

RNase H2 dependent PCR Amplification for Next Generation Sequencing (*rhAMPSeq*®), validates off-target editing with multiplexed PCR and targeted off-target sequencing. Table reflects the number of off-target editing loci tested (denominator) and the number of validated off-target (numerator).

CONCLUSIONS

CRISPR/Cas9 off-target detection with whole genome sequencing would require >1,000X coverage to achieve the off-target sensitivity of a biochemical genome-wide off-target discovery assay like *SITE-Seq*®. Furthermore, bioinformatics prediction allowing up to 6 mismatches and non-canonical PAMs identifies >80,000 potential off-target sites. Therefore, empirical off-target discovery assays facilitate the discovery of potential off-target editing loci for validation and quantification with targeted off-target sequencing in edited cells. The cell-based assay GUIDE-Seq is less sensitive than biochemical off-target discovery assays like *SITE-Seq*® because cell-based assays face DNA editing limitations in a cellular context. Furthermore, cell-based off-target discovery assays are restricted by tissue type and executed in *in vitro* cell culture systems, while biochemical off-target discovery assays are devoid of CRISPR/Cas9 enzymatic restrictions and serve as a species specific off-target discovery assay.

REFERENCES

[1]P Cameron et al. Mapping the genomic landscape of CRISPR-Cas9 cleavage. (2017) *Nature Methods* 14; 600-606.

[2]S Tsai et al. GUIDE-seq enables genome-wide profiling of off-target cleavage by CRISPR-Cas nucleases. (2015) *Nature Biotech* 33; 187-197.