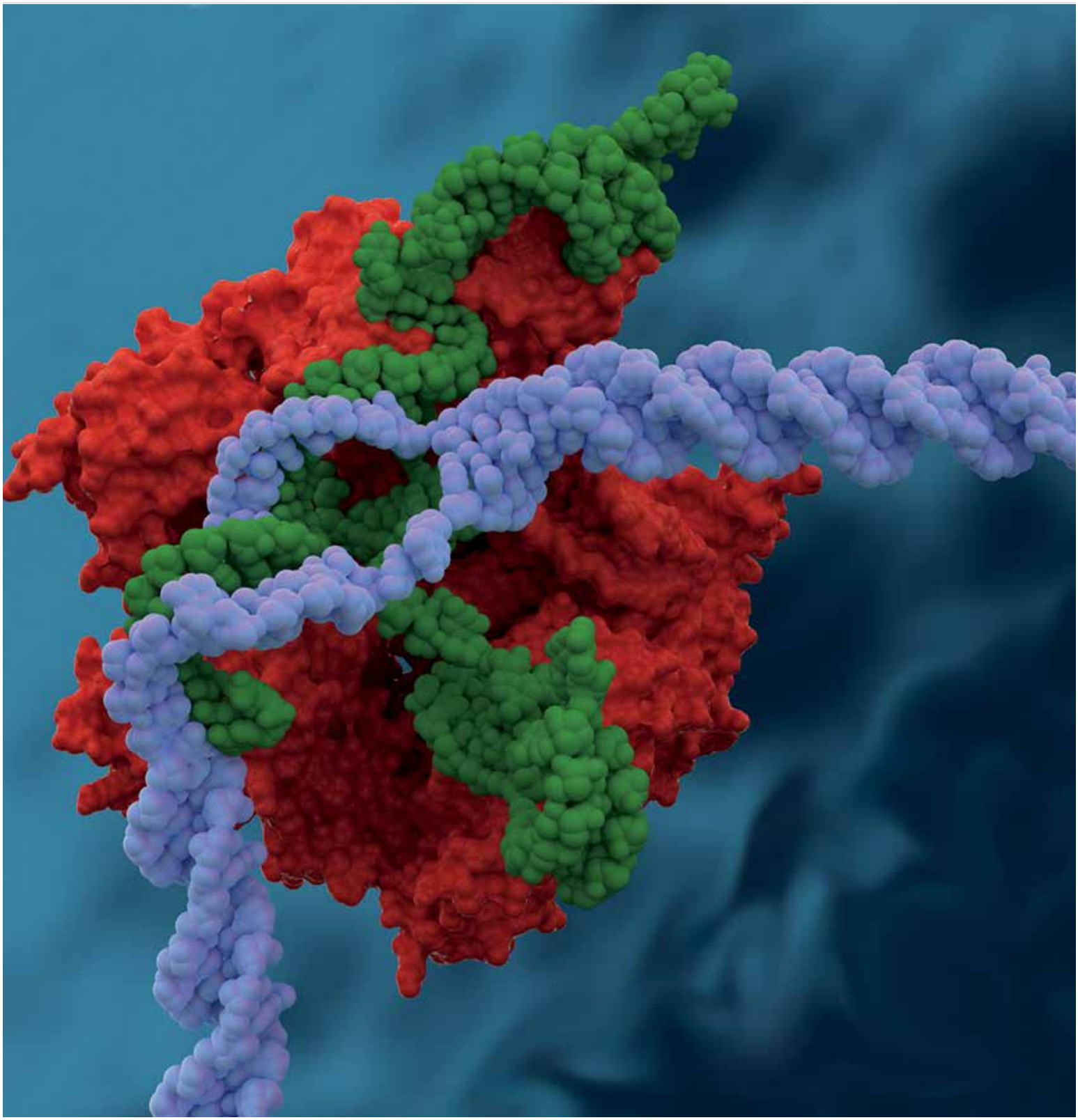
Gene Editing Research Review

An Overview of Recent Gene Editing Research Publications Featuring Illumina® Technology



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This document highlights recent publications that demonstrate the use of Illumina technologies in single-cell research. To learn more about the platforms and assays cited, visit www.illumina.com.

Table 4. Unbiased Methods to Detect Off-Target Effects [148, 149]

Method	Type	Description	Advantage	Limitation	References
Integrase-defective lentiviral vector (IDLV) capture	Cell-based (live cells)	Integration of IDLVs with a selectable marker into DSB sites, recovery of integration sites through LAM-PCR, high-throughput sequencing	Can detect DSBs in living cells	Relatively insensitive	150, 151, 152
Genome-wide unbiased identification of DNBs enabled by sequencing (GUIDE-Seq)	Cell-based (live cells)	Integration of an end-protected dsODN into DSBs sites in living cells, tag-specific amplification, high-throughput sequencing	Simple, efficient, and precise, straightforward protocol, and availability of open-source software for data analysis	The efficient delivery of dsODN may be potentially harmful to cells, and it has not been tested *in vivo*	153, 154
High-throughput genome-wide translocation sequencing (HGTST)	Cell-based (live cells)	Generation of a prey and bait DSB through the expression of 2 nucleases, use of a biotinylated primer against the bait DSB junction, LAM-PCR to recover translocations between prey and bait, streptavidin-based enrichment, high-throughput sequencing	Requires the delivery of only the editing complex and can potentially be used *in vivo*	Nuclease-induced translocations are rare events and tend to occur between sites on the same chromosome	155, 156
Breaks labeling, enrichment on streptavidin and next-generation sequencing (BLESS)	Cell-based (fixed cells)	Isolation and fixation of treated cells, isolation and permeabilization of intact nuclei, *in situ* ligation of adapters to transient nuclease-induced DSBs, enrichment, high-throughput sequencing	Has been used in tissues where Cas9 had been delivered in vivo, independent from endogenous DNA repair machinery	Technically challenging, only allows identification of DSBs present at a specific moment, cannot detect DSBs before permeabilization	157
Digested genome sequencing (Digenome-Seq)	*In vitro*	Isolation of genomic DNA from *in vitro* treated cells, ligation of sequencing adapters, whole-genome sequencing	No limitations related to cell-based factors	Sequencing-inefficient, high background noise	158, 159

Frock R. L., Hu J., Meyers R. M., Ho Y. J., Kii E., et al. Genome-wide detection of DNA double-stranded breaks induced by engineered nucleases. *Nat Biotechnol*. 2015;33: 179-186.
In this study, the authors leverage a previously described emulsion PCR method to perform high-throughput, genome-wide, translocation sequencing (HTGTS) to detect DNA DSBs generated by nucleases across the human genome. This method is based on the identification of translocations between nuclease-induced and off-target DSBs. The application of HTGTS revealed that off-target hotspot numbers for given nucleases ranged from a few or none to dozens or more, and it extended the number of known off-target sites for certain previously characterized nucleases more than tenfold. The authors were also the first to detect translocations between nuclease targets on homologous chromosomes.

Illumina Technology: MiSeq Sequencer

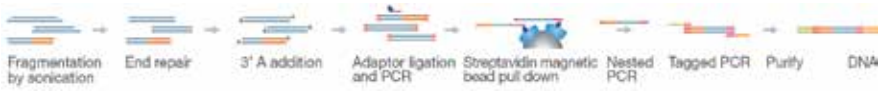

Figure 6. Schematic overview of HTGTS.

Tsai S. Q., Zheng Z., Nguyen N. T., Liebers M., Topkar V. V., et al. GUIDE-seq enables genome-wide profiling of off-target cleavage by CRISPR-Cas nucleases. *Nat Biotechnol*. 2015;33: 187-197.
The authors describe GUIDE-Seq, a method for the genome-wide identification of DNA DSBs introduced by CRISPR RNA-guided nucleases (RGNs). In the first stage, the method uses an end-joining process to tag RGN-induced DSBs in human cells by inserting a dsODN. The second stage uses unbiased amplification and NGS to map the dsODN integration sites. The application of GUIDE-Seq to 13 RGNs in human cell lines revealed wide variability in RGN off-target activities, most of which had not been identified with previously existing methods. GUIDE-Seq also identified RGN-independent genomic breakpoint hotspots. Finally, the authors also observed that truncated guide RNA led to a reduction in the number of RGN-induced off-target DSBs.

Illumina Technology: MiSeq Sequencer

145. Kim D, Kim S, Kim S, Park J and Kim JS. Genome-wide target specificities of CRISPR-Cas9 nucleases revealed by multiplex Digenome-seq. *Genome Res*. 2016;26:406-415.
146. Tsai SQ and Joung JK. Defining and improving the genome-wide specificities of CRISPR-Cas9 nucleases. *Nat Rev Genet*. 2016;17:300-312.
147. Tycko J, Myer VE and Hsu PD. Methods for Optimizing CRISPR-Cas9 Genome Editing Specificity. *Mol Cell*. 2016;63:355-370.
148. Tsai SQ and Joung JK. Defining and improving the genome-wide specificities of CRISPR-Cas9 nucleases. *Nat Rev Genet*. 2016;17:300-312.
149. Tycko J, Myer VE and Hsu PD. Methods for Optimizing CRISPR-Cas9 Genome Editing Specificity. *Mol Cell*. 2016;63:355-370.
150. Gabriel R, Lombardo A, Arens A, et al. An unbiased genome-wide analysis of zinc-finger nuclease specificity. *Nat Biotechnol*. 2011;29:816-823.
151. Osborn MJ, Webber BR, Knipping F, et al. Evaluation of TCR Gene Editing Achieved by TALENs, CRISPR/Cas9, and megaTAL Nucleases. *Mol Ther*. 2016;24:570-581.
152. Wang X, Wang Y, Wu X, et al. Unbiased detection of off-target cleavage by CRISPR-Cas9 and TALENs using integrase-defective lentiviral vectors. *Nat Biotechnol*. 2015;33:175-178.

Kleinstiver B. P., Tsai S. Q., Prew M. S., Nguyen N. T., Welch M. M., et al. Genome-wide specificities of CRISPR-Cas Cpf1 nucleases in human cells. Nat Biotechnol. 2016;34: 869-874.
The authors found that AsCpf1 and LbCpf1 have on-target editing accuracies in human cells that are comparable to that of *Streptococcus pyogenes* Cas9. Through GUIDE-Seq and targeted deep sequencing analysis performed on both nucleases, they found no off-target effects for most of the crRNAs used. Their results suggest that AsCpf1 and LpCpf1 are highly specific in human cells.

Illumina Technology: MiSeq Sequencer

Komor A. C., Kim Y. B., Packer M. S., Zuris J. A. and Liu D. R. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature*. 2016;533: 420-424.
CRISPR-Cas9 genome editing relies on the generation of targeted DSBs and 1 of 2 repair mechanisms: either NHEJ or HDR, in the presence of a homologous template. These mechanisms are a limitation when it is necessary to correct a point mutation, as in many genetic diseases. In this study, the authors developed an approach that enables the direct conversion of a target DNA base to another in a programmable manner, without requiring DSBs or a donor template. To do so, they fused CRISPR-Cas9 and a cytidine deaminase enzyme that can mediate the direct conversion of cytidine to uridine, thereby enabling C -> T (or G -> A) substitutions.

Illumina Technology: MiSeq Sequencer

Nishida K., Arazoe T., Yachie N., Banno S., Kakimoto M., et al. Targeted nucleotide editing using hybrid prokaryotic and vertebrate adaptive immune systems. *Science*. 2016; .
In this study, the authors engineered the nuclease-deficient type II CRISPR-Cas9 and the activation-induced cytidine deaminase (AID) ortholog PmCDA1 to create a synthetic complex (Target-AID). They used it to demonstrate the targeted single-nucleotide substitution of DNA. Using deep sequencing, they showed the high specificity of Target-AID in performing targeted mutagenesis. Target-AID induced specific point mutations mainly at cytidines within the target range of 5 bases. The authors further improved efficiency by using uracil DNA glycosylase inhibitor (UGI) to suppress indel formation.

Illumina Technology: MiSeq Sequencer

Slaymaker I. M., Gao L., Zetsche B., Scott D. A., Yan W. X., et al. Rationally engineered Cas9 nucleases with improved specificity. *Science*. 2016;351: 84-88.
The authors used structure-guided engineering to increase the specificity of *Streptococcus pyogenes* Cas9 by inserting individual alanine substitutions at 31 positively charged residues within the non–target-strand groove of the enzyme. They then used both targeted and whole-genome deep sequencing (breaks labeling, enrichment on streptavidin and NGS, or BLESS) to assess on-target activity and specificity. They demonstrated that the "enhanced specific" eSpCas9 variants they created were able to edit genomes in human cells while maintaining a robust on-target activity.

Illumina Technology: TruSeq Nano LT Kit

Zetsche B., Volz S. E. and Zhang F. A split-Cas9 architecture for inducible genome editing and transcription modulation. *Nat Biotechnol*. 2015;33: 139-142.
Strategies using catalytically inactive Cas9 are useful to direct the protein to the correct target and regulate transcription. In this study, the authors created a split-Cas9 system in which the 2 fragments can be chemically induced to dimerize and are activated in presence of rapamycin. They then tested the system in HEK293 cells and observed that it reduces off-target effects when the split enzyme is expressed from an integrated low-copy lentiviral vector.

Illumina Technology: MiSeq Sequencer

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Tsai S. Q. and Joung J. K. Defining and improving the genome-wide specificities of CRISPR-Cas9 nucleases. *Nat Rev Genet*. 2016;17: 300-312

Tycko J., Myer V. E. and Hsu P. D. Methods for Optimizing CRISPR-Cas9 Genome Editing Specificity. *Mol Cell*. 2016;63: 355-370

Shalem O., Sanjana N. E. and Zhang F. High-throughput functional genomics using CRISPR-Cas9. *Nat Rev Genet*. 2015;16: 299-311

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