CRISPR GLOSSARY:

- **Cas9 (n.)** CRISPR-associated protein #9
- **CRISPR (n., adj.)** Clustered, regularly-interspaced, short palindromic repeat
- **crRNA (n.)** CRISPR RNAs
- **DSB (n.)** Double-stranded break
- **dsDNA (n.)** Double-stranded DNA
- **GMO (n.)** Genetically modified organism
- **HDR (n.)** Homology-directed repair
- **HNH (n.)** A nuclease domain that cuts the target strand
- **Indel (n.)** Insertion or deletion
- **NHEJ (v.)** Nonhomologous end joining
- **NUC (n.)** Nuclease lobe of Cas9
- **PAM (n.)** Protospacer-adjacent motif
- **REC (n., adj.)** Recognition lobe of Cas9
- **RuvC (n.)** A nuclease domain that cuts the noncomplementary strand
- **sgRNA (n.)** Single guide RNA
- **ssDNA (n.)** Single-stranded DNA
- **tracrRNA (n.)** Trans-activating crRNA

GENOME EDITING

With CRISPR/Cas9 – An Introduction

Discovered nearly 30 years ago, the CRISPR/Cas9 system has gone from a form of bacterial innate immunity to a game-changing genome editing technology, completely revolutionizing molecular biology within the past five years. Learn more about the mechanism, protocol, and groundwork that have brought this method to laboratories across the globe.

1987 "Unusual structure" in *E. coli iap* gene identified by Ishino et al. [1]

2000 Short Regularly Spaced Repeats (SRSR) proposed [2]

2002 CRISPR acronym first used to define Cas gene clusters [3]

2005 First evidence of Cas protein [7]

2005 Spacer sequences matched to foreign DNA [4-6]

2006 Adaptive immunity function of CRISPR proposed [8]

2007 CRISPR/Cas9 secondary structure classification [11]

2007 CRISPRdb becomes available [10]

2008 crRNA processing pathway defined [14, 15]

2008 The term "protospacer" coined [13]

2007 First experimental evidence of CRISPR adaptive immunity [9]

2008 CRISPR cuts DNA target [12]

2009 First use of "protospacer-adjacent motif" (PAM) terminology [16]

2009 RNA-guided RNA cleavage described [17]

2010 Mechanism for self vs. non-self identified [18, 19]

2010 crRNA guides Cas9 cleavage of DNA via DSBs [20]

2011 Evidence of tracrRNA complexing with crRNA and Cas9 [21]

2011 Type II CRISPR genes expressed in other organisms [22]

2012 Single-protein requirement for type II Cas9 revealed [23, 24]

2013 CRISPR/Cas9 used in human cells [25-27]

2013 CRISPR/Cas9 used in plant cells [28, 29]

2014 PAM/Cas9 interaction assists in local strand separation & R-loop formation [31]

2014 PAMs revealed as key driver of target interrogation [30]

2014 Direct delivery of Cas9 & sgRNA to cells [32]

2015 Germline editing prompts a global ethical discussion [34]

2015 CRISPR first used in human embryos [33]

2016 USDA determines edited crops will not be regulated as GMOs [35]

2016 NIH approves first human trial [36]

2001 2003 2005 2007 2009 2011 2013 2015

2008 2010 2012 2014 2016

REPAIR MECHANISMS:

Nonhomologous End Joining (NHEJ)

Error prone · Random mutation of 1-10 indels · High-frequency, sgRNA-dependent event

Homology-directed Repair (HDR)

dsDNA or ssDNA donor DNA required · Either corrects known mutations, adds foreign DNA, or replaces defined fragments · Low-frequency event (<5%)

CONFIRM YOUR CRISPR EFFICIENCY WITH HCA:

Heteroduplex cleavage assays (HCA)

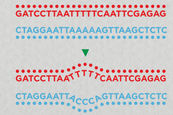
Simple method for determining CRISPR efficiency · Works for both NHEJ- and HDR-based events

Equations for determining the cleavage efficiency of pooled cells:

$$\text{Fraction cleaved} = \frac{\text{Concentration of Fragment 1} + \text{Concentration of Fragment 2}}{\left(\frac{\text{Concentration of Fragment 1} + \text{Concentration of Fragment 2}}{2}\right) + \text{Concentration of Intial Amplicon}}$$

$$\text{Fraction Mutated} = 1 - (1 - \text{Fraction Cleaved})^{0.5}$$

Schematic of HCA



▲ T7 Endonuclease 1

BASIC CRISPR WORKFLOW:

1. Design sgRNA(s) and/or donor DNA fragment(s)
2. Introduce Cas9 and sgRNA into your cells
3. Induce Cas9 and sgRNA expression; add donor DNA
4. Allow cells to recover
5. Analyze cells for gene-editing events, either individually or in pools

1. Y. Ishino et al., *J Bacteriol* 169:5429-33, 1987. 2. F.J. Mojica et al., *Mol Microbiol*; 36:244-46, 2000. 3. R. Jansen et al., *Mol Microbiol*; 43:1565-75, 2002. 4. A. Bolotin et al., *Microbiology* 151:2551-61, 2005. 5. F.J. Mojica et al., *J Mol Evol*; 60:174-82, 2005. 6. C. Pourcel et al., *Microbiology* 151:653-63, 2005. 7. N.F. Saunders et al., *J Phytopathol Res*; 6:4642-72, 2005. 8. K.S. Makarova et al., *Biol Direct* 1:7, 2006. 9. R. Barrangou et al., *Science* 315:1709-12, 2007. 10. I. Grissa et al., *BMC Bioinformatics* 8:172, 2007. 11. V. Kunin et al., *Genome Biol Evol* 1:1-8, 2007. 12. L.A. Marraffini et al., *Science* 322:1843-45, 2008. 13. H. Deveau et al., *J Bacteriol* 190:1390-400, 2008. 14. S.J. Brouns et al., *Science* 321:960-64, 2008. 15. J. Carte et al., *Genes Dev* 22:3489-96, 2008. 16. F.J. Mojica et al., *Microbiology* 155:733-40, 2009. 17. C.R. Hale et al., *Cell* 139:945-56, 2009. 18. P. Horvath, et al., *Science* 327:167-70, 2010. 19. L. Marraffini et al., *Nature* 463:568-71, 2010. 20. J.E. Garneau et al., *Nature* 468:67-71, 2010. 21. E. Deltcheva et al., *Nature* 471:602-07, 2011. 22. R. Sapranauskas et al., *Nucleic Acids Res* 39: 9275-82, 2011. 23. M. Jinek et al., *Science* 337:816-21, 2012. 24. G. Gasiunas et al., *PNAS* 109: E2579-86, 2012. 25. L. Cong et al., *Science* 339:819-23, 2013. 26. P. Mali et al., *Science* 339:823-26, 2013. 27. M. Jinek et al., *ELife* 2:e00471, 2013. 28. J.F. Li et al., *Nat Biotechnol* 31:688-91, 2013. 29. S.A. Shalem et al., *Science* 343:84-87, 2014. 30. C. Anders et al., *Nature* 513:569-73, 2014. 31. E. Richardson et al., *Genome Res* 24:1020-27, 2014. 32. F. Liang et al., *Protein Cell* 6:363-72, 2015. 33. P. Liang et al., *Protein Cell* 6:363-72, 2015. 34. D. Baltimore et al., *Science* 348:36-38, 2015. 35. E. Waltz, *Nature* 532:293, 2016. 36. S. Reardon, *Nature*; doi:10.1038/nature.2016.20212, 2016.

...THE POSSIBILITIES WITH AUTOMATED CRISPR ANALYSIS...

...with the Fragment Analyzer™

You can utilize the Fragment Analyzer throughout your CRISPR workflow. Efficiently assess the size, quality and purity of *in vitro* transcribed guide RNAs for improved gene targeting.Then accurately evaluate gene editing events generated through NHEJ or HDR using our sensitive and validated heteroduplex cleavage assay. Simultaneously run 12, 48, or 96 samples using your choice of parallel capillary arrays, reducing your time to results for quicker decision making.

More at www.aati-us.com/JustImagineCRISPR

CRISPR is becoming the main procedure to knock-in or knock-out genes or alter genetic sequences. Due to its simplicity, multiplexing capability and reagent availability, researchers are exploring the limits of its capabilities in model systems and for clinical applications. Efficient screening and detection of gene editing events is critical to successfully generating edited cell lines or organisms.

Advanced Analytical Technologies, Inc. is a world leader in parallel capillary electrophoresis instruments for automating genomics discovery. Our award winning instrument, the Fragment Analyzer™ Automated CE System, is the premier instrument for analyzing nucleic acid fragments and smears and has become the industry standard for most genomics applications. Created to streamline laboratory workflow and decrease time to results, the Fragment Analyzer can:

- Reliably quantify and qualify DNA fragments, NGS libraries, genomic DNA, total RNA, and small RNA.
- Analyze gene editing events generated through CRISPR/Cas9 mutagenesis.
- Accurately size all gene editing events, in both pooled and individual samples.
- Assess the relative quantity to determine the mutation frequency percentage and zygosity.
- Generate results with minimal effort through the aid of a specially designed CRISPR software package.

The CRISPR experimental design dictates whether a few or many cells with edits are generated from a population of pooled cells. Editing is dependent on many factors including: the expression or delivery of the guide RNA(s)/Cas9 complex, delivery or expression of donor DNA, effectiveness of the guide RNA(s) and efficiency of repair mechanisms. These factors all have an impact on gene editing mutation frequency yield. The Fragment Analyzer can be used to screen the pooled cell populations post-editing. Measuring the overall effectiveness of the experimental strategy helps determine how many cells need to be evaluated in order to choose and propagate cells with the desired modifications. To assist in winnowing the cells with known edits, the Fragment Analyzer can also be used to identify which individual cells have edits at one or more than one of the alleles, essentially zygosity determination. Being able to identify monoallelic from diallelic events in diploid organisms greatly reduces the amount and costs associated with sequencing to identify the organism with the desired nucleotide changes.

As CRISPR becomes the premier gene editing procedure, doesn't it make sense to couple it with the premier instrument for fragment analysis? Find out more at www.aati-us.com/crispr

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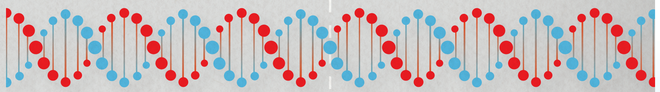

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JUST IMAGINE...

Fragment Analyzer™ is the only automated instrument for the analysis of CRISPR/Cas9 gene editing events.
Accelerate your scientific discovery using a streamlined process for easy identification of both individual and pooled gene mutations.

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Available Reagent Kits for Heteroduplex Cleavage

DNF-910-1000CP - CRISPR Discovery Gel dsDNA Reagent Kit
- Reagents for separating heteroduplexed cleaved fragments

DNF-440-1000CP AccuCleave^{CE} T7 Kit
- T7 based enzyme kit for automated digestion of heteroduplex fragments
- Optimized for use on the Fragment Analyzer

DNF-441-0015CP - CRISPR 15 Control DNA Kit
- Fifteen Control DNAs for optimizing cleavage
- Kit contains 15 DNA fragments - intact, ± 1, ± 2, ± 10 and 8 point mutations.

DNF-443-0002CP - CRISPR 2 Control DNA Kit
- Control DNAs for optimizing and testing T7 digestions
- Kit contains 2 DNA fragments - intact and -2 deletion fragment