Introduction to CRISPR/Cas, a Powerful Technology for Genome Editing

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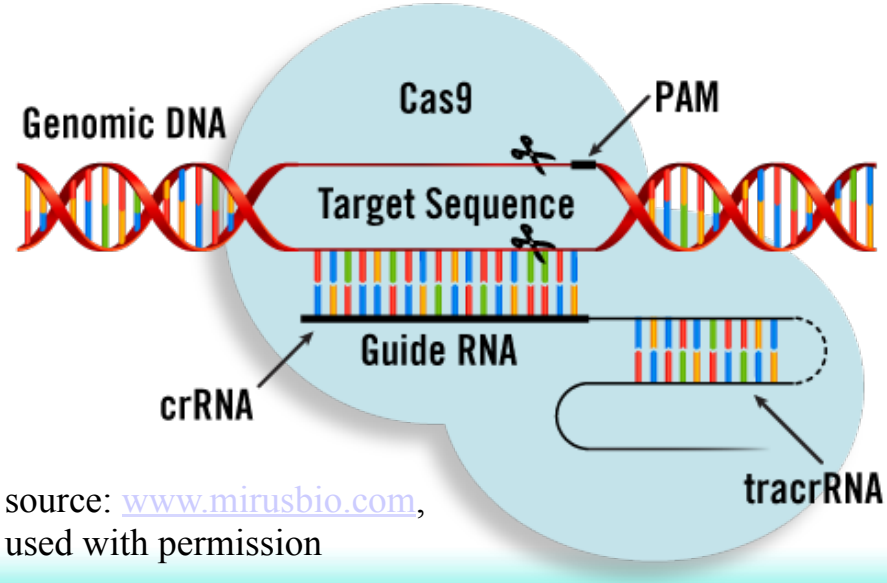
What is CRISPR?

- CRISPR stands for clustered regularly interspaced short palindromic repeats (CRISPR), genetic elements harbored in bacteria and archaea genomes as part of a RNA-based adaptive immune system that protects against invading viruses and plasmids
- CRISPR RNAs (crRNAs) function with trans-activating crRNA (tracrRNA) and CRISPR-associated nuclease (Cas) to introduce double-stranded breaks in target DNA
- CRISPR-Cas9 has now been developed into a powerful technology for genome editing at very high specificity and efficiency in bacteria, yeast, plants, animals and human

How Does CRISPR-Cas9 Work?

- By expressing or delivering the Cas9 nuclease and sgRNA (a single guide RNA containing both crRNA and tracrRNA) in/into a cell, the cell's genome can be cut and altered at a desired location
- Target cleavage by Cas9 requires base-pairing between the crRNA and its target DNA (protospacer)
- sgRNA determines the specific cleavage site
- Target site has to be located next to the nucleotides 5'-NGG-3' (called a protospacer-adjacent motif, PAM) on the opposite DNA strand



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CRISPR-Cas9 Applications

- CRISPR-Cas9: creates double-stranded breaks (DSBs) and activate cellular DSB repair mechanisms
  - DSBs are repaired by non-homologous end joining (NHEJ), introducing insertion or deletion, thus disrupting the target gene
  - In the presence of DNA sequences homologous to the target site, DSBs can be repaired by homology-directed repair (HDR), resulting in gene replacement or restoration
- Cas9D10 with only nickase activity: cleavages only one DNA strand, does not activate NHEJ. Paired Cas9D10 complexes can create adjacent nicks for gene replacement using homologous DNA through HDR
- Nuclease-deficient dCas9: lost cleavage activity, but still binds to target DNA. By fusing with various effector domains, dCas9 can be fused with various effector domains for gene silencing or activation

CRISPR-Cas9 for Microbial Strain Development

- CRISPR-Cas9 technology is much simpler and faster than other standard methods in any host cells
  - Cas9 and dCas9 function with crRNAs to inactivate multiple copies of a gene of interest
  - Cas9 and dCas9 can operate with a large number of different crRNAs to simultaneously alter multiple targets (alteration to over 60 genes at once has been demonstrated in animal)
- Several companies sell CRISPR-Cas9 kits or reagents for research
- Inscripta released a new, unique CRISPR Gene-Editing Enzyme (MAD7). It is IP and cost free to use for all academic or commercial researchers.

Contract Research Service at Bio-Technical Resources

Scientists at Bio-Technical Resources (BTR) have experiences in applying CRISPR technologies for microbial genetic engineering and strain development. Please contact us for further discussion for your needs and we look forward to working with you.

CRISPR-Cas9 Reference

- www.mirusbio.com
- www.NEB.com
- www.IDT.com
- Nature doi:10.1038/nature.2015.18525