

CRISPR-Cas9: A Microbial Defense System

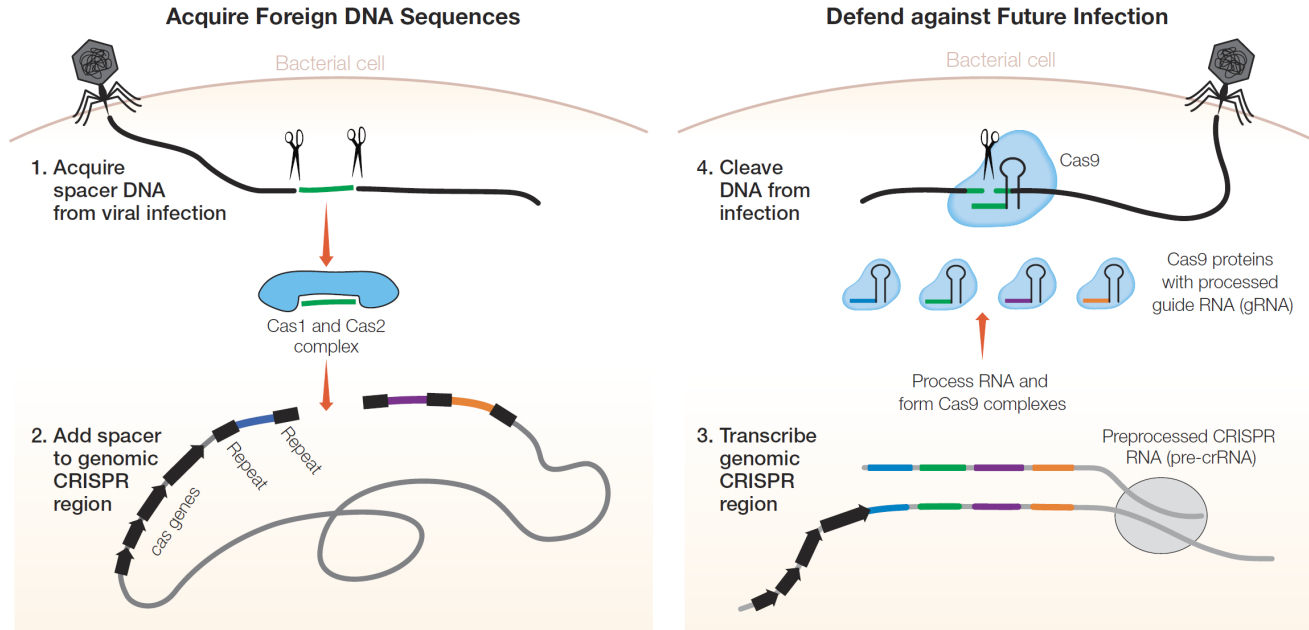
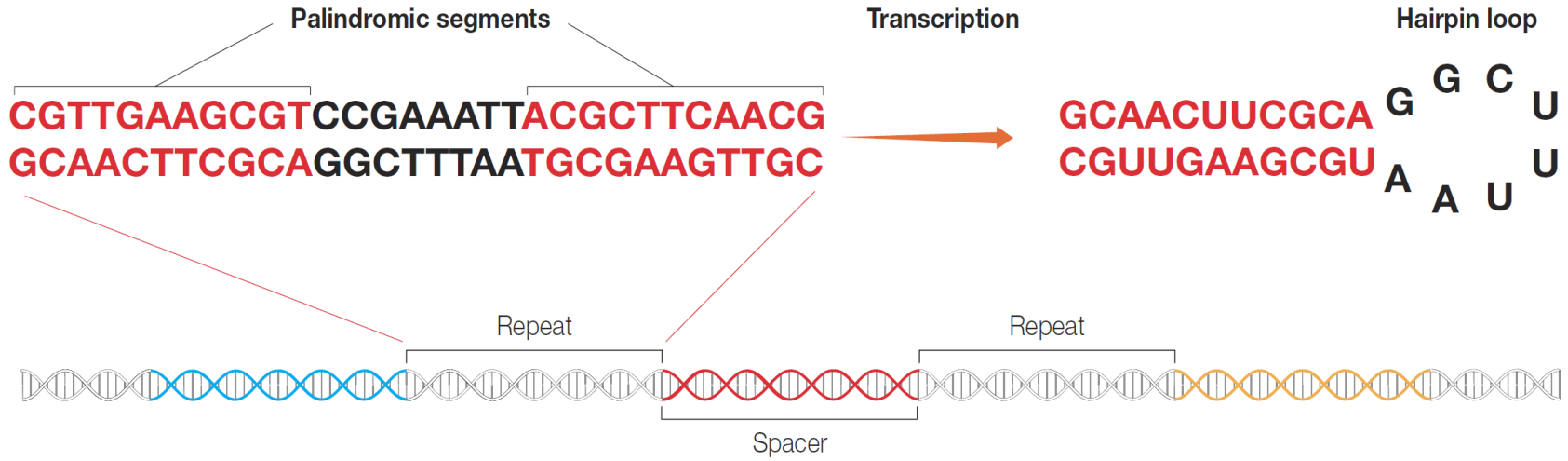
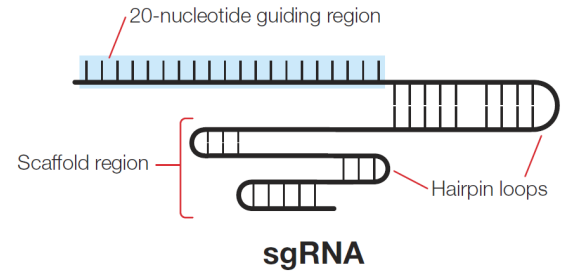
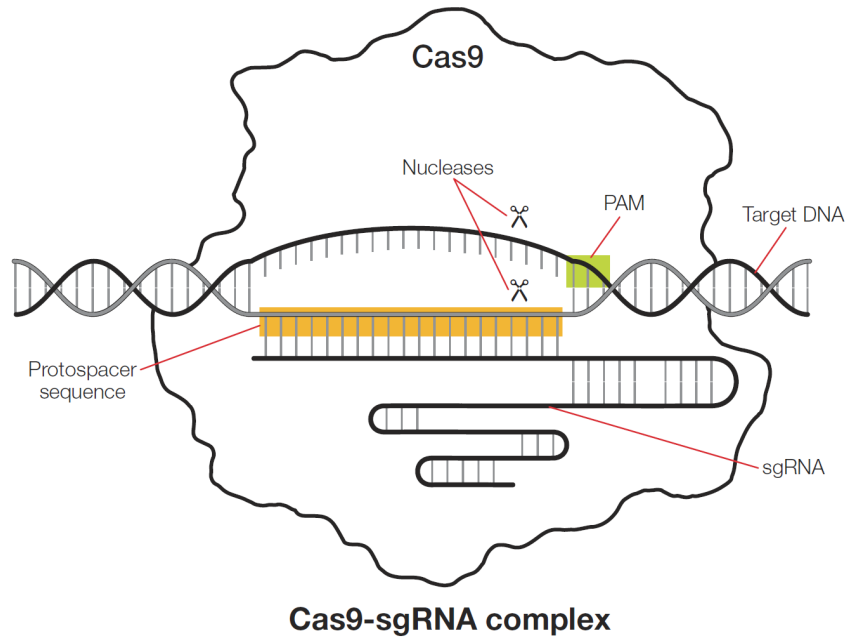


Fig. 4. The CRISPR-Cas9 microbial defense system. **1**, the Cas1-Cas2 enzymes of the microbe recognize foreign DNA and cut out a segment. **2**, the Cas1-Cas2 enzymes insert the DNA segment into the CRISPR region of its own genome as a spacer. **3**, a spacer sequence is transcribed and then linked to a Cas9 protein. **4**, upon reinfection by the same invader, the CRISPR-Cas9 complex can recognize the foreign DNA sequence and cut it to prevent complete infection.

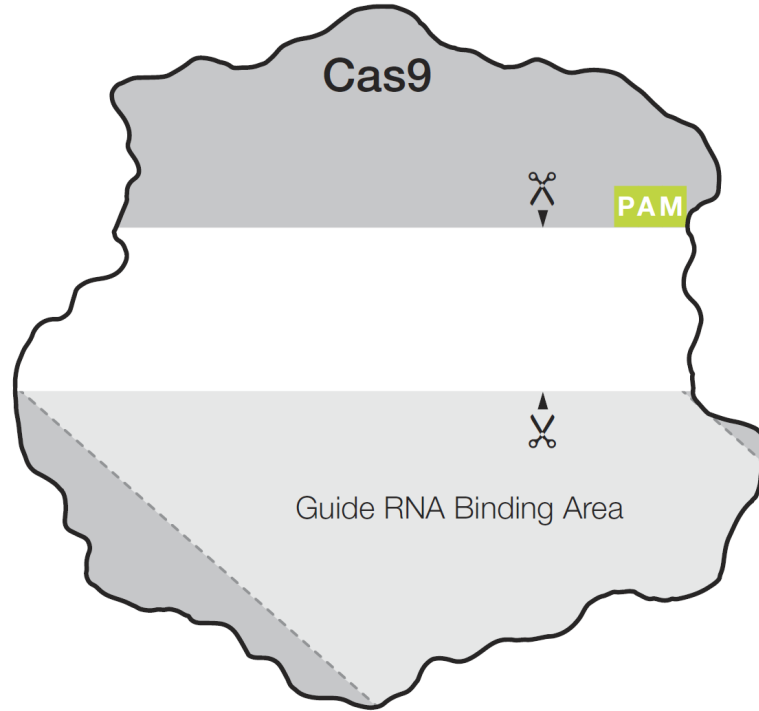
CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats



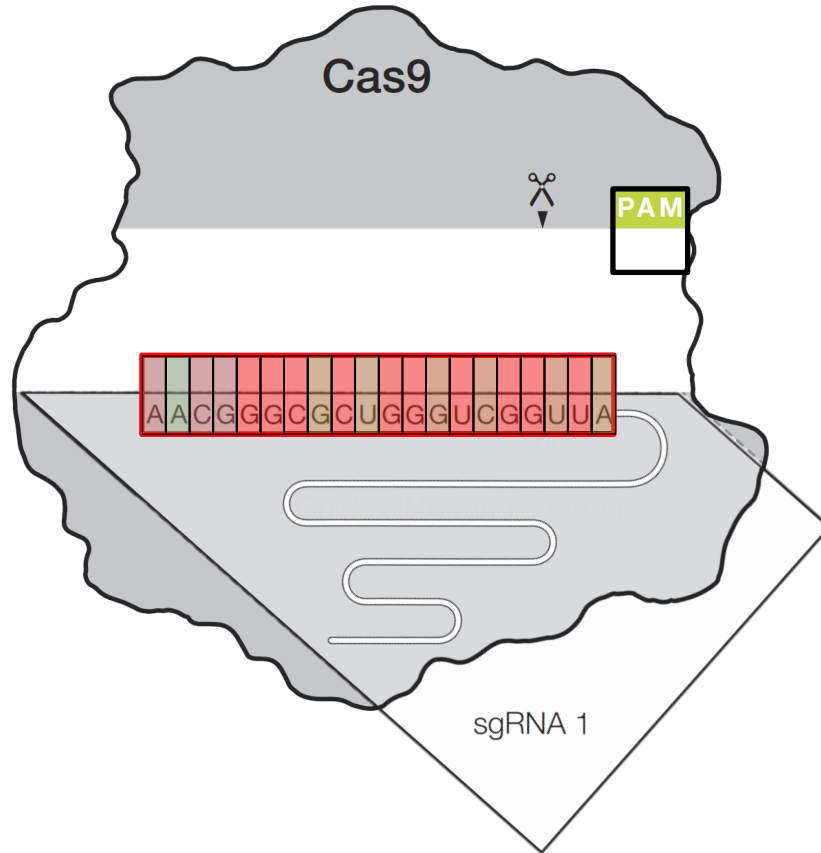
CRISPR+Cas9: Targeted Cutting of DNA



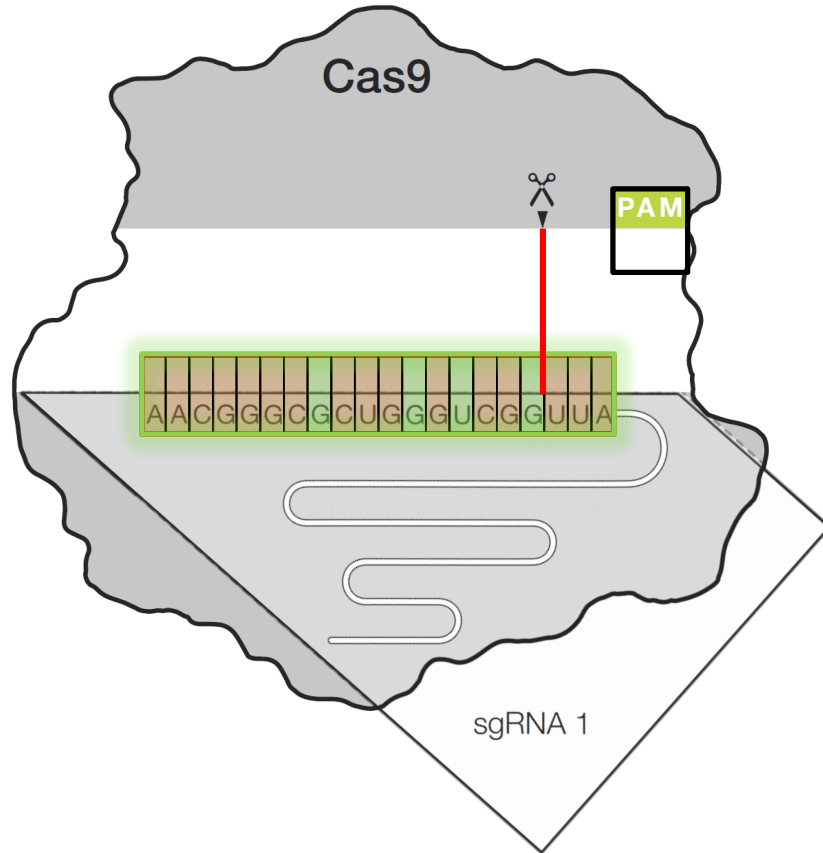
Activity 1.1



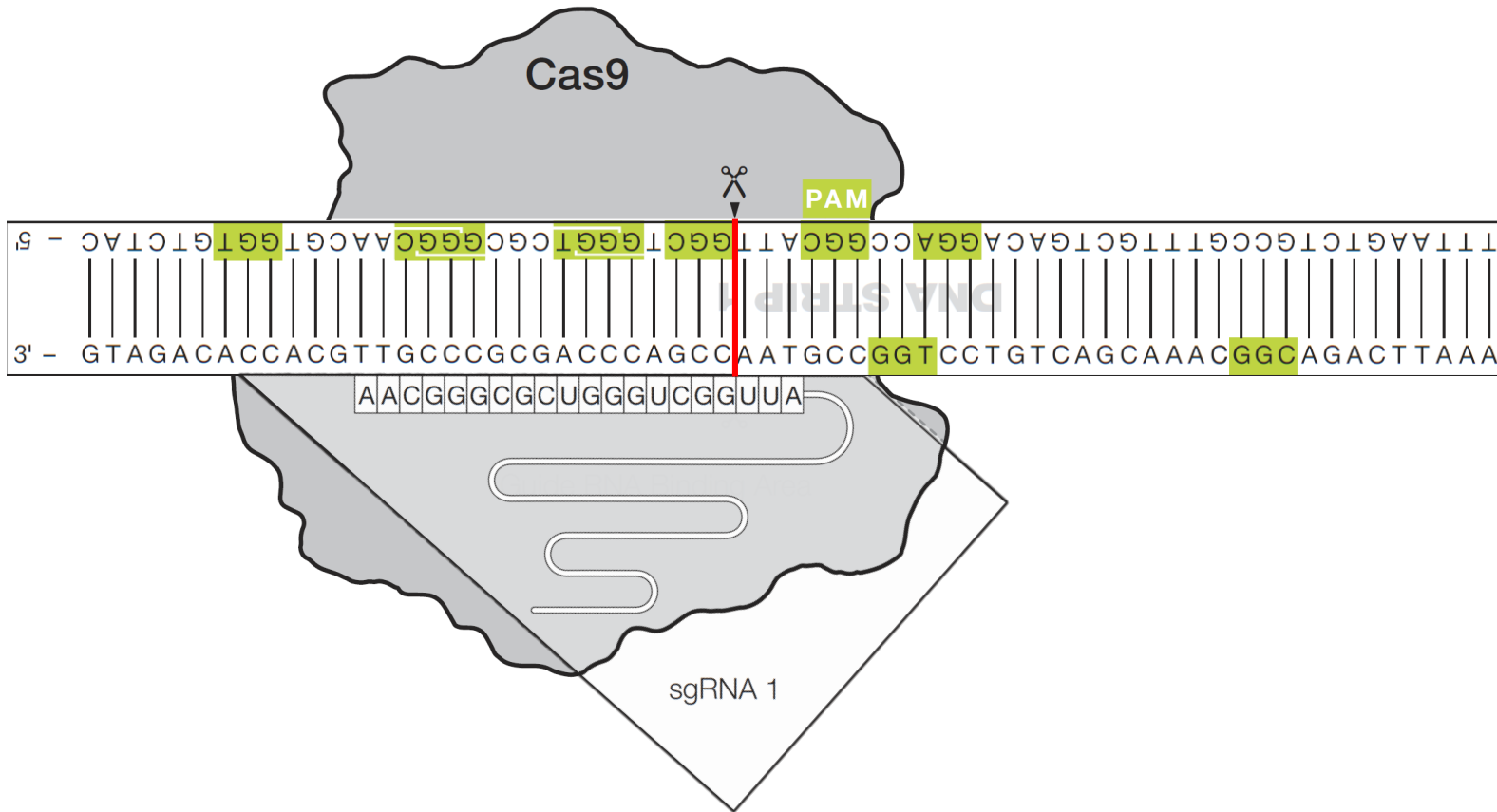
Activity 1.1



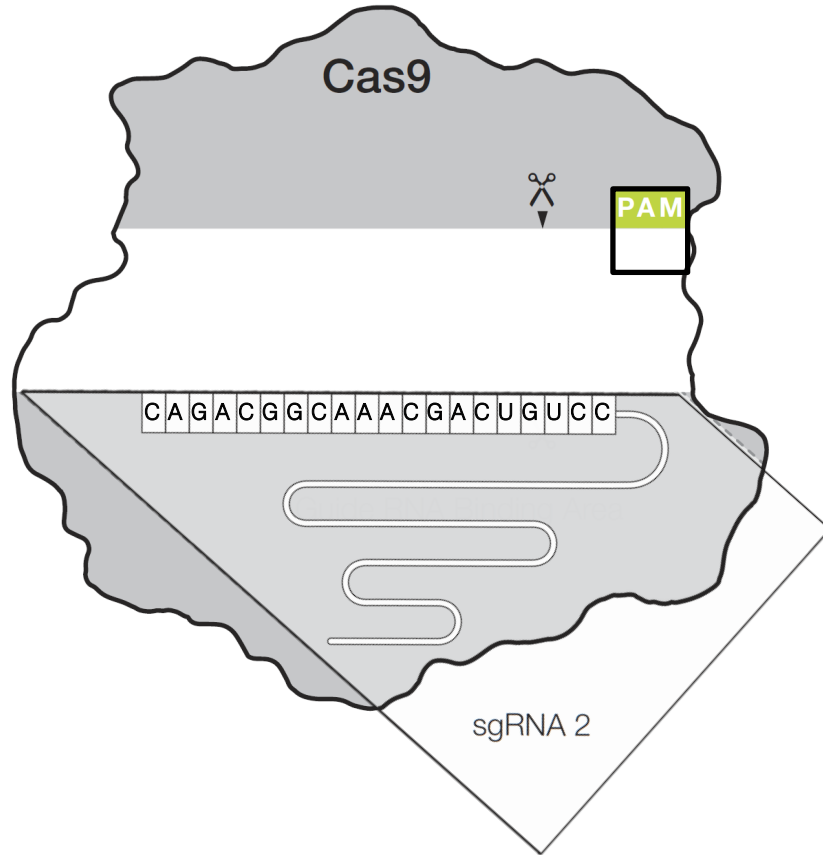
Activity 1.1



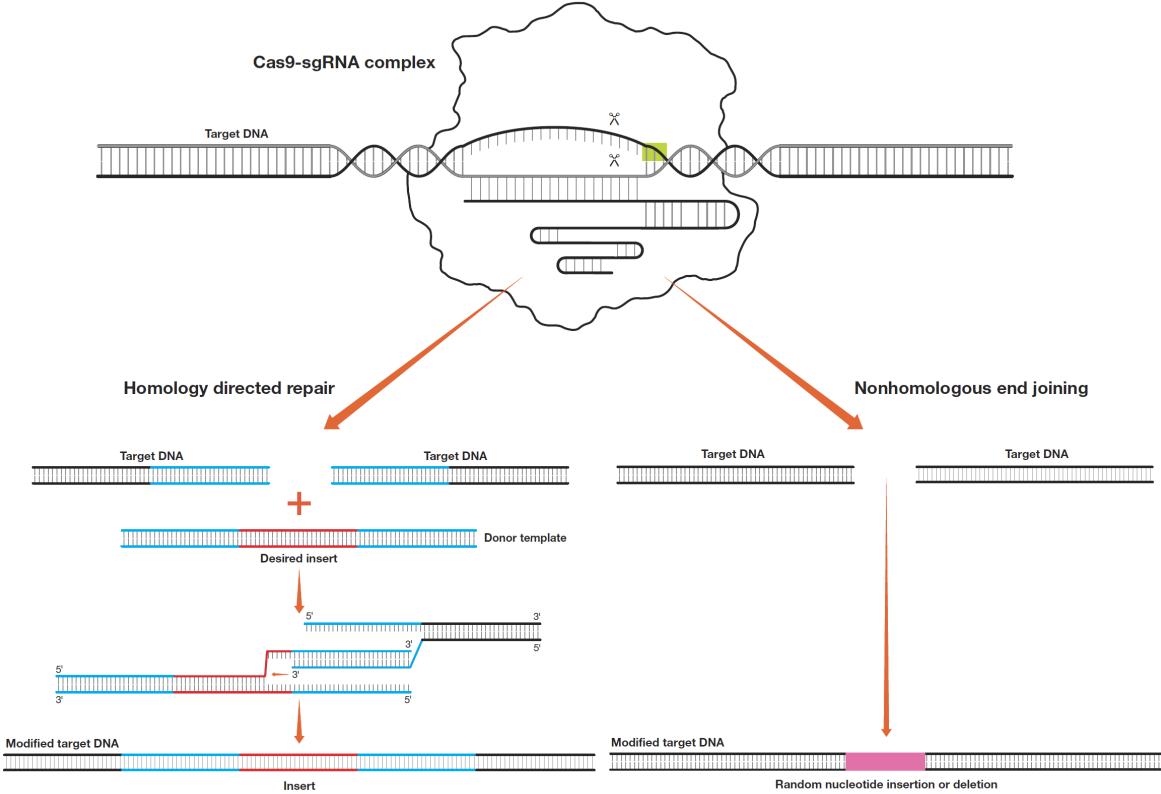
Activity 1.1



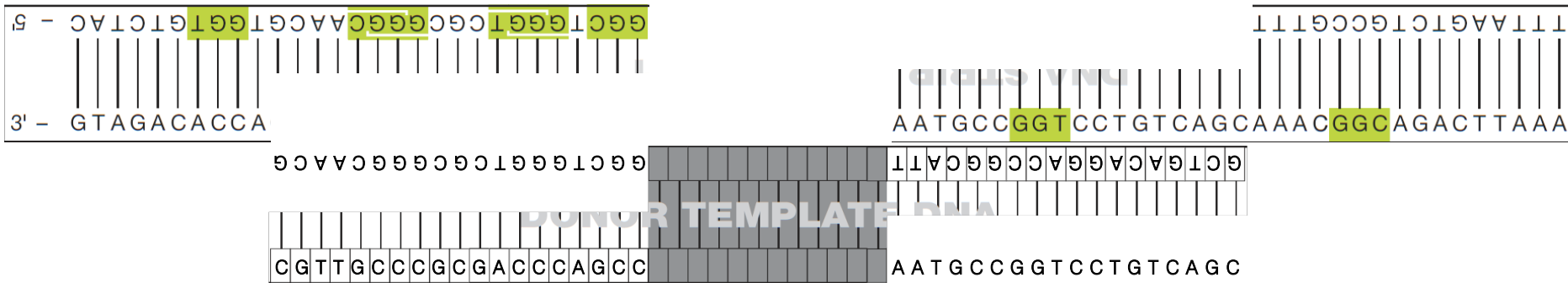
Activity 1.2



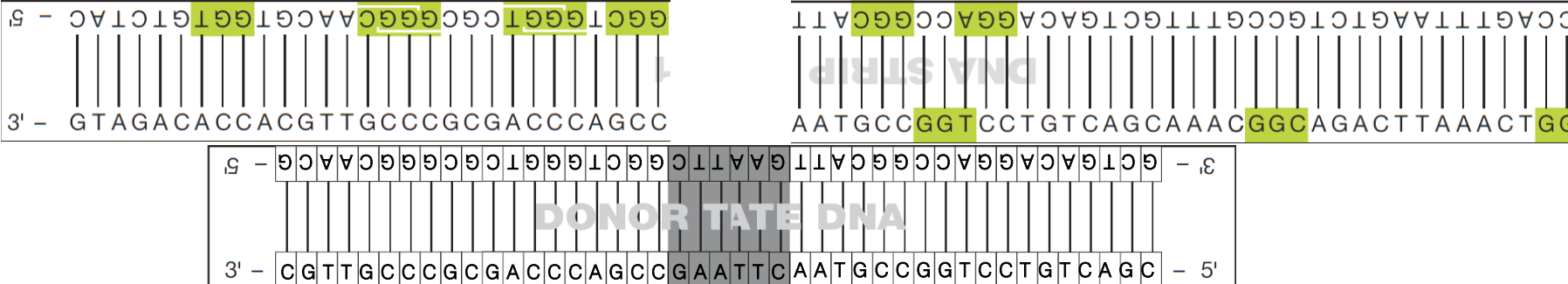
DNA Repair: HDR vs NHEJ



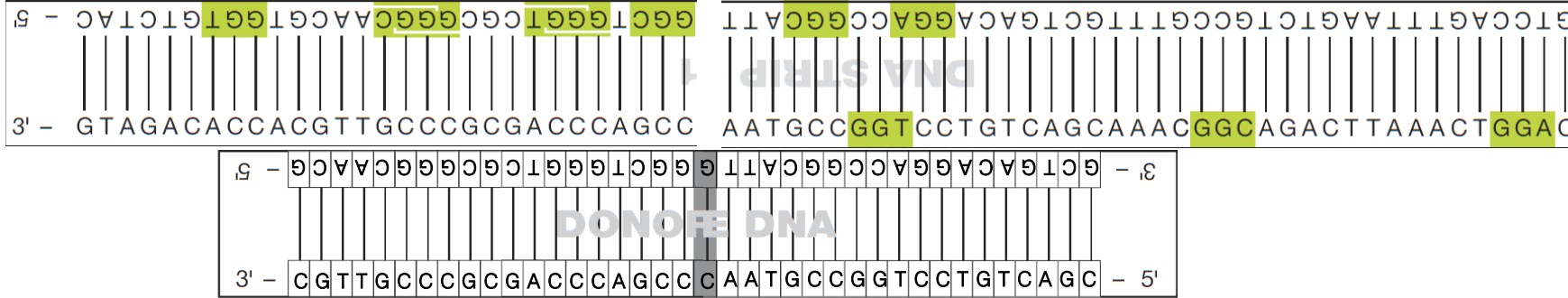
Activity 1.4.1



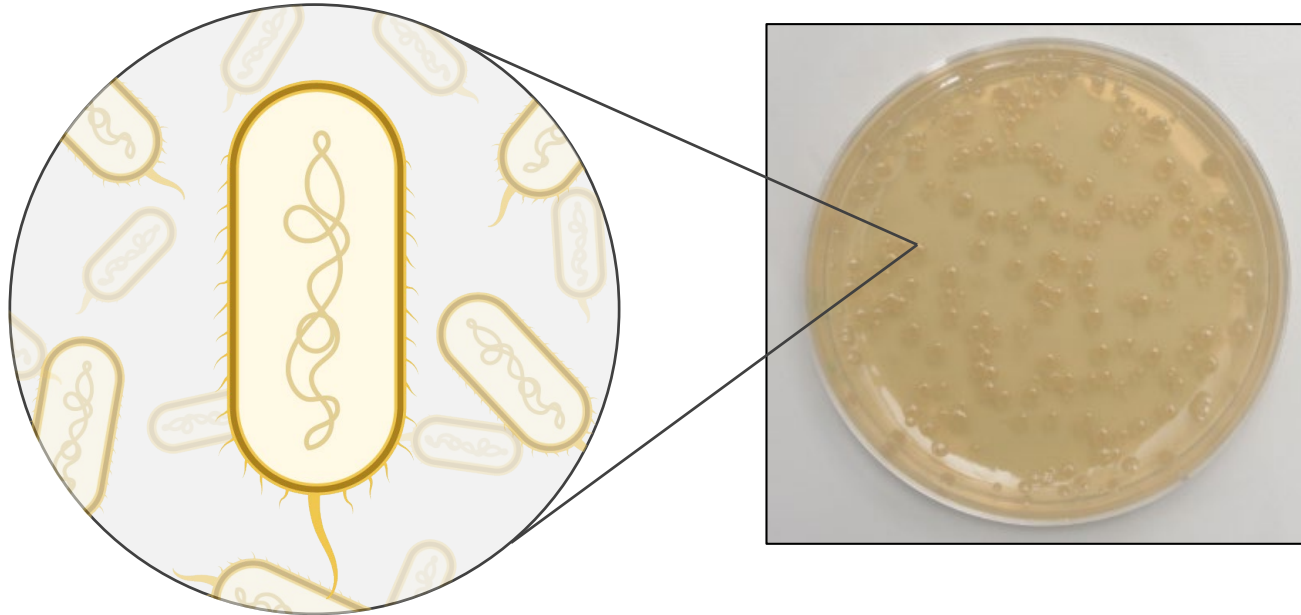
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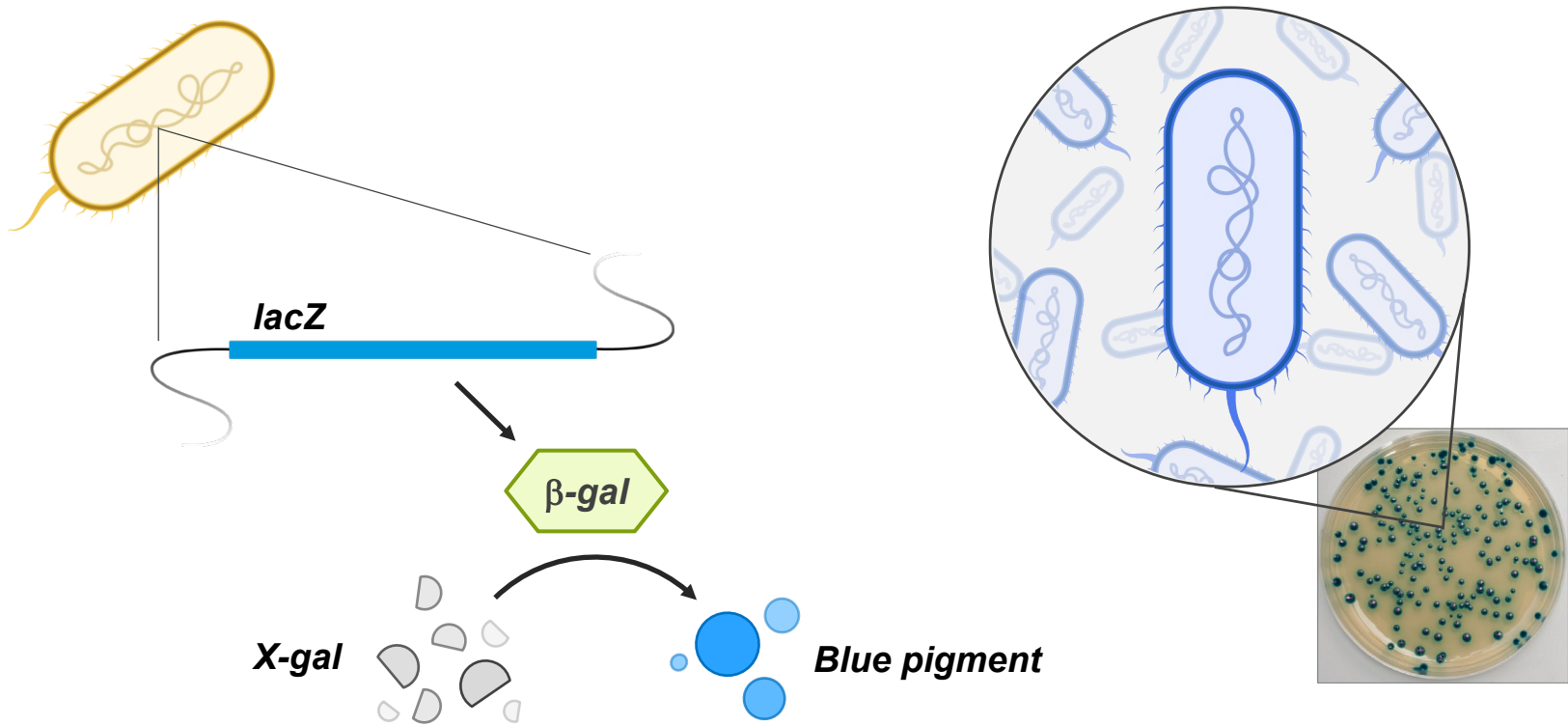


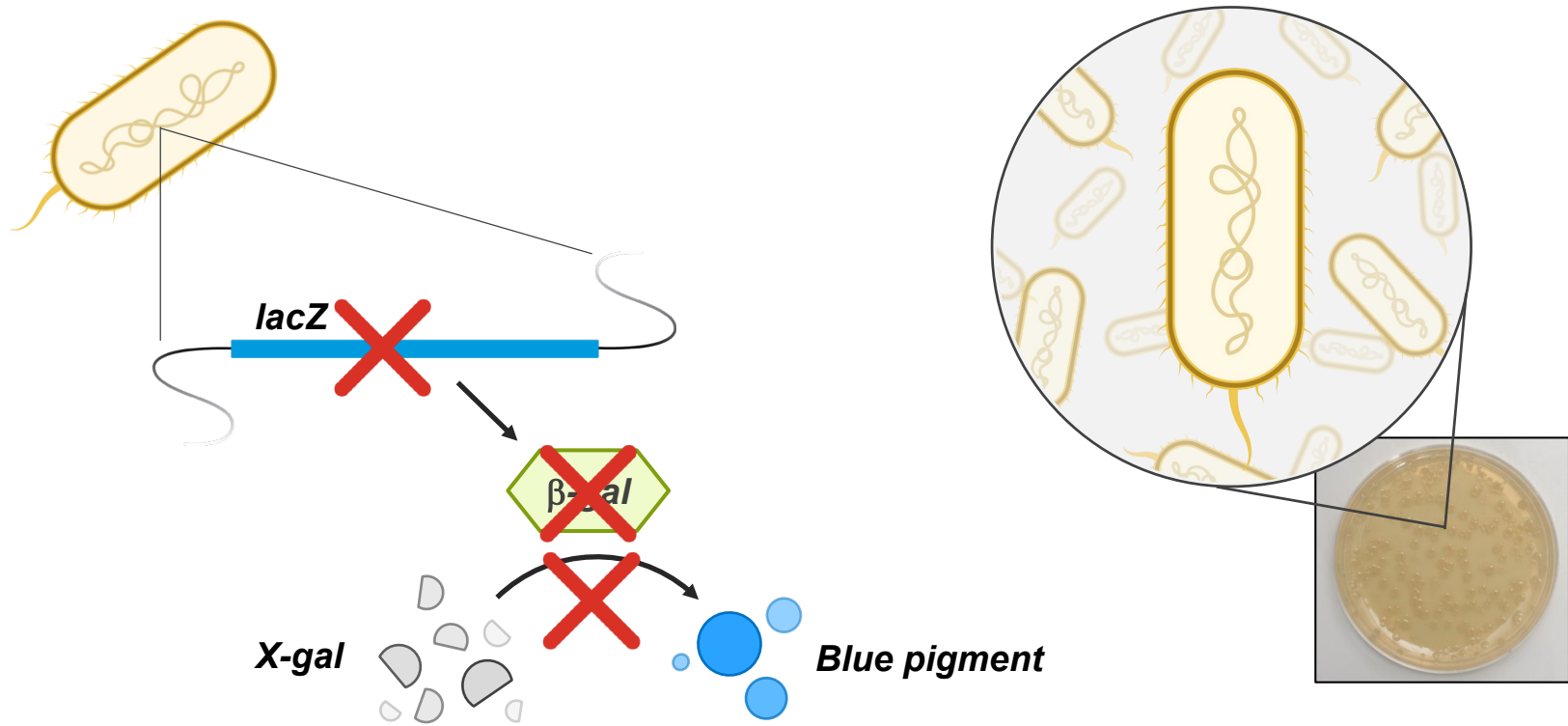
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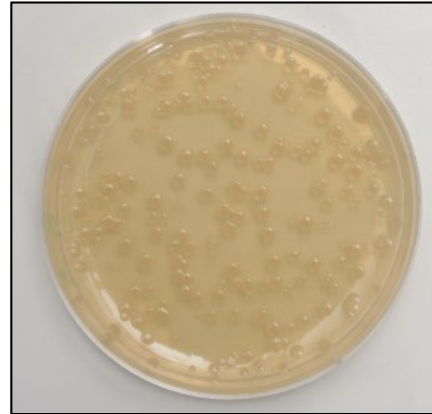
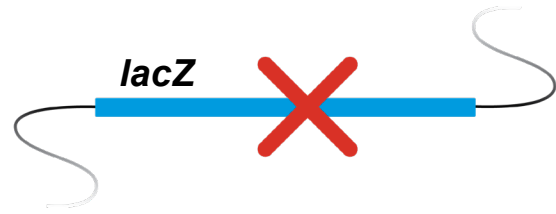
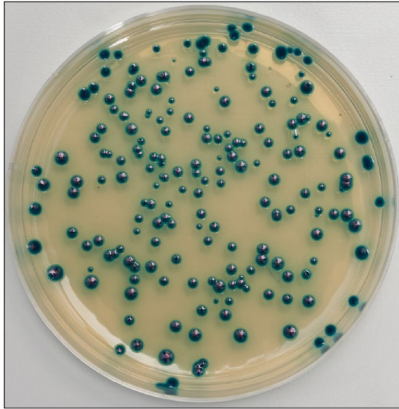


Wild type *E. coli*





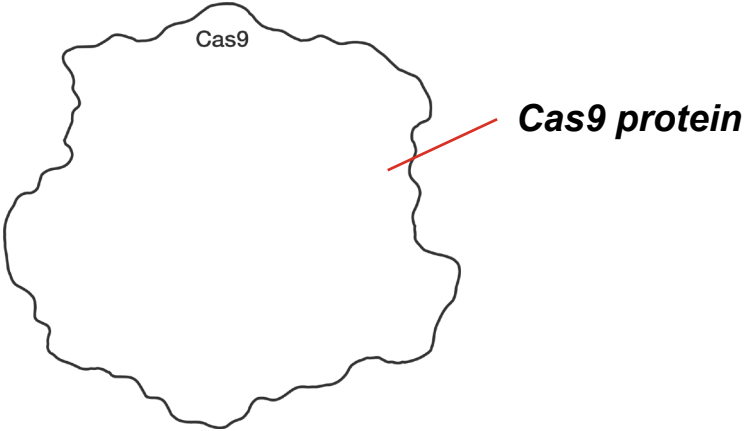




CRISPR-Cas9 Gene Editing

Cut

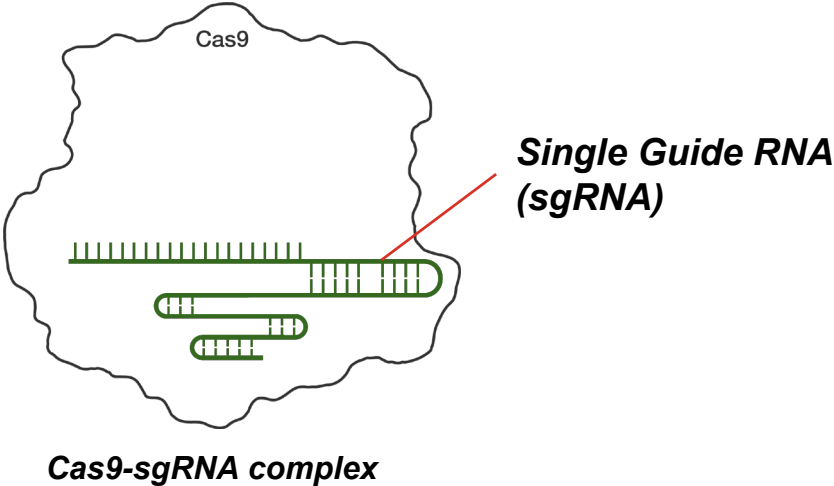
Repair



CRISPR-Cas9 Gene Editing

Cut

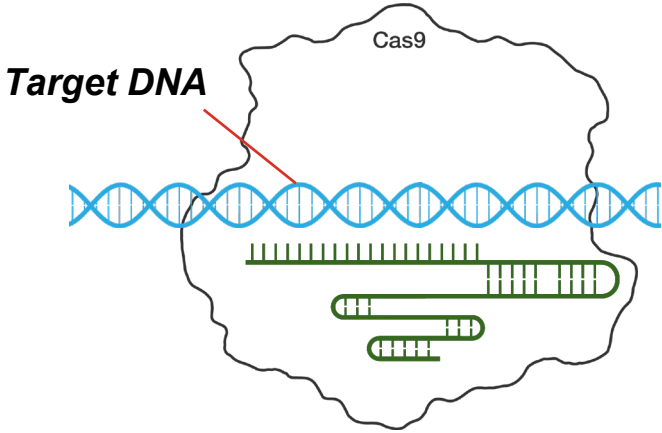
Repair



CRISPR-Cas9 Gene Editing

Cut

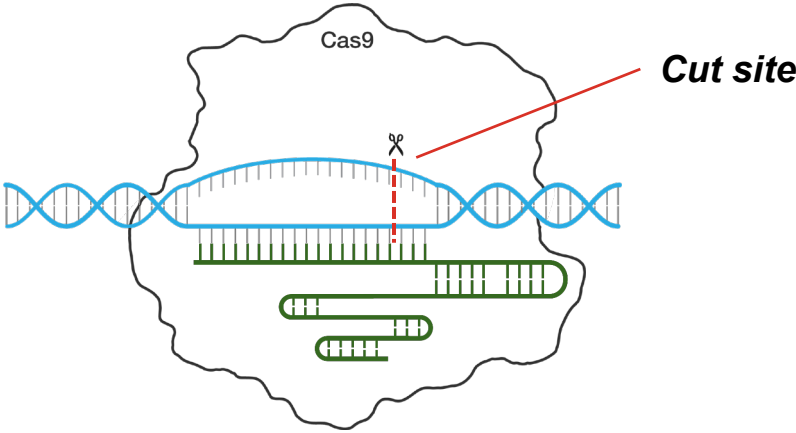
Repair



CRISPR-Cas9 Gene Editing

Cut

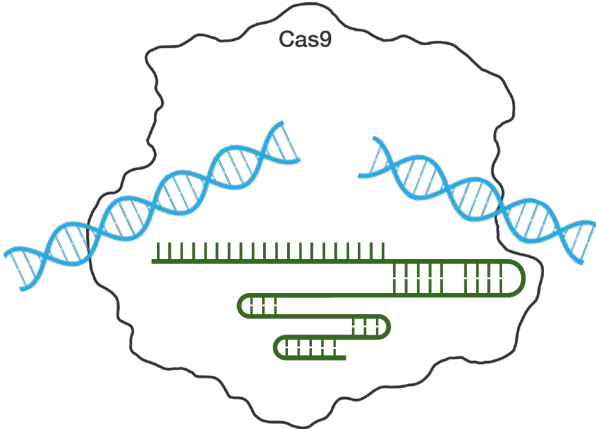
Repair



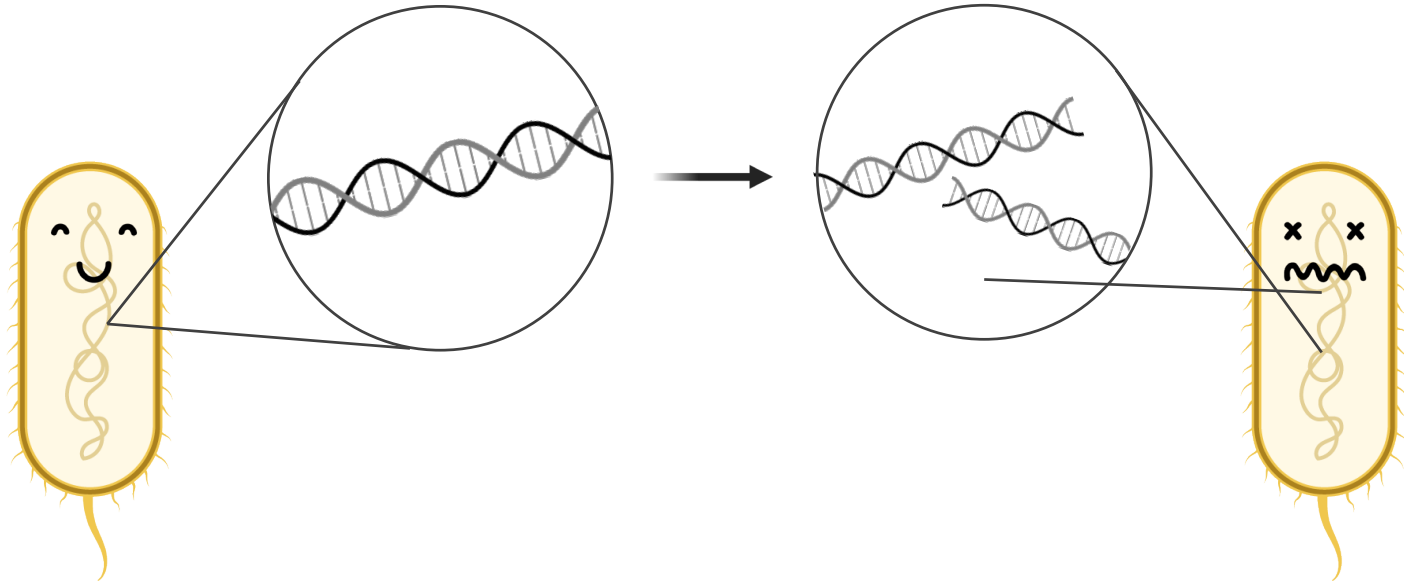
CRISPR-Cas9 Gene Editing

Cut

Repair



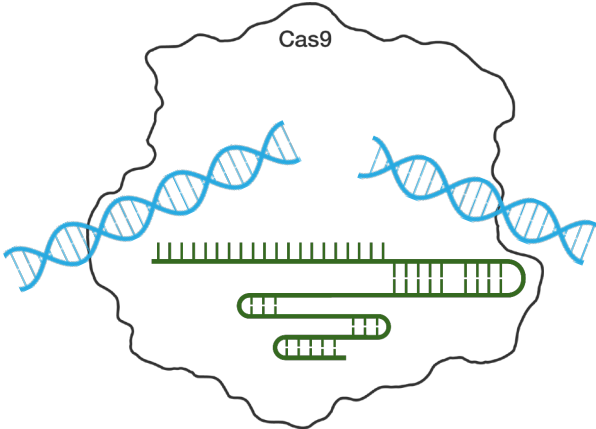
Lethal Double-Stranded Breaks



CRISPR-Cas9 Gene Editing

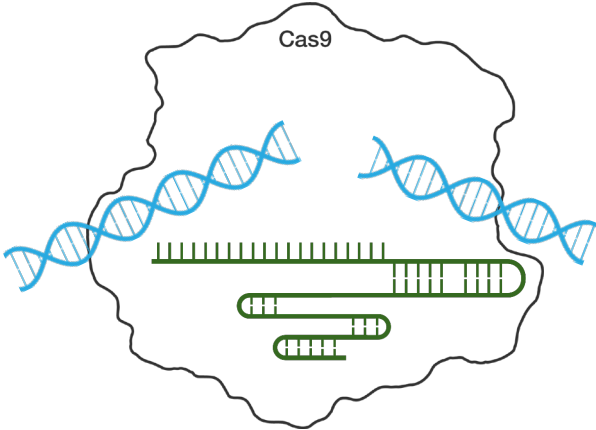
Cut

Repair



CRISPR-Cas9 Gene Editing

Cut

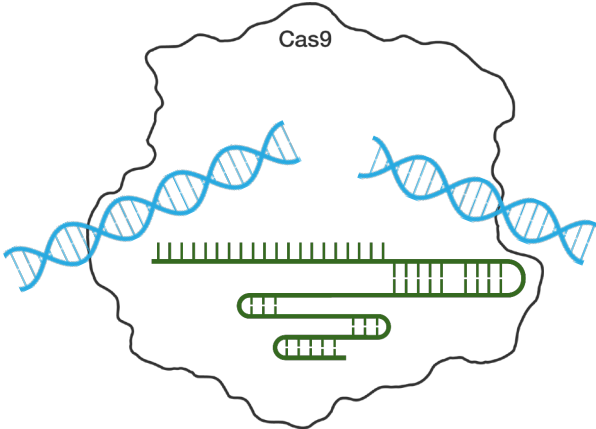


Repair

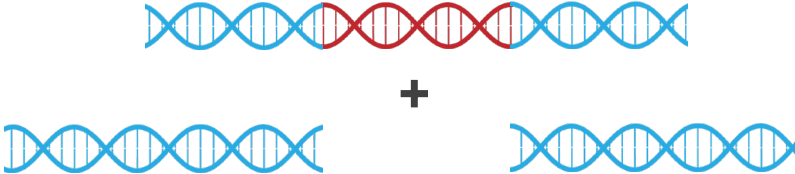


CRISPR-Cas9 Gene Editing

Cut

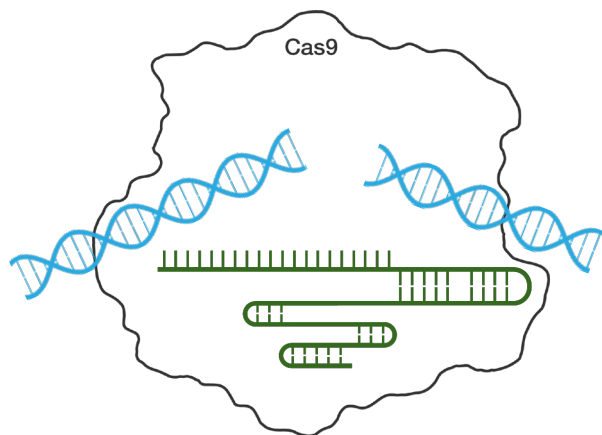


Repair

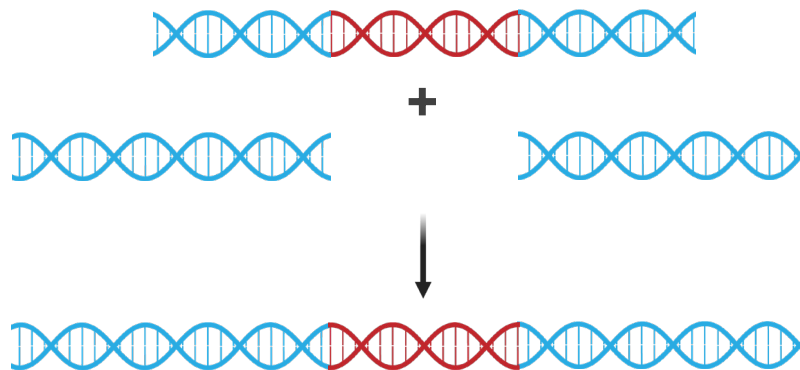


CRISPR-Cas9 Gene Editing

Cut



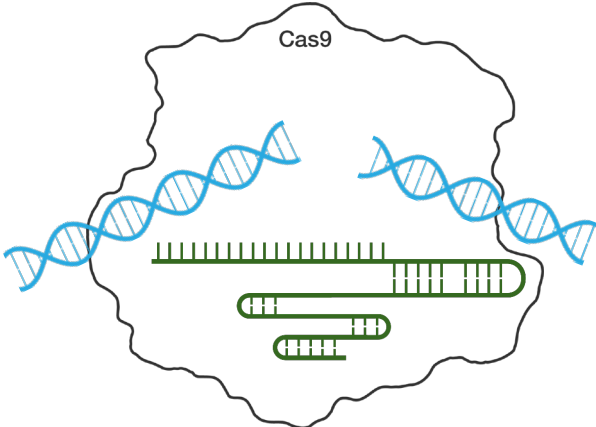
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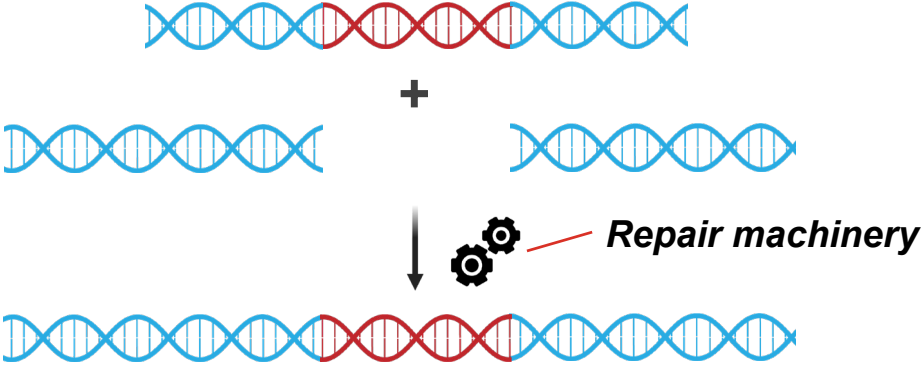
Homology directed repair

CRISPR-Cas9 Gene Editing

Cut

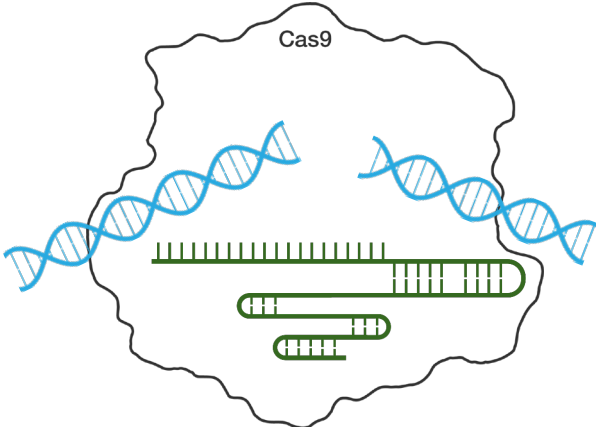


Repair

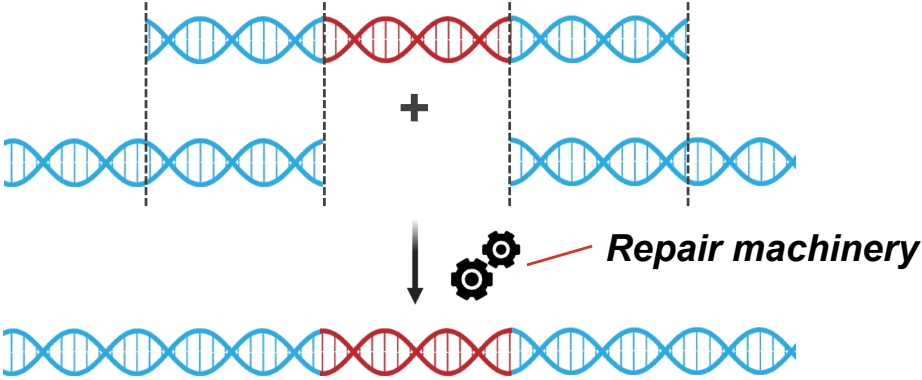


CRISPR-Cas9 Gene Editing

Cut



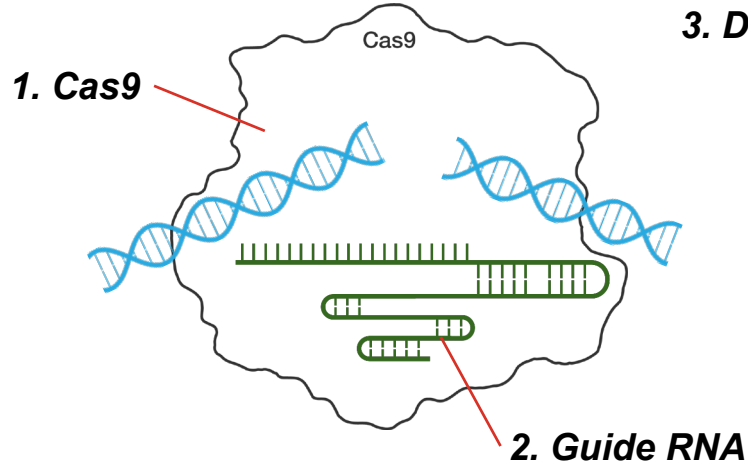
Repair



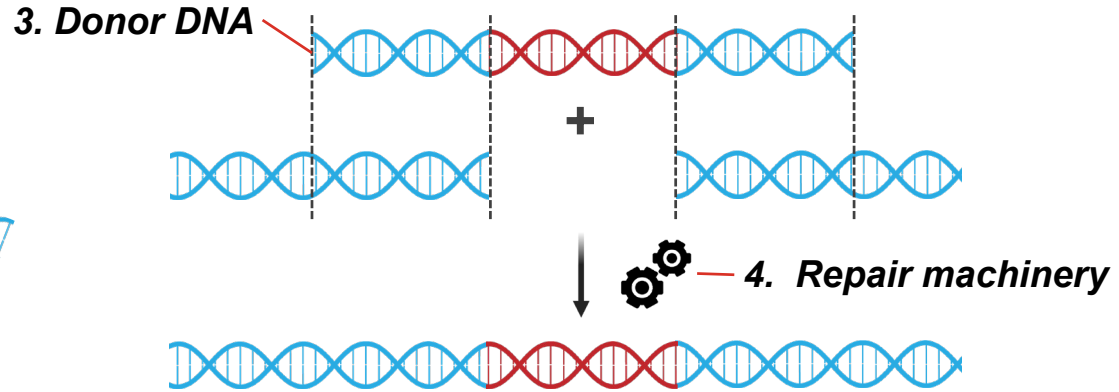
Homology directed repair

CRISPR-Cas9 Gene Editing

Cut

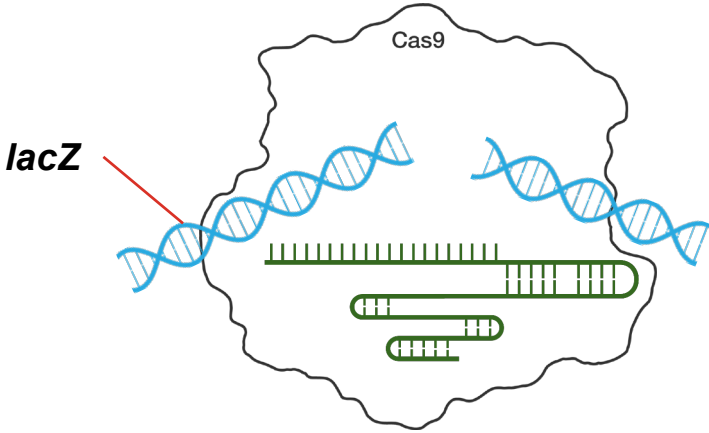


Repair

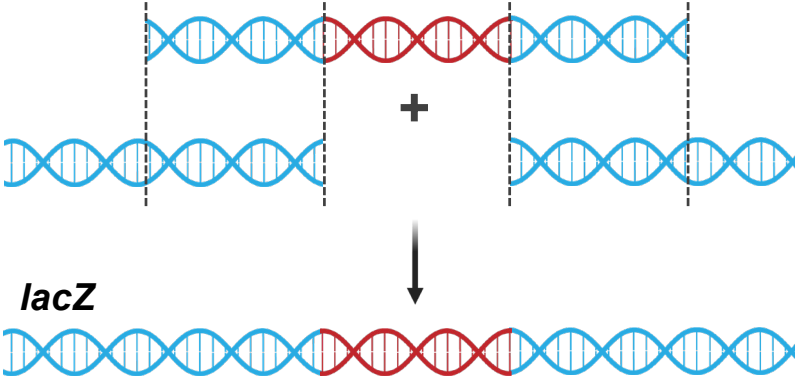


CRISPR-Cas9 Gene Editing

Cut



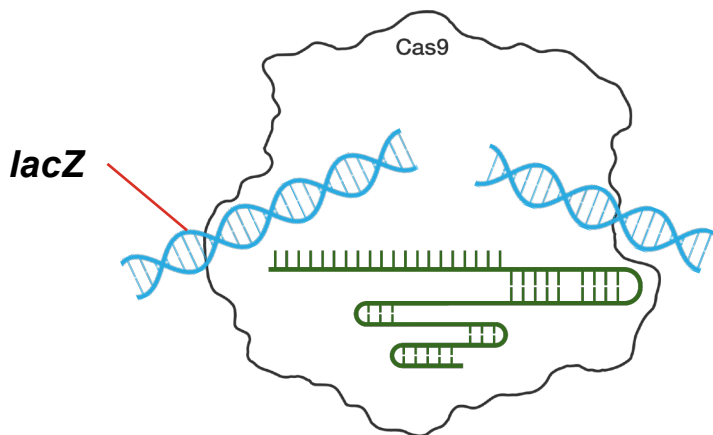
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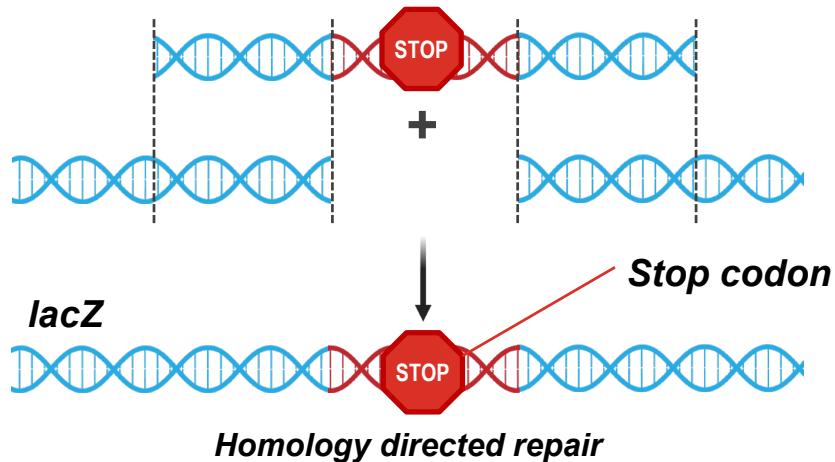
Homology directed repair

CRISPR-Cas9 Gene Editing

Cut

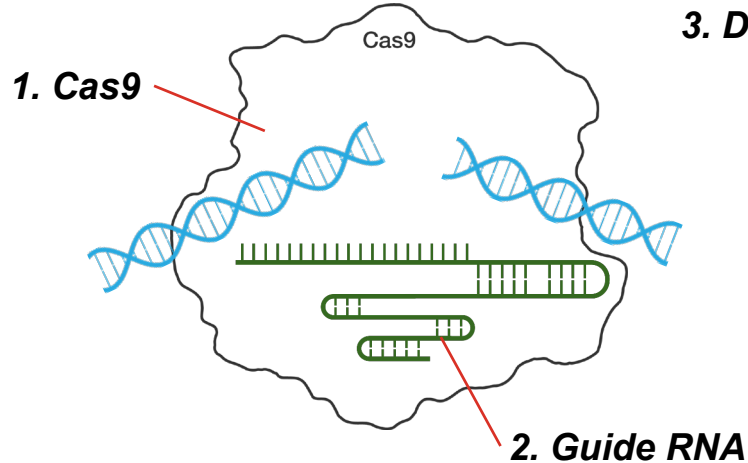


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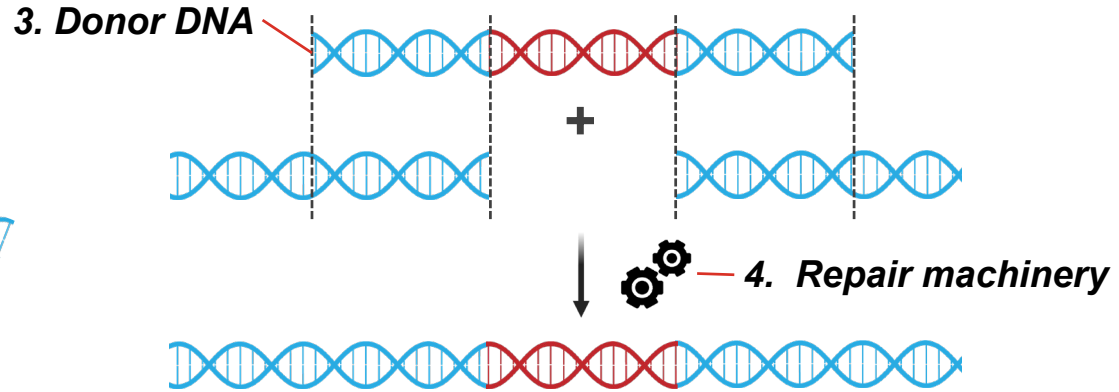


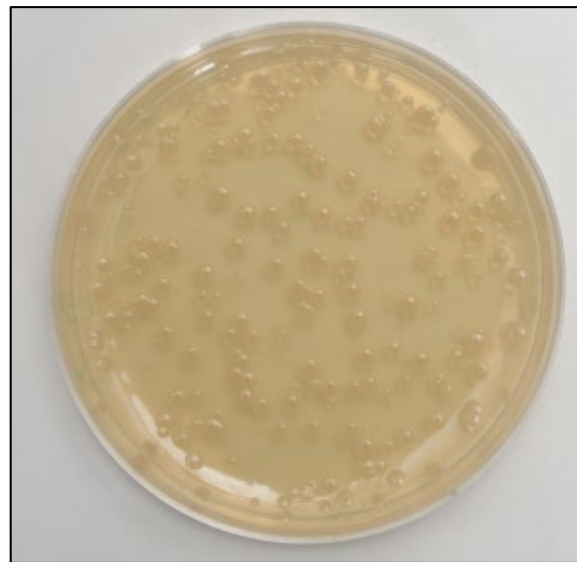
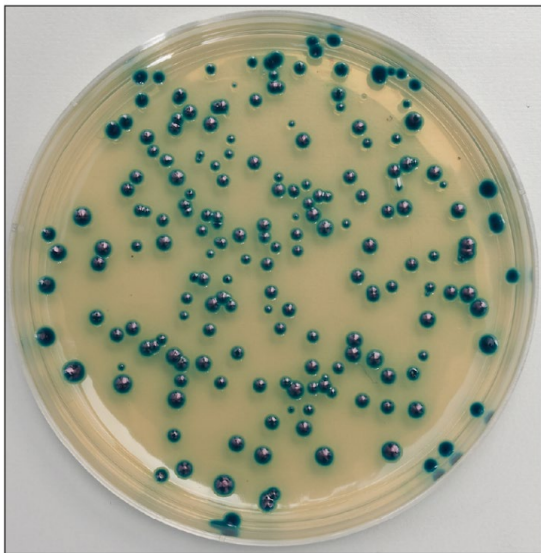
CRISPR-Cas9 Gene Editing

Cut



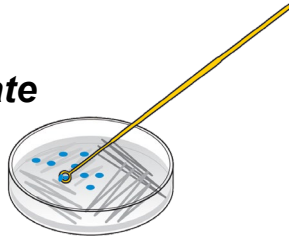
Repair





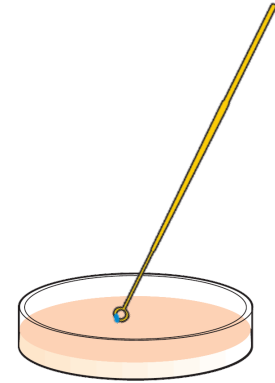
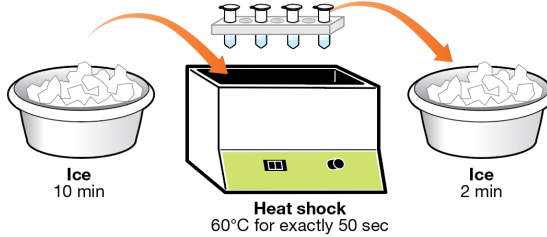
Protocol Overview

**Starter plate
bacteria**

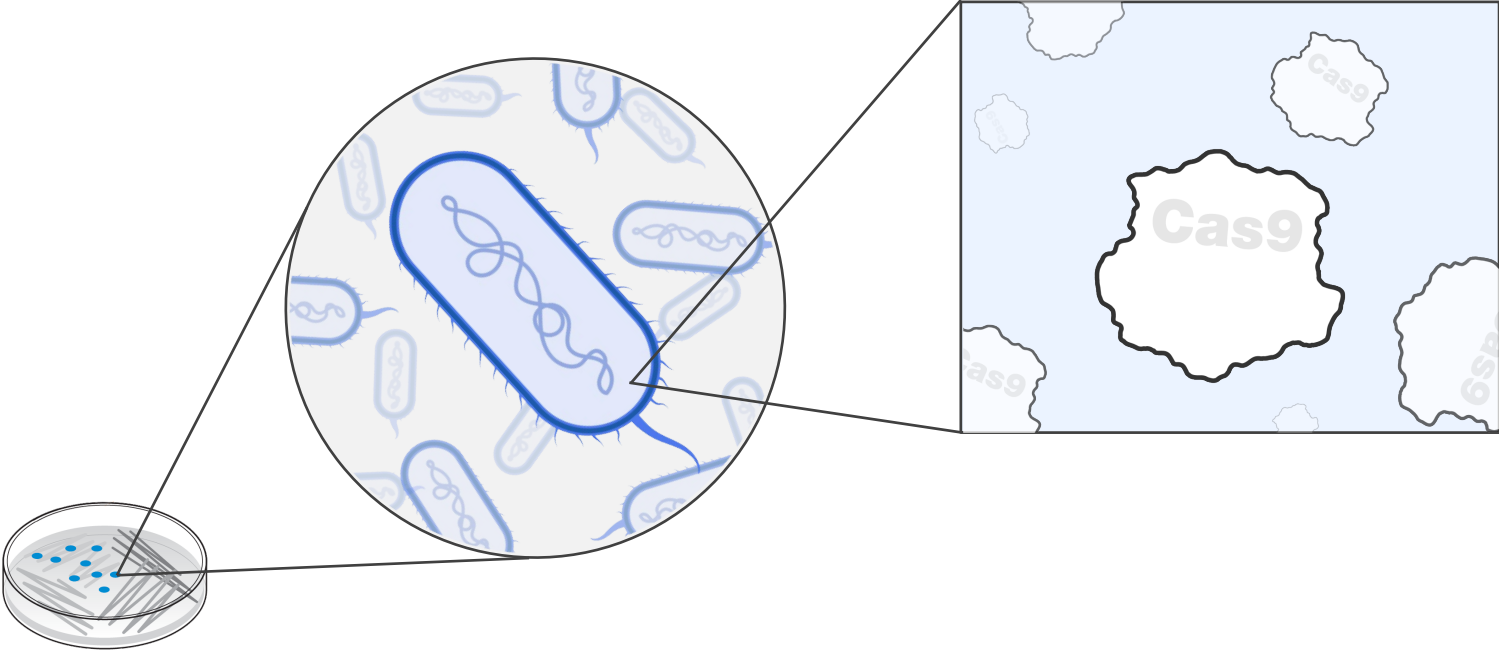


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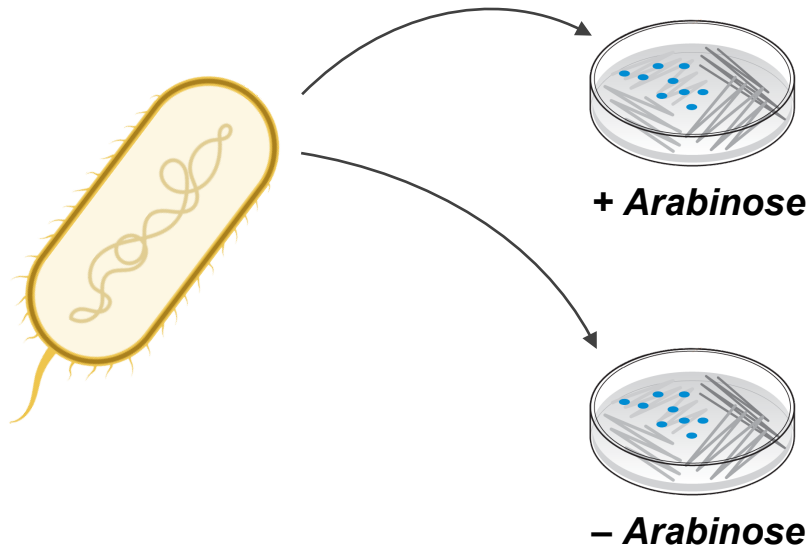
Plasmid



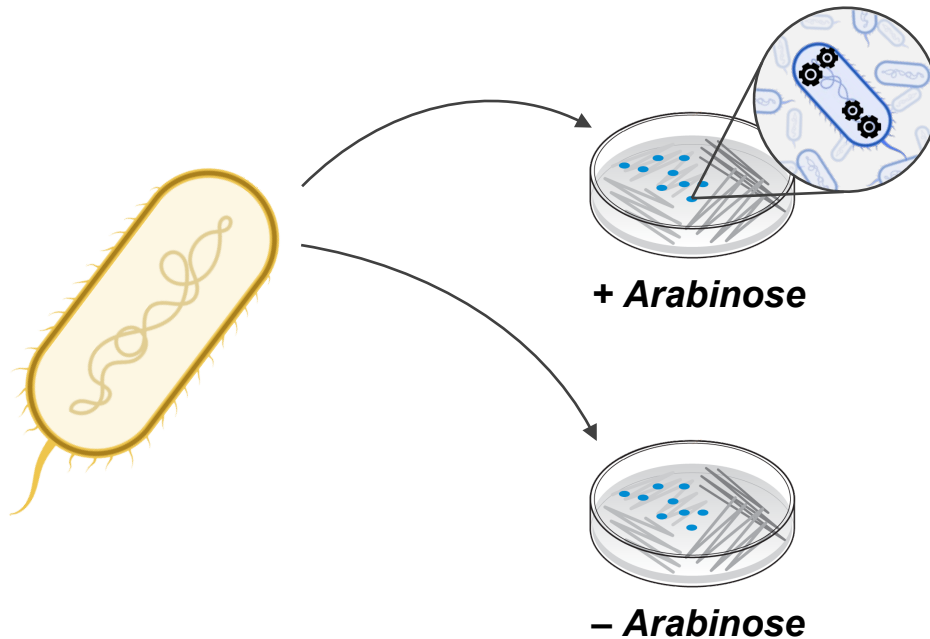
Cas9 expression



Arabinose Control of Repair Machinery



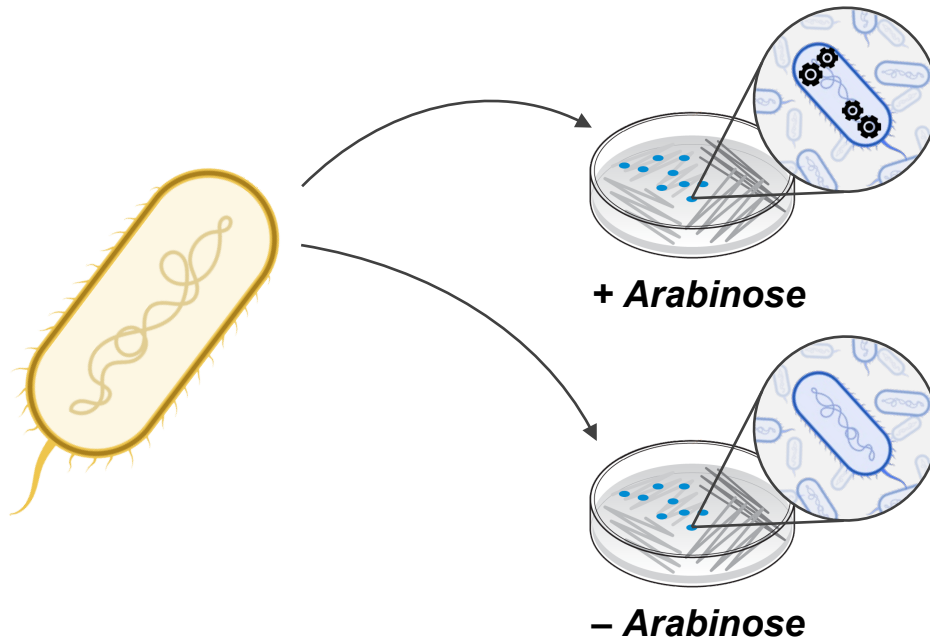
Arabinose Control of Repair Machinery



Repair machinery ON



Arabinose Control of Repair Machinery



Repair machinery ON



Repair machinery OFF



Available Plasmids

pLZDonorGuide
(pDG)



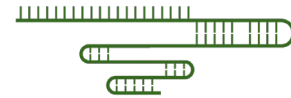
pLZDonor
(pD)

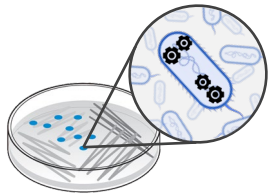

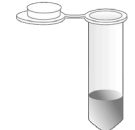
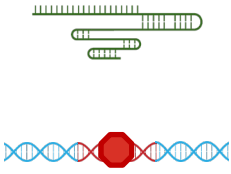


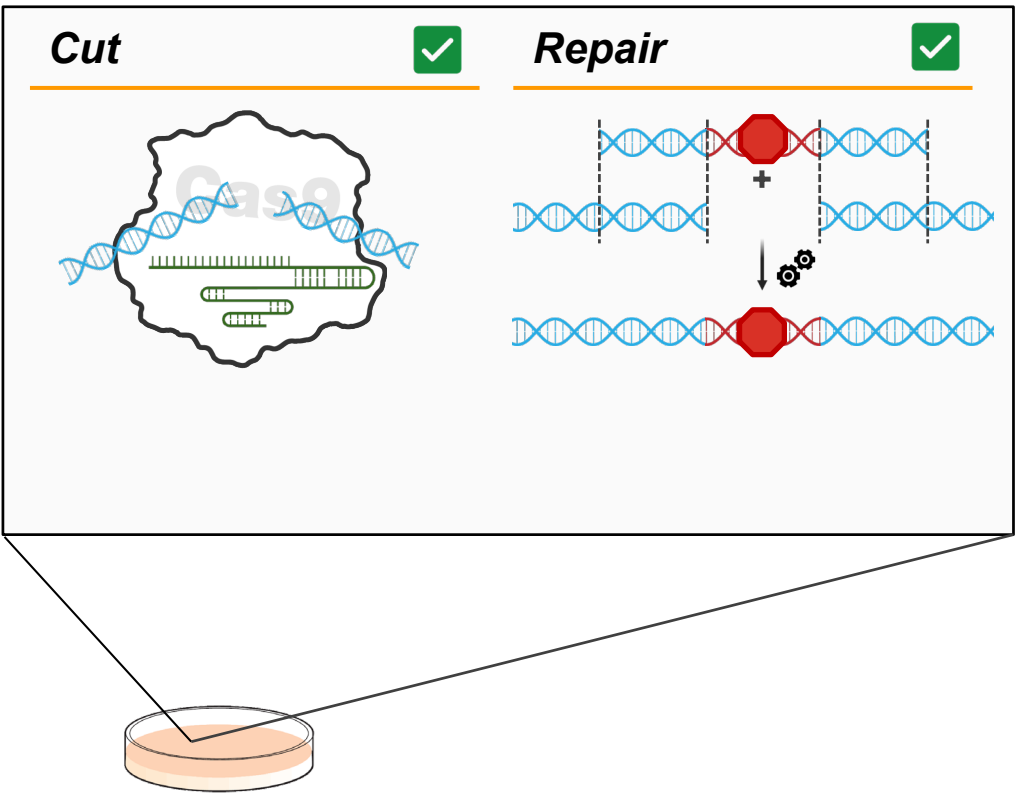
Donor DNA



sgRNA



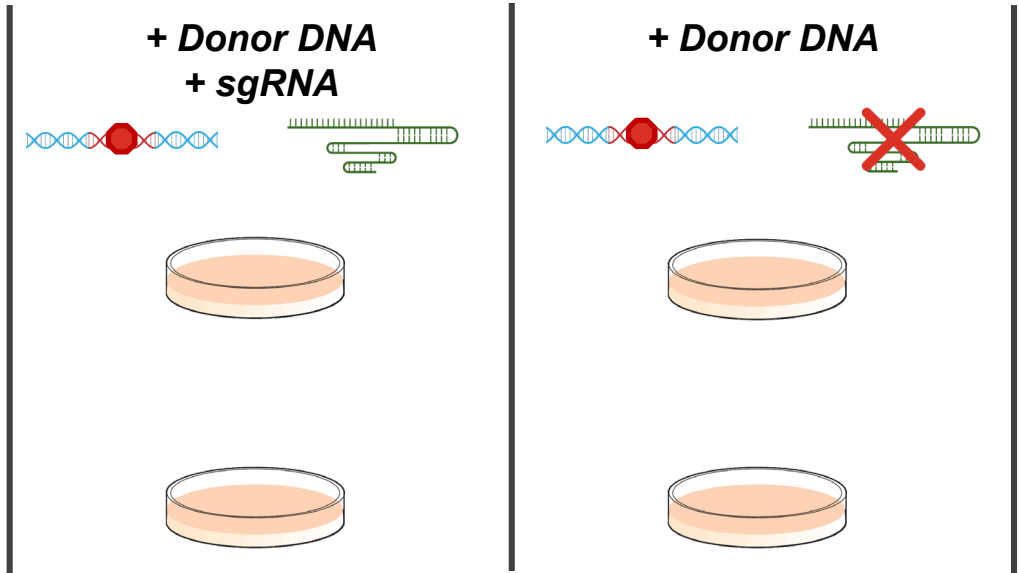
Source	Component
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 <p data-bbox="154 775 425 829">pDG plasmid</p>	



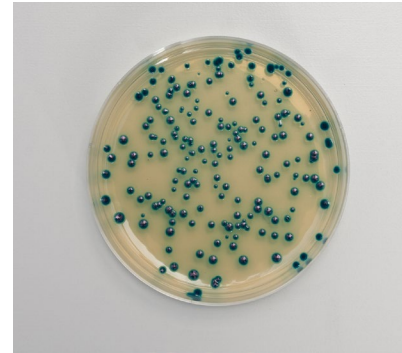
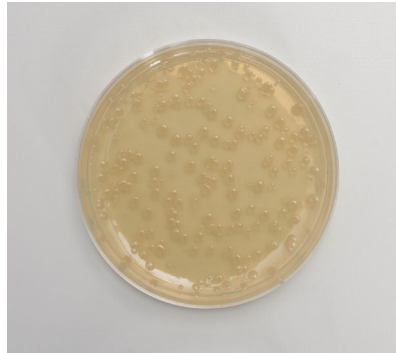
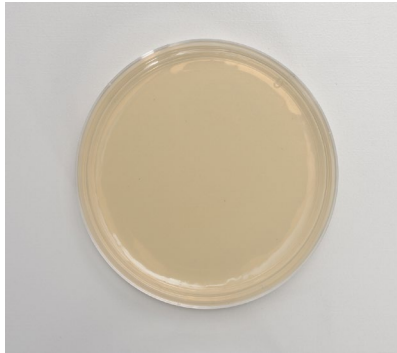
Experimental setup

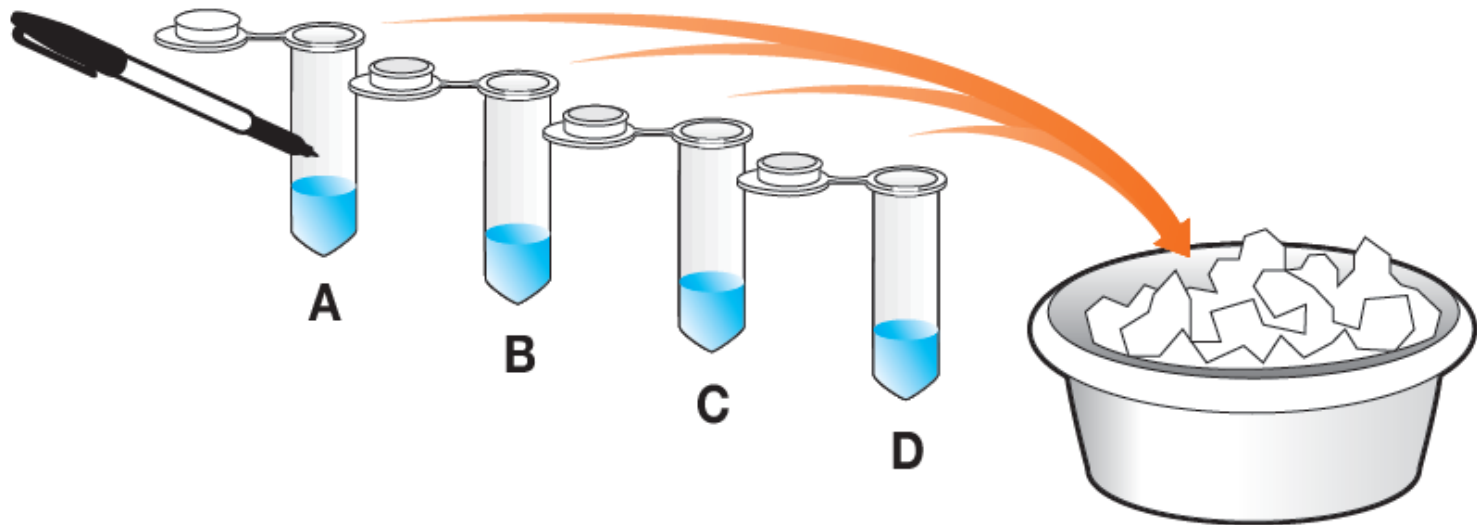
+ *Repair machinery* 

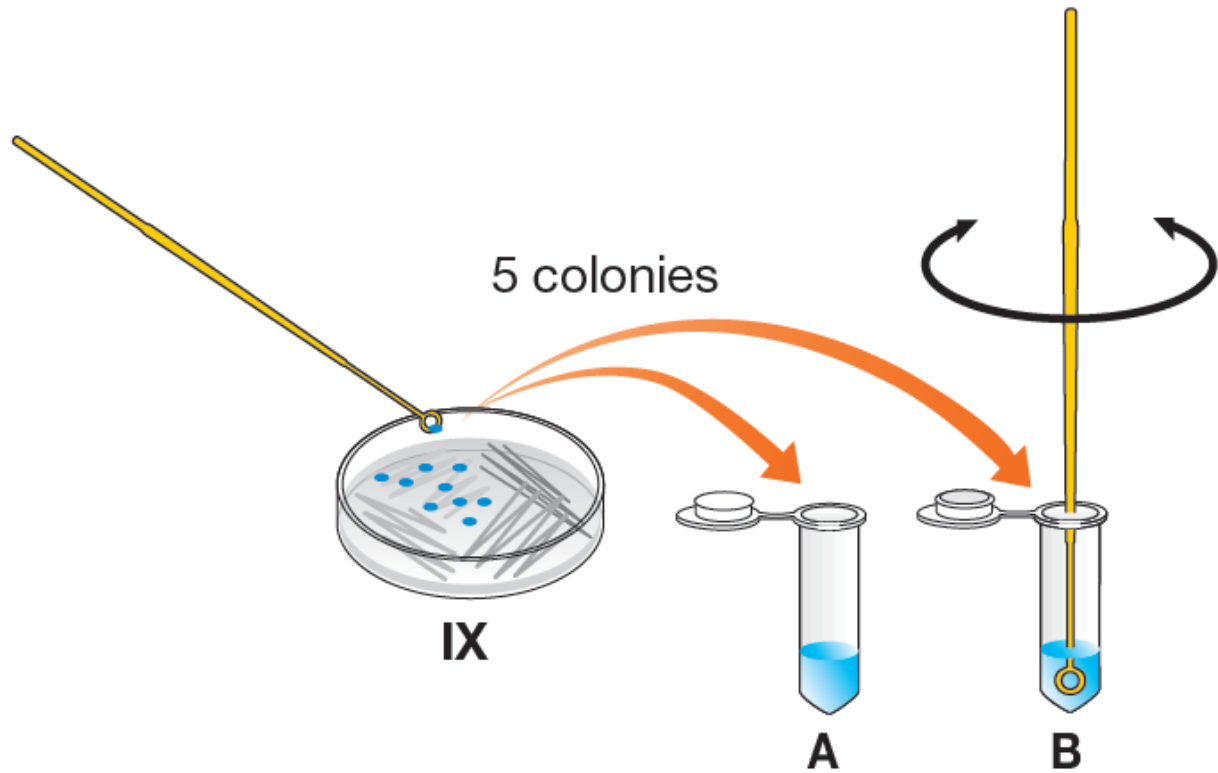
- *Repair machinery* 

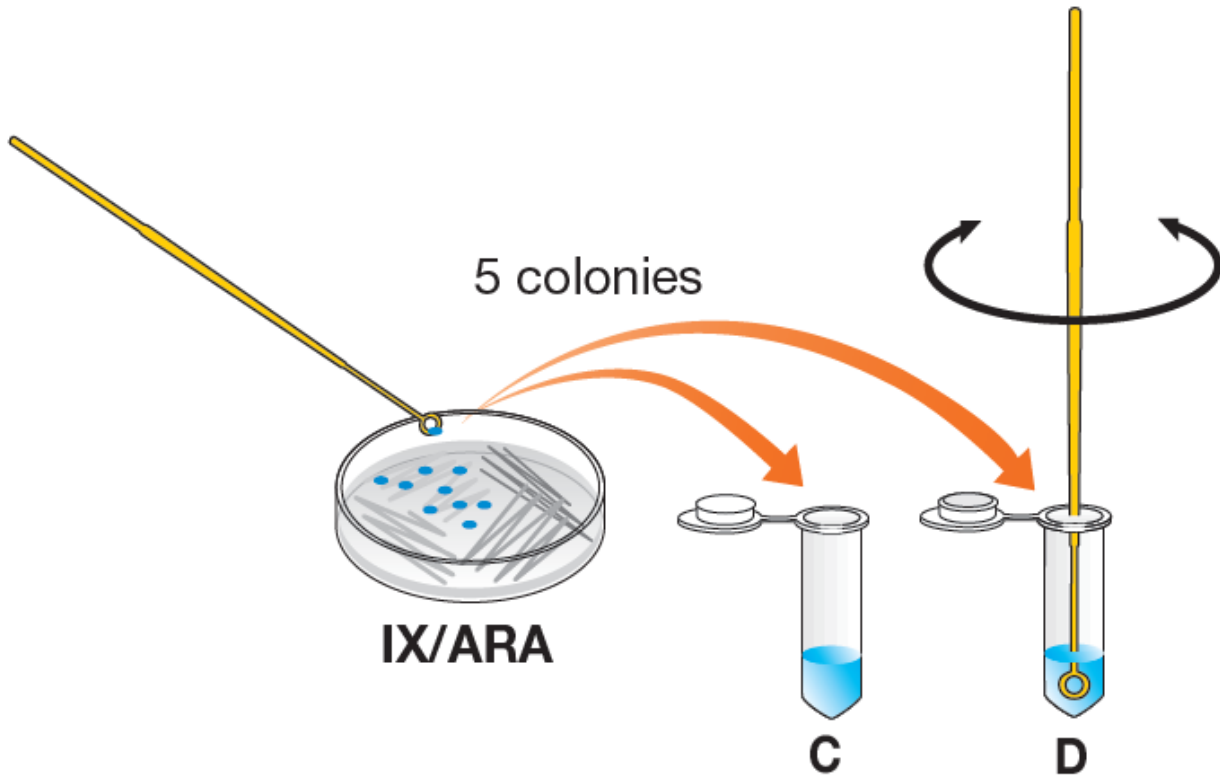


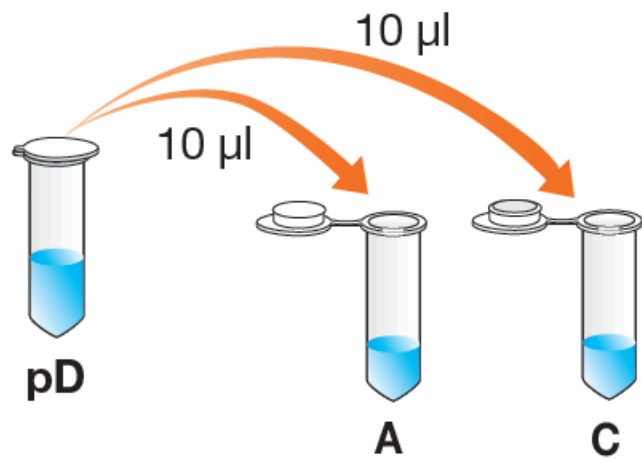
Example Results

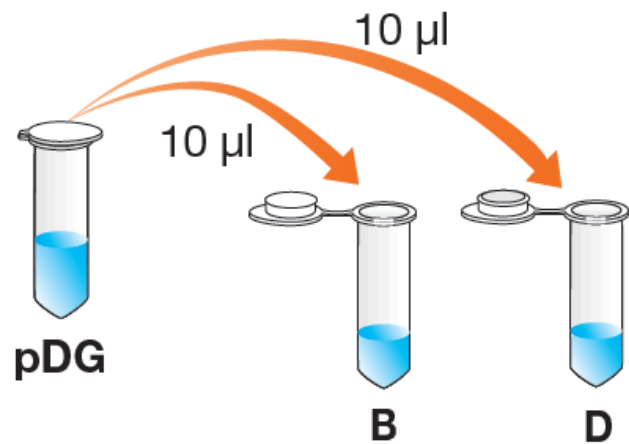






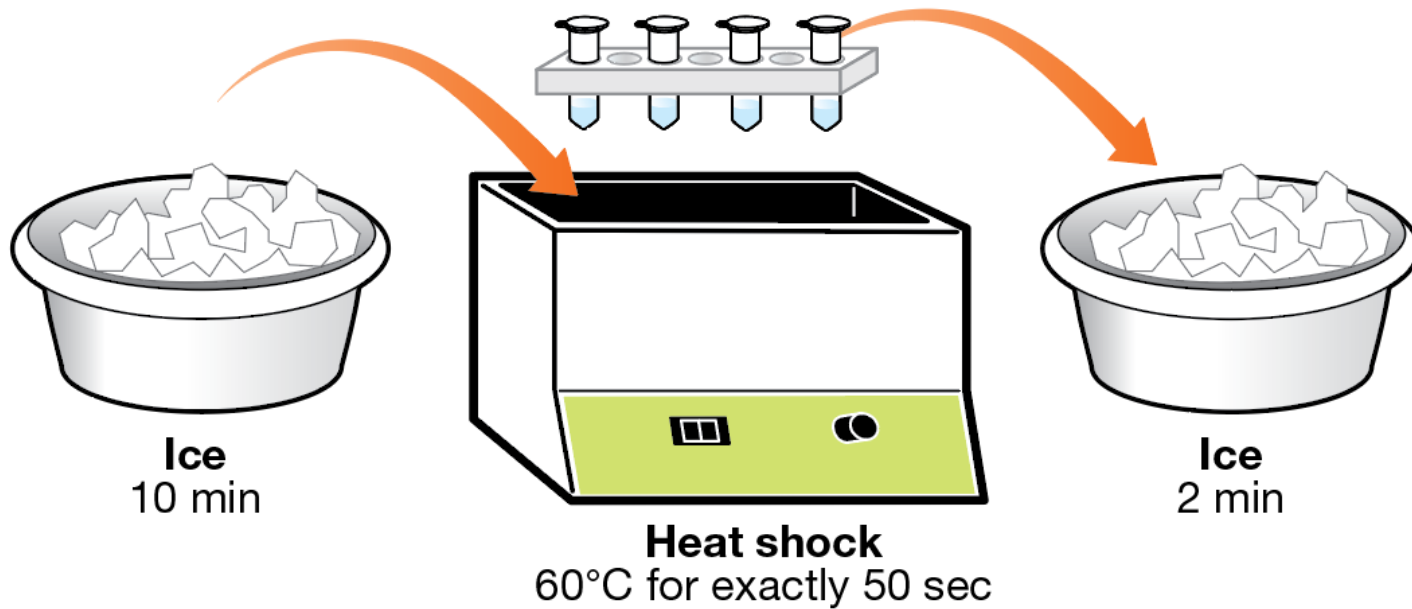


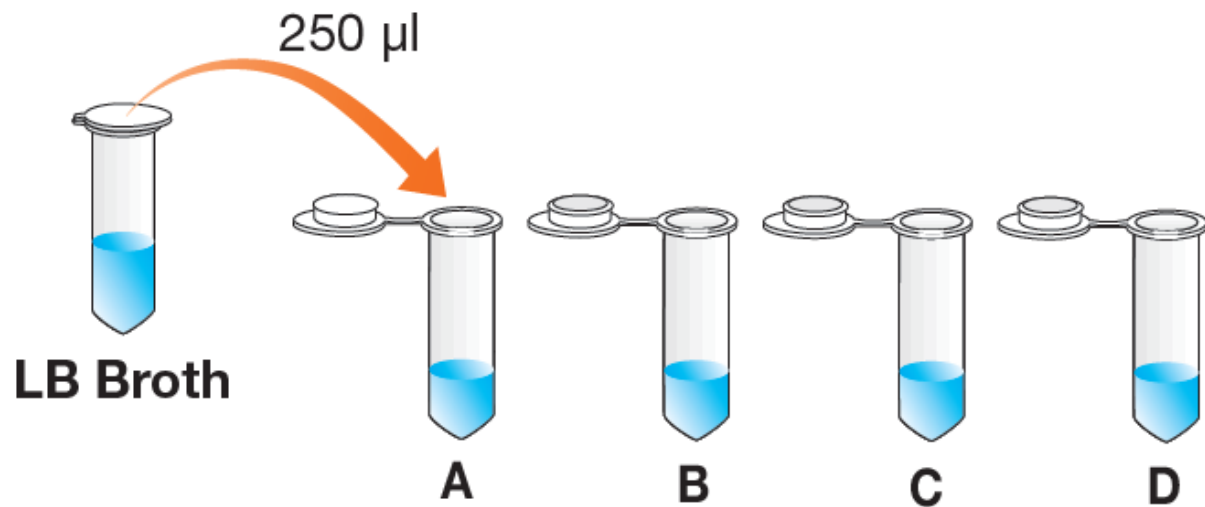






Ice
10 min







A



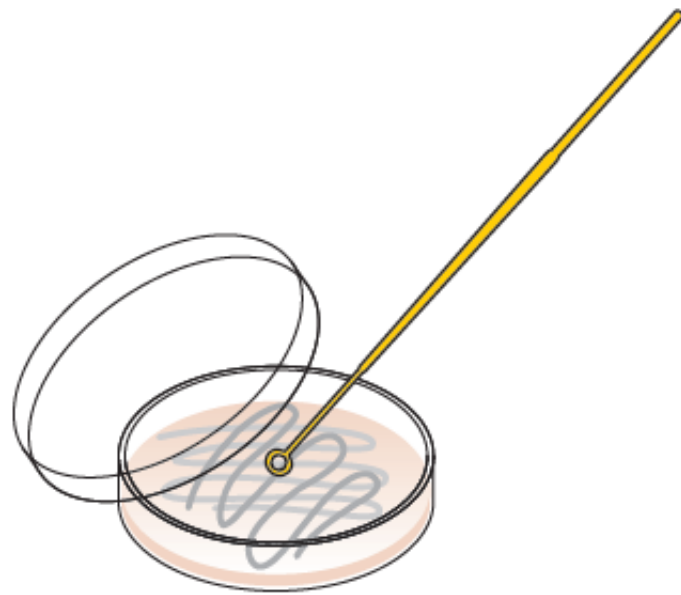
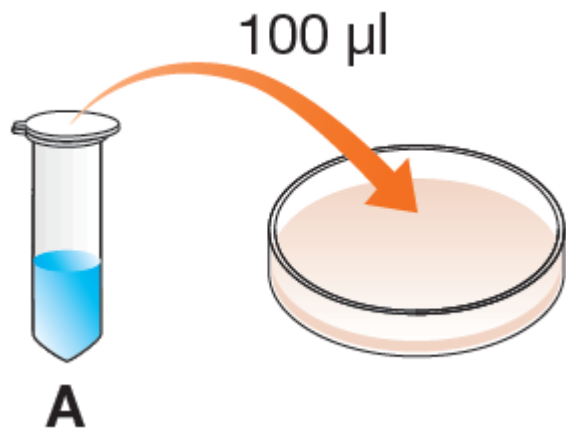
B

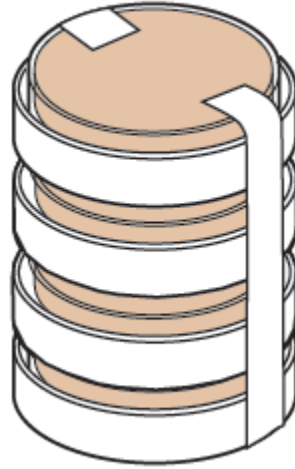


C



D







Example Results

