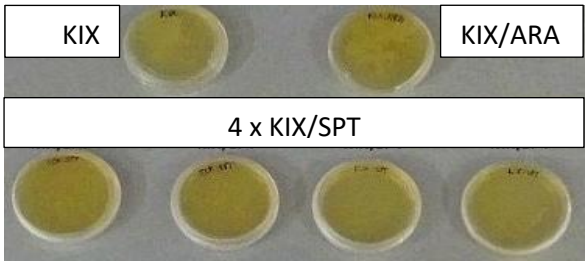
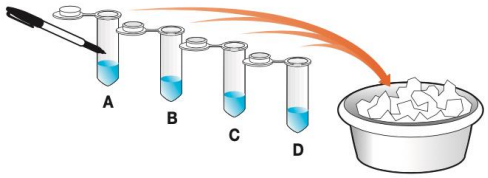
# Protocol Geneediting by CRISPR/Cas9

**Cultures and Nutrients:**

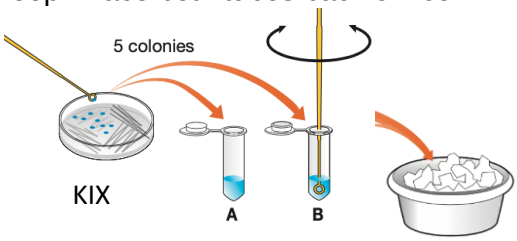



## 1. Geneediting by CRISPR/Cas9

1.1 Pipet 250 µL ice cold transformation solution (TS) in each of 4 labeled tubes A – D. Place 4 tubes back on ice.

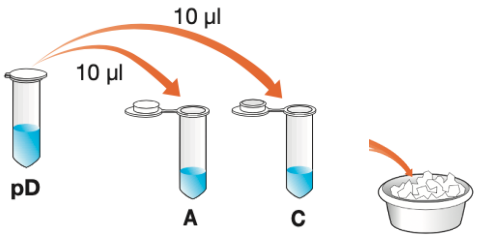



1.2 With an inoculation loop pick 5 *E. coli* colonies from **KIX**-starter plate. Swirl the loop in tube A until all bacteria are dispersed in transformation solution. No bacteria should remain on the loop. Repeat the step for tube B with a new loop. Place both tubes back on ice.




1.3 With an inoculation loop pick 5 *E. coli* colonies from **KIX/Ara**-starter plate. Swirl the loop in tube C until all bacteria are dispersed in transformation solution. No bacteria should remain on the loop. Repeat the step for tube D with a new loop. Place both tubes back on ice.

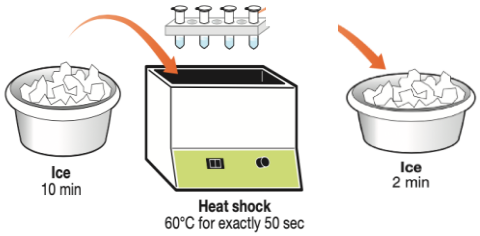
1.4 Pipet in each tubes A and C 10 µL *pLZDonor Plasmid (**pD**)* and mix it by pipetting up and down. Immediately place both tubes back on ice.




1.5 Pipet in each tubes B and D 10 µL pLZDonor Guide Plasmid (**pDG**) and mix it by pipetting up and down. Place both tubes back on ice.

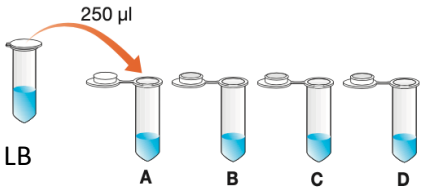
1.6 Incubate tubes A – D for 10 minutes on ice.

1.7 Heat shock ice cold tubes A - D on a swimmer for exactly 50 seconds in a 60 °C hot water bath.

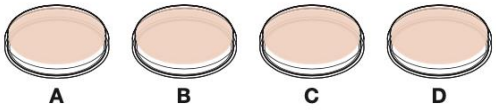



1.8 Immediately return 4 tubes to ice for 2 minutes.

1.9 Pipet in each 4 tubes A – D 250 µL LB nutrient broth and mix it by snipping. Incubate 4 tubes for 20 minutes at room temperature.




1.10 Near the edges, label the bottoms of 4 **KIX/SPT** selection plates with A – D and your group number.




1.11 Resuspend bacteria in tube A by snipping.

1.12 Pipet 50 µL of tube A on plate A and spread liquid evenly by a Drigalski-spatula. Rotate the plate several times in the process.

1.13 Repeat steps 1.11 and 1.12 with tubes B, C and D and plates B, C and D.

1.14 Incubate plates bottom upside down for 24 hours at 37 °C.

## Evaluation of Experiment

1. Check your plates for color development.
2. Explain blue and white color of colonies in the different plates.