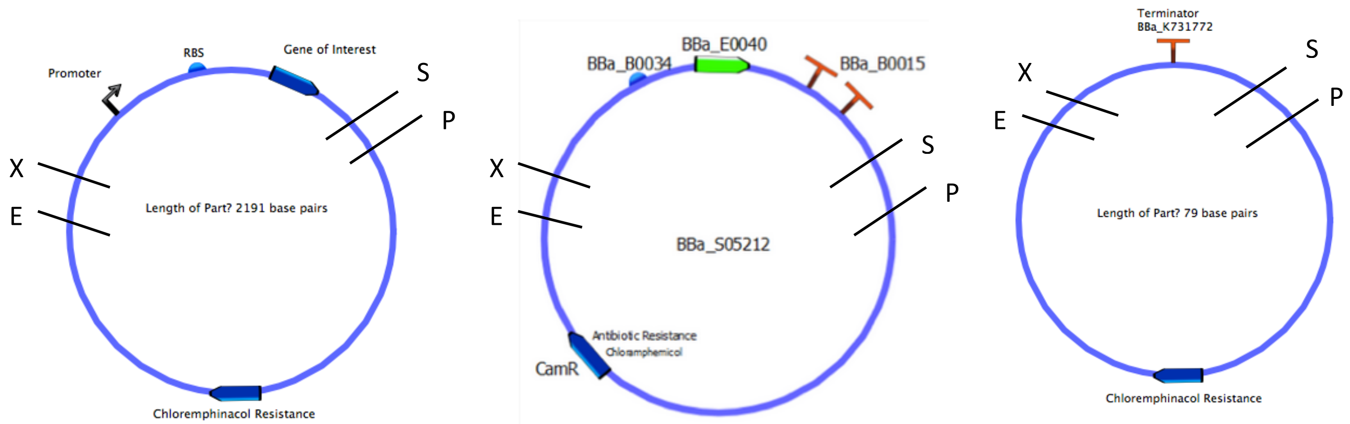
RESTRICTION ENZYME DIGEST

Pick two of the plasmids below, and circle which restriction enzyme cut sites you would use to combine the two parts.



Amount of DNA insert wanted: **500 ng**

DNA Insert Concentration: _____ **ng/µL**

CALCULATE *how many µL of DNA insert to add given the concentration above:*

Amount of DNA vector wanted: **250 ng**

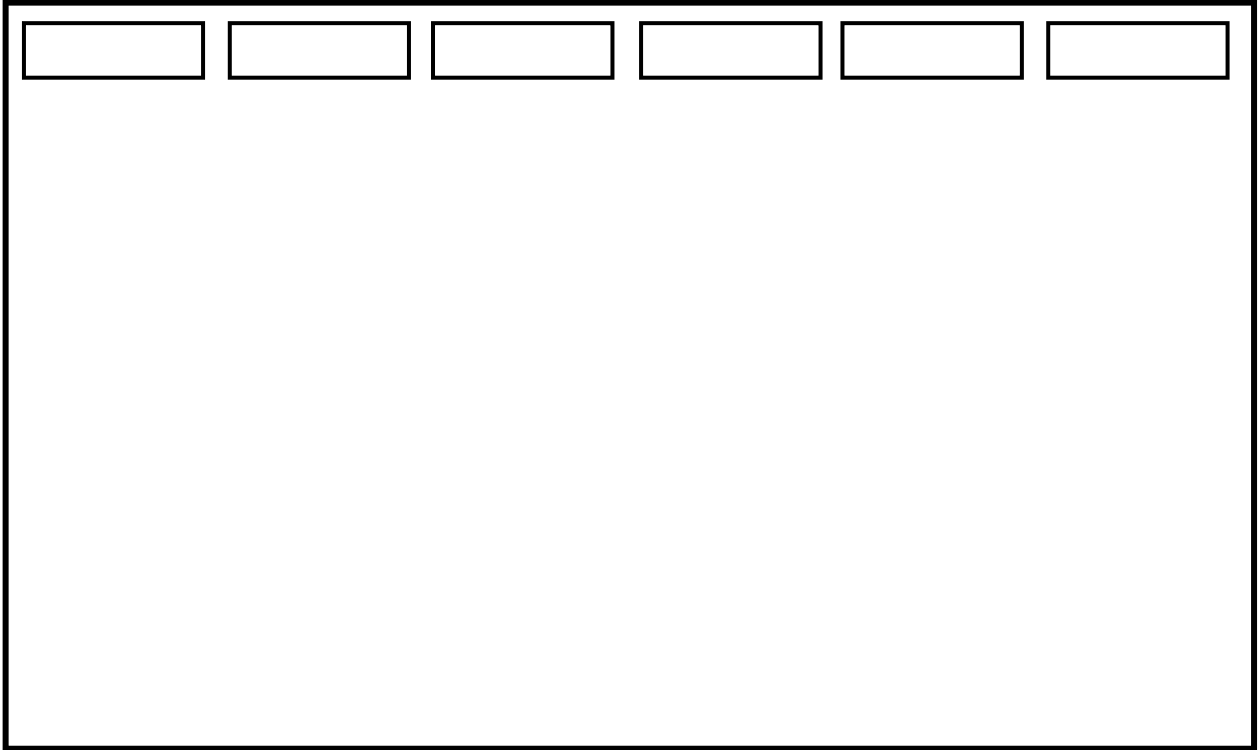
DNA Vector Concentration: _____ **ng/µL**

CALCULATE *how many µL of DNA vector to add given the concentration above:*

ORDER ADDED	MATERIAL	SAMPLE 1 (insert DNA)	SAMPLE 2 (vector DNA)
1	MilliQ Water (bring total volume to 20 µL)		
2	10x Corresponding Buffer	2 µL	2 µL
3	10x Cutsmart BSA	2 µL	2 µL
4	DNA (insert, vector, or plasmid)		
5	Enzyme (0.5 µL per enzyme)		
TOTAL		20 µL	20 µL

GEL ELECTROPHORESIS

LABEL *the wells in which you are loading your products.*



TRANSFORMATION AND PLATING PROTOCOL

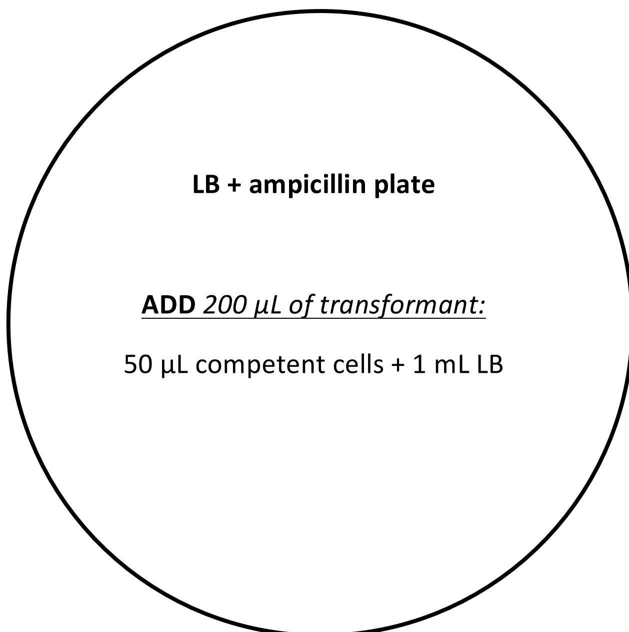
Transformation

1. Add 5uL of ligation product to 50uL of competent cells.
2. Gently flick the cells 6 times to properly mix the cells and the ligation products.
   *Be sure not to displace the cells all over the tube.
3. Incubate on ice for up to 5 minutes (NO LONGER). Then heat shock the samples at 42 C for 1 minute using one of the water baths. Place the samples back on ice for 1 minute, then place them in a tube rack (at room temperature). The bacterial cells do not tolerate the agarose well so keeping them on ice for too long can lead to disastrous results.
4. Add 1 mL of LB liquid to each sample.

Plating

1. Remove LB+antibiotic plates from 4°C and bring to room temperature.
2. Pipet 200uL of transformant onto a room temperature LB+antibiotic plate.
3. Use a glass pipet as a spreader and spread the pipetted transformants around the plate.

TRANSFORMATION CONTROL



EXPERIMENTAL