

Operon 3




- 20 steps
- 'high' setting
- 5 restriction digests
- 5 ligations
- 5 Transformations
- 5 plasmid extractions

Parts

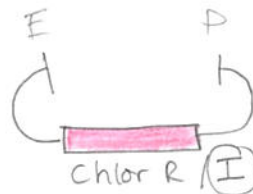
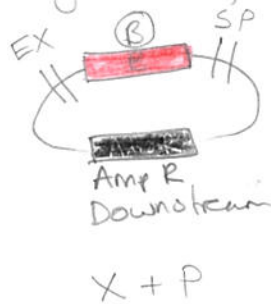
- (A) Bba_K418003 - lac repressor production circuit & promoter repressed by lacI (Amp)^{? ordering}
- (B) Bba_B0033 - (Weaker) RBS (Amp)
- (C) Bba_C0040 - Tet R gene (to repress operon 1) (Amp)
- (D) Bba_C0072 - Mnt gene (to repress operon 2) (Kan)
- (E) Bba_E010 - RFP gene (Kan)
- (F) Bba_B0015 - Terminator (Amp/Kan)
- (G) pSB1A3 - Ampicillin resistant
- (H) pSB1K3 - Kanamycin resistant
- (I) pSB1C3 - Chloramphenicol resistant

Plasmid Backbones

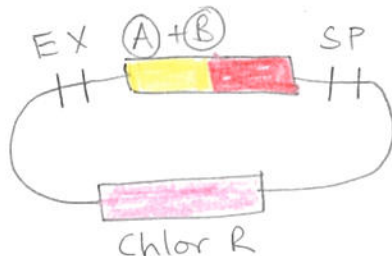
1) Transform all parts into competent cells to grow up on plates & multiply the plasmid with the biobrick on it for use. Plasmid extractions before use

Amp R = 
 Kan R = 
 Chlor R = 

2) & 3) Digest & Ligation of (A) + (B) onto (I)

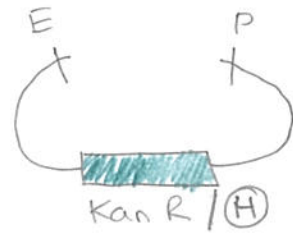
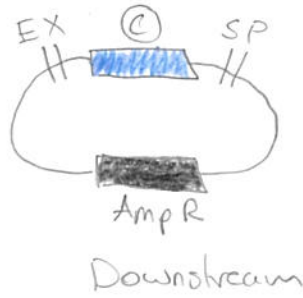
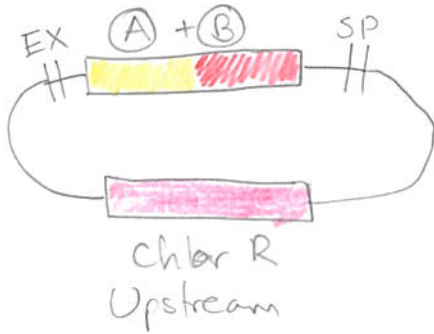


Ligation



4) & 5) Transformation of plasmid above into competent cells to multiply it. Plasmid extraction & Purification

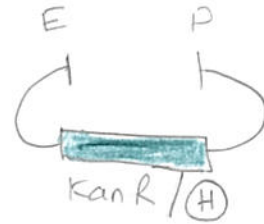
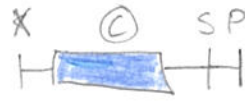
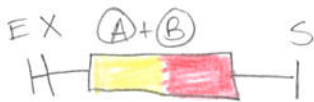
6) & 7) Digest & Ligation of ((A&B)) + (C) onto (H)



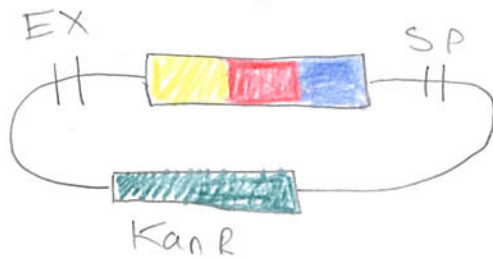
E & S

X & P

E & P

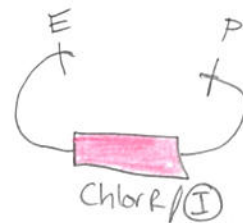
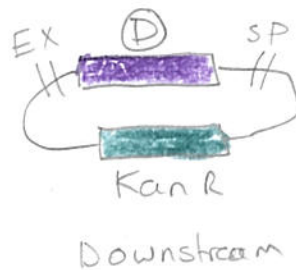
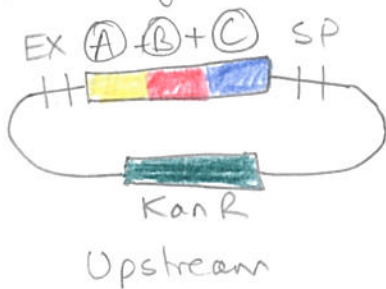


Ligation



8) & 9) Transformation of above plasmid into competent cells to multiply it. Plasmid extraction & purification

10) & 11) Digestion & Ligation of ((A&B&C)) + (D) onto (I)



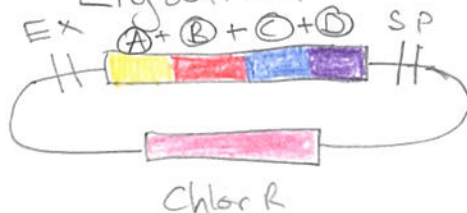
E & S

X & P

E & P

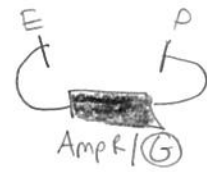
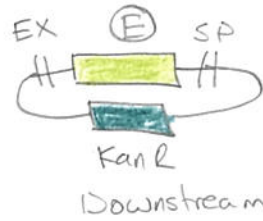
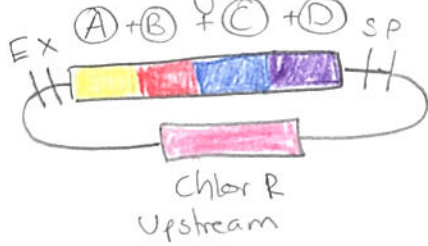


Ligation



12) & 13) Transformation of above plasmid into competent cells to multiply it. plasmid extraction & purification

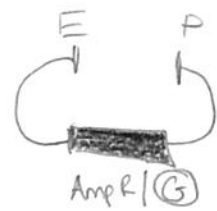
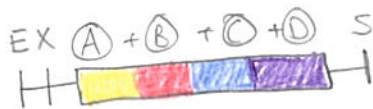
14) & 15) Digestion & ligation of (A & B & C & D) + E onto G



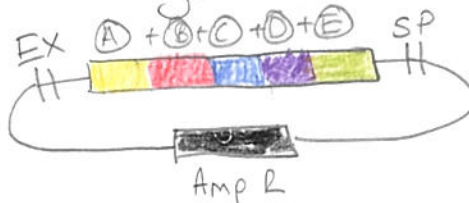
E & S

X & P

E & P

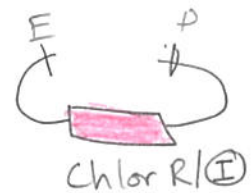
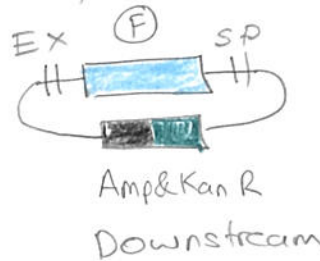
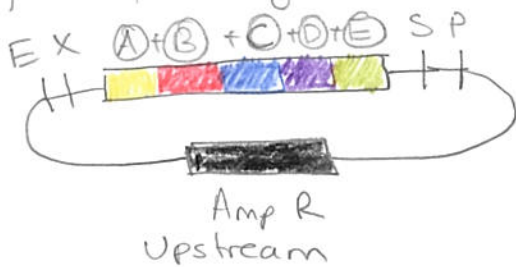


Ligation



16) & 17) Transformation of the above plasmid into competent cells to multiply it. plasmid extraction & purification.

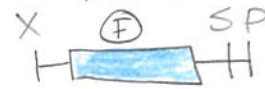
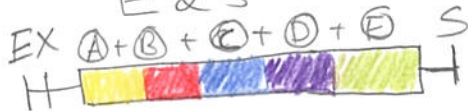
18) & 19) Digestion & ligation of (A & B & C & D & E) + F onto I



E & S

X & P

E & P



Ligation



20) Transformation of finished plasmid into competent cells to multiply it.