Operon 2

- 16 steps
- 'low' setting
- 4 restriction digests
- 4 ligations
- 4 transformations
- 4 plasmid extractions

015 (+)
040 (C)

Parts

- (J) BBa_K091104 (Amp) - Promoter, repressed by Mnt & lacI
- (K) BBa_B0034 (Amp) - (Normal) RBS
- (C) BBa_C0040 (Amp) - Tet R gene (to repress operon 1)
- (L) BBa_E0030 (Amp/Kan) - YFP Gene
- (F) BBa_B0015 (Amp/Kan) - Terminator

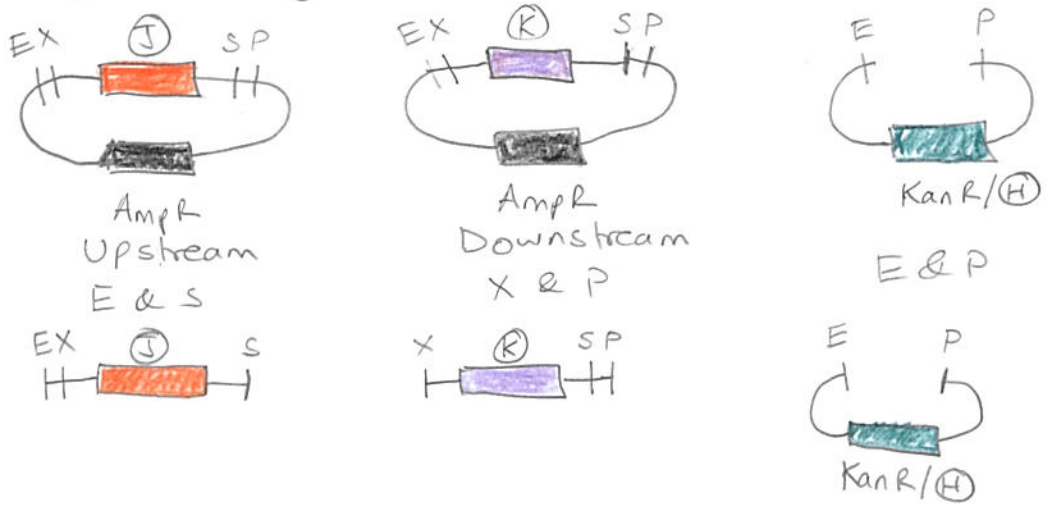
Plasmid Backbones

- (H) pSB1K3 - Kanamycin resistant
- (I) pSB1C3 - Chloramphenicol resistant
- (M) pSB1T3 - Tetracycline resistant

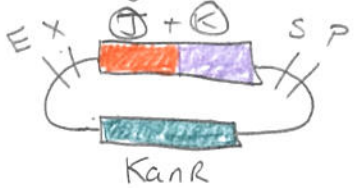
AmpR =
KanR =
ChlorR =
TetR =

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

2)&3) Digest & Ligation of (J) + (K) onto (H)

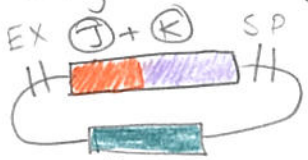


Ligation



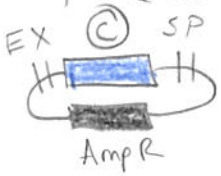
4)&5) Transform the above plasmid into competent cells to multiply it. Plasmid extraction & purification

6) & 7) Digest & Ligation of (J&K) + C onto I



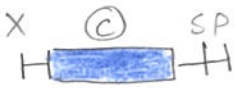
Upstream

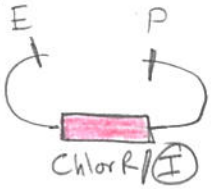
E & S



Down Stream

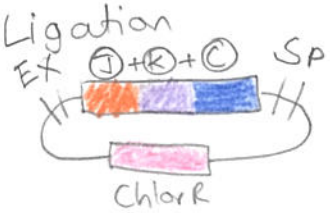
X & P





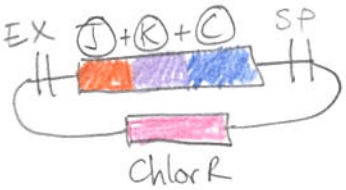
E & P

Ligation



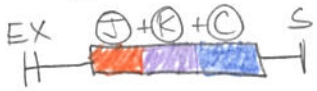
8) & 9) Transform the above plasmid into competent cells to multiply it. Plasmid extraction & purification

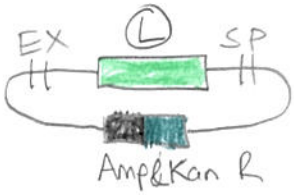
10) & 11) Digest & Ligation of (J&K & C) + L onto M



Upstream

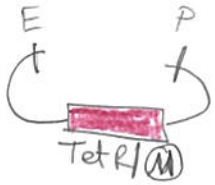
E & S





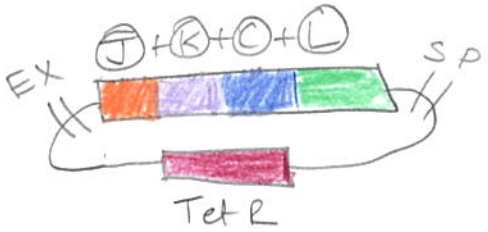
downstream

X & P



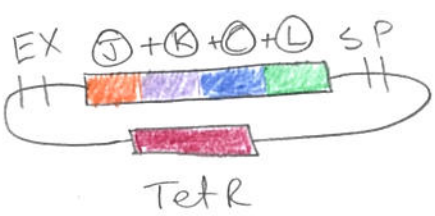
E & P

Ligation



12) & 13) Transform the above plasmid into competent cells to multiply it. Plasmid extraction & purification.

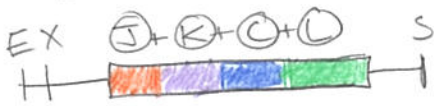
14) & 15) Digest & ligation of (J & K & C & L) + F onto I

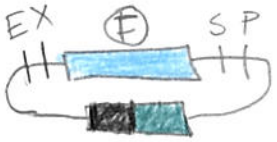


Tet R

Upstream

E & S

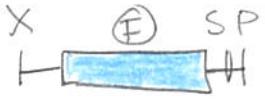




Amp & Kan R

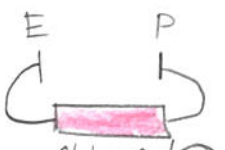
Downstream

X & P



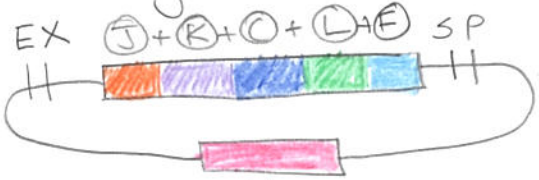
Chlor R/I

E & P



Chlor R/I

Ligation



Chlor R

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