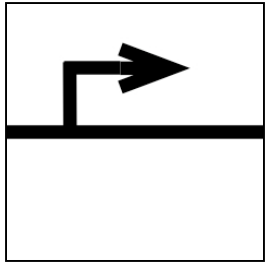
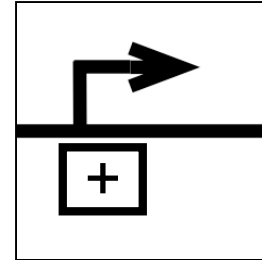


Construction Plan Overview

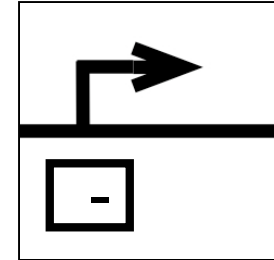
Quick vBOL Key



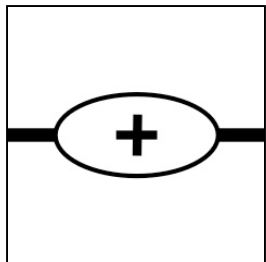
Forward Promoters



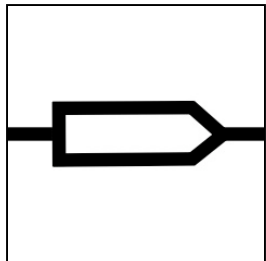
Inducible



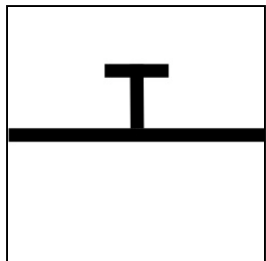
Repressible



Forward Ribosome Binding Sight



Open Reading Frame (gene)



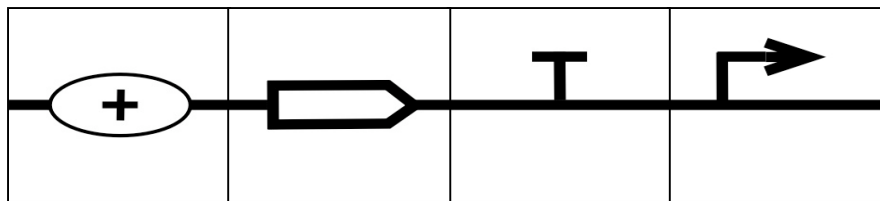
Terminator

Device 1

First Round of Cloning

1.1a SoxR/S Promoter + GFP tag

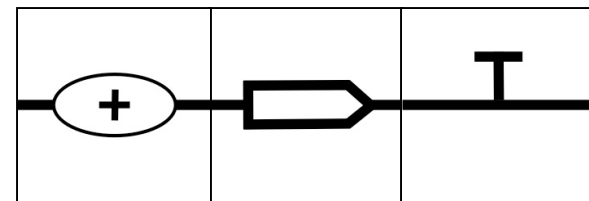
(Synthesized part # 54) + (BioBrick part # I13504)



B0034 + k223000 + B0015 + k2230001
(RBS) (SoxR gene) (TT) (SoxS Promoter)

Cut w/ EcoR1/Spe1

+



B0034 + E0040 + B0015
(RBS) (GFP) (TT)

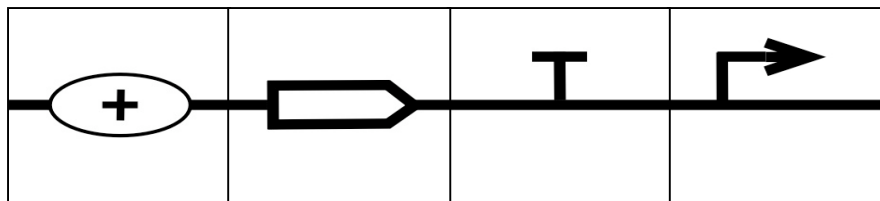
Cut w/ EcoR1/XbaI

Device 1

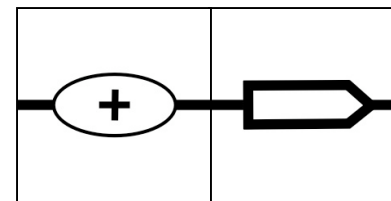
First Round of Cloning

1.1b SoxR/S Promoter +
(Synthesized part # 54) +

Blh parts
(Synthesized part # 57)



+



B0034 + k223000 + B0015 + k2230001
(RBS) (SoxR gene) (TT) (SoxS Promoter)

Cut w/ EcoR1/Spe1

B0034 + k223035
(RBS) (Blh gene)

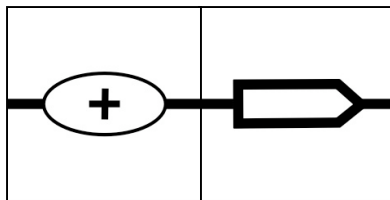
Cut w/ EcoR1/XbaI

Device 1

First Round of Cloning

1.1c Blh gene + Terminator

(Synthesized part # 57) + (BioBrick part # B0015)

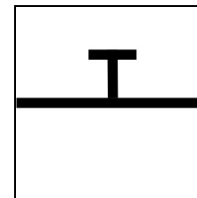


B0034 + k223035

(RBS)

(Blh gene)

+



B0015

(TT)

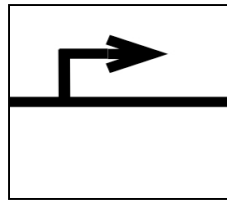
Cut w/ EcoR1/Spe1

Cut w/ EcoR1/XbaI

Device 1

First Round of Cloning

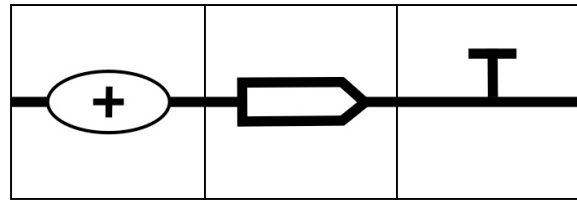
1.1d Promoter + Blh gene w/ Terminator
(# J23100) + (Part from Cloning 1.1c)



J23100

Promoter)

+



B0034 + k223035 + B0015

(RBS) (Blh gene) + (TT)

Cut w/ Spe1/Pst1

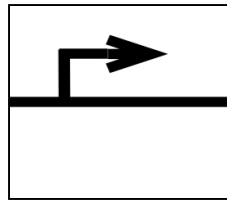
Cut w/ Xba1/Pst1

Device 1

First Round of Cloning

1.1e Promoter + Crt Cluster (Beta-Carotene Producers)

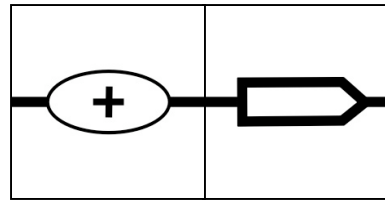
(# J23100) + (Synthesized Part on way)



J23100

Promoter)

+



B0034 + (?)

(RBS)

(crt genes = B,E, I, Y, and X)

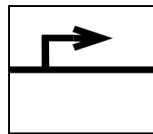
Cut w/ Spe1/Pst1

Cut w/ Xba1/Pst1

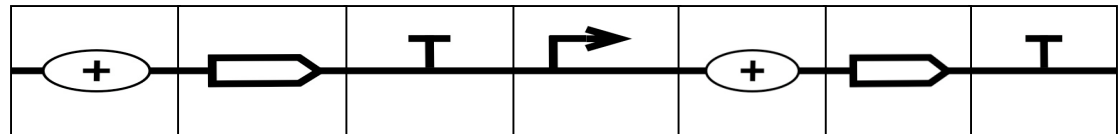
Device 1

Second Round of Cloning

1.2a Promoter + SoxR/S with GFP tag
(J23100) + (Made From cloning step 1.1a)



+



J23100

Promoter)

B0034 + k223000 + B0015 + k2230001+ B0034 +E0040 + B0015

(RBS)

(SoxR gene)

(TT)

(SoxS Promoter)

(RBS)

(GFP)

(TT)

Cut w/ Spe1/Pst1

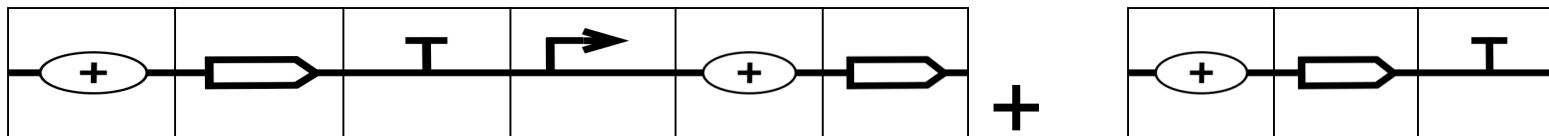
Cut w/ Xba1/Pst1

Device 1

Second Round of Cloning

1.2b SoxR/S w/ Blh + RALDH II group

(Cloned part from 1.1b) + (Synthesized part # 52)



B0034 + k223000 + B0015 + k2230001 + B0034 + k223035
(RBS) (SoxR gene) (TT) (SoxS Promoter) (RBS) (Blh gene)

B0034 + k223036 + B0015
(RBS) (RALDH II gene) (TT)

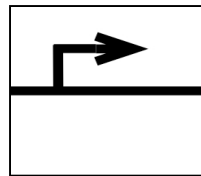
Cut w/ Spe1/Pst1

Cut w/ Xba1/Pst1

Device 1

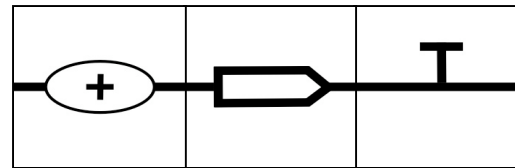
Second Round of Cloning

1.2c Promoter + RALDH II group
(Part # J23100) + (Synthesized part # 52)



J23100
Promoter

+



B0034 + k223036 + B0015
(RBS) (RALDH II gene) (TT)

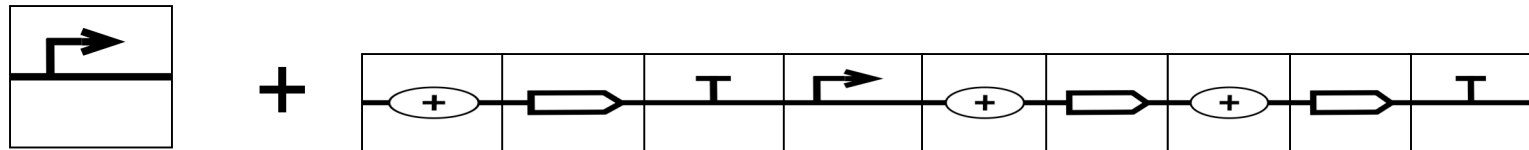
Cut w/ Spe1/Pst1

Cut w/ Xba1/Pst1

Device 1

Third Round of Cloning

1.3a Promoter + SoxR/S w/ Blh and RALDH II groups
(Part #J23100) + (Cloned from step 1.2b)



J23100

Promoter

B0034 + k223000 + B0015 + k2230001 + B0034 + k223035 + B0034 + k223036 + B0015

(RBS) (SoxR gene) (TT) (SoxS Promoter) (RBS) (Blh gene) (RBS) (RALDH II gene) (TT)

Cut w/ Spe1/Pst1

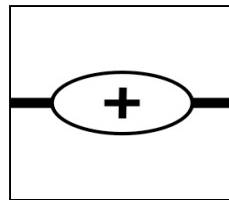
Cut w/ Xba1/Pst1

Device 2

First Round of Cloning

2.1a RBS + Il-6 and HlyAs linker

(# B0034) + (Synthesized part # 53)

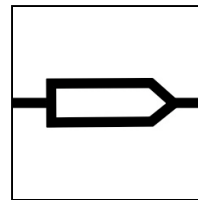


B0034

(RBS)

Cut w/ Spe1/Pst1

+



K223022

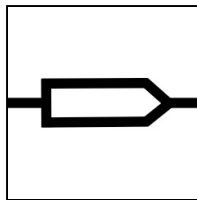
(Il-6 + HlyAs linker)

Cut w/ Xba1/Pst1

Device 2

First Round of Cloning

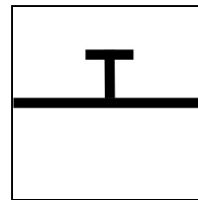
2.1b Il-6 w/ HlyAs linker + Terminator
(Synthesized part # 53) + (#B0015)



K223022

(Il-6 + HlyAs linker)

+



B0015

(TT)

Cut w/ EcoR1/Spe1

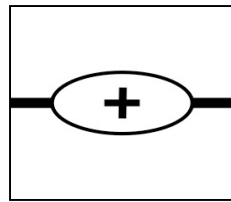
Cut w/ EcoR1/Xba1

Device 2

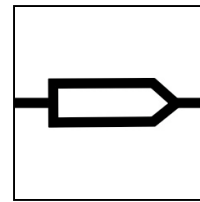
First Round of Cloning

2.1c RBS + TolC gene

(# B0034) + (Synthesized part)



+



B0034

(RBS)

K223024

(TolC gene)

Cut w/ Spe1/Pst1

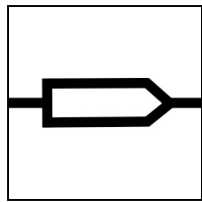
Cut w/ Xba1/Pst1

Device 2

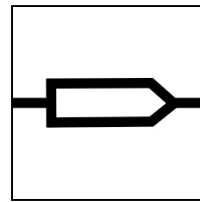
First Round of Cloning

2.1d HlyA signal + YFP

(will be registered) + (BioBricks part# I75700)



+



#

(HlyA signal0)

I75700

(YFP)

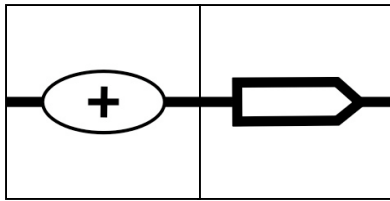
Cut w/ NgoMIV/AgeI

Cut w/ NgoMIV/AgeI

Device 2

Second Round of Cloning

2.2a RBS w/ Il-6 and HlyA + Terminator
(Cloned from step 2.1a) + (#B0015)

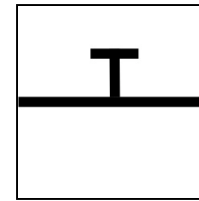


B0034 + k223022

(RBS)

(Il-6 and HlyAs linker)

+



B0015

(TT)

Cut w/ EcoR1/Spe1

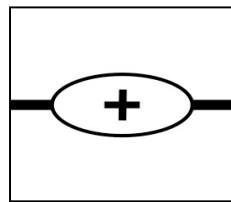
Cut w/ EcoR1/XbaI

Device 2

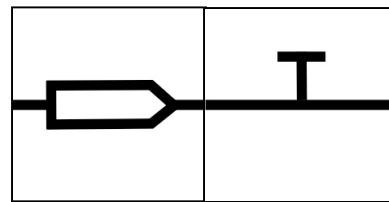
Second Round of Cloning

2.2b RBS + Il-6 and HlyAs linker w/ TT

(# B0034) + (Cloned from step 2.1b)



+



B0034

(RBS)

K223022 + B0015

(Il-6 + HlyAs linker) (TT)

Cut w/ Spe1/Pst1

Cut w/ Xba1/Pst1

Device 2

Second Round of Cloning

2.2c RBS w/ TolC

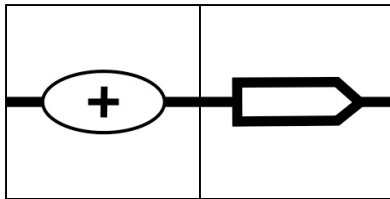
+

Hly R,B,D,C cluster

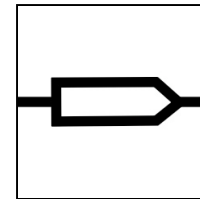
(Cloned from step 2.1c)

+

(Receiving in Plasmid from another lab)



+



B0034 + k223024

(RBS)

(TolC gene)

k223025

(Hly R,B,D,C)

Cut w/ EcoR1/Spe1

Cut w/ EcoR1/XbaI

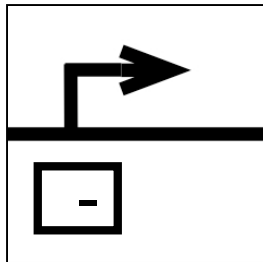
Device 2

Third Round of Cloning

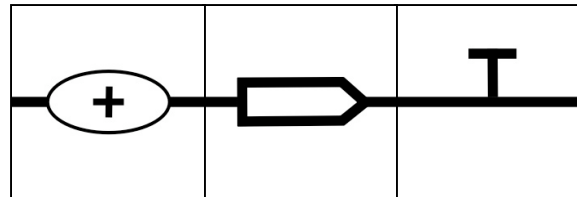
2.3a Trp Promoter + Rbs w/ Il-6 and HlyAs w/ TT

(?) +

(Cloned from step 2.2a)



+



k223023

(Trp Promoter)

B0034 + k223022 + B0015

(RBS) (Il-6 and HlyAs linker) (TT)

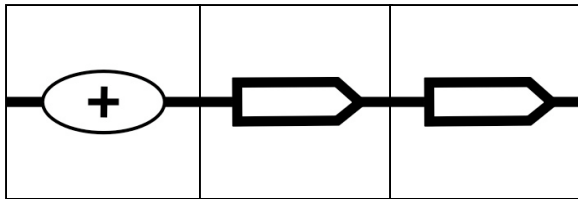
Cut w/ EcoR1/Spe1

Cut w/ EcoR1/XbaI

Device 2

Third Round of Cloning

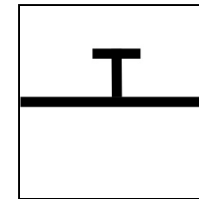
2.3b RBS w/ TolC and HlyR,B,D,C + Terminator
(Cloned from step 2.2c) + (#B0015)



B0034 + k223024 + k223025
(RBS) (TolC) (HlyR,B,D,C)

Cut w/ EcoR1/Spe1

+



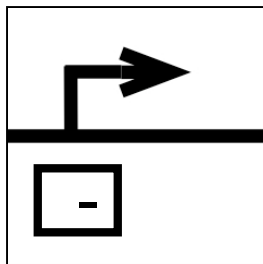
B0015
(TT)

Cut w/ EcoR1/XbaI

Device 2

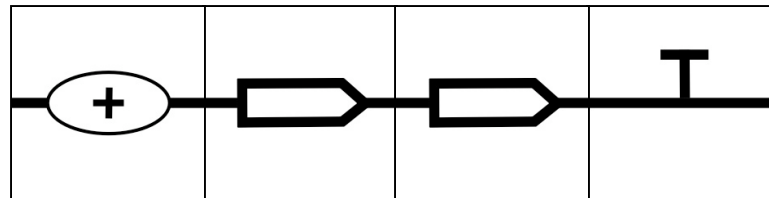
Fourth Round of Cloning

2.4a Trp Promoter + Rbs, TolC and HlyR,B,D,C, TT
(Going to be ordered) + (Cloned from step 2.3b)



k223023
(Trp Promoter)

+



B0034 + k223024 + k223025 + B0015
(RBS) (TolC) (HlyR,B,D,C) (TT)

Cut w/ EcoR1/Spe1

Cut w/ EcoR1/XbaI

