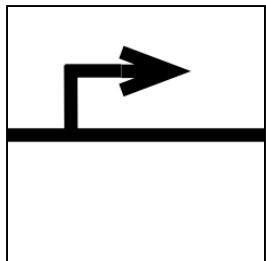
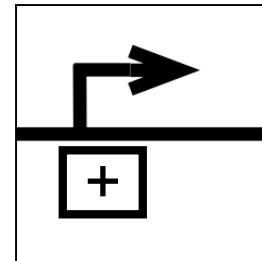


# Construction Plan Overview

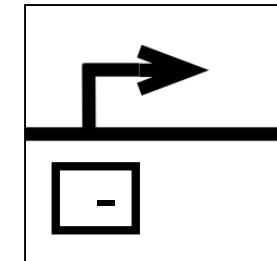
# Quick vBOL Key



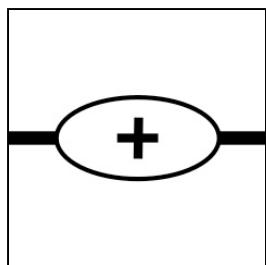
Forward Promoters



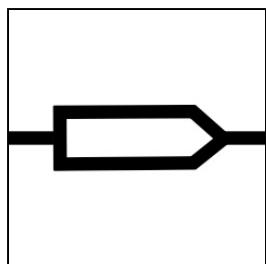
Inducible



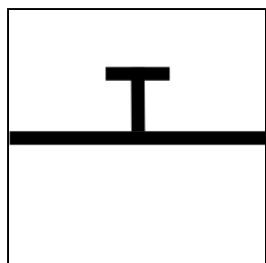
Repressible



Forward Ribosome Binding Sight



Open Reading Frame (gene)



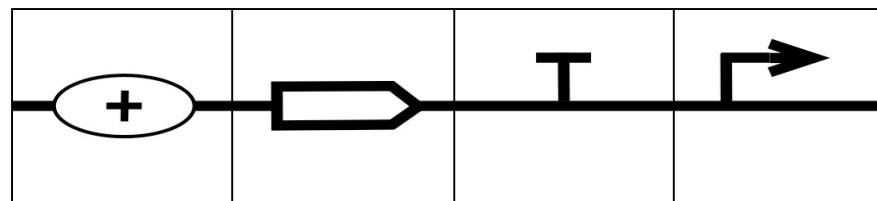
Terminator

# Device 1

## First Round of Cloning

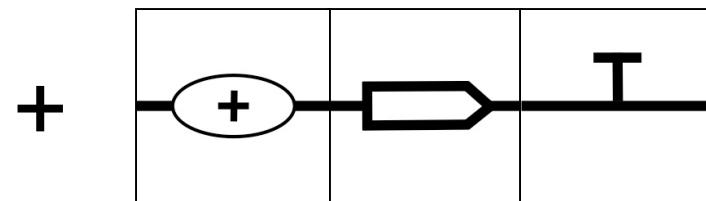
1.1a SoxR/S Promoter + GFP tag

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



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

Cut w/ EcoR1/Spe1



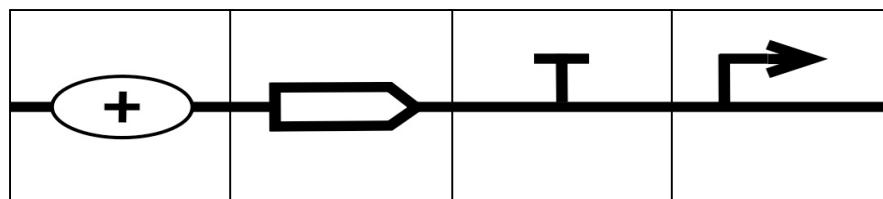
B0034 + E0040 + B0015  
(RBS) (GFP) (TT)

Cut w/ EcoR1/Xba1

# Device 1

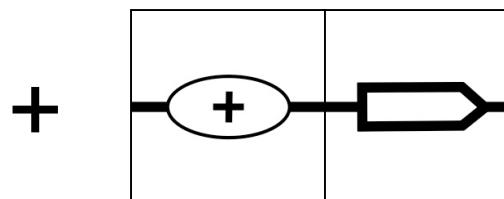
## First Round of Cloning

1.1b     SoxR/S Promoter +     Blh parts  
(Synthesized part # 54) +     (Synthesized part # 57)



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

Cut w/ EcoR1/Spe1



B0034 + k223035  
(RBS)     (Blh gene)

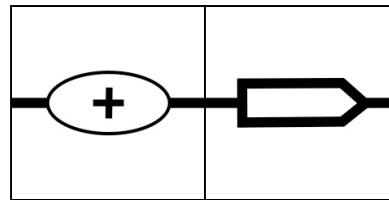
Cut w/ EcoR1/Xba1

# Device 1

## First Round of Cloning

1.1c Blh gene + Terminator

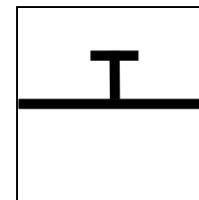
(Synthesized part # 57) + (BioBrick part # B0015)



B0034 + k223035  
(RBS) (Blh gene)

Cut w/ EcoR1/Spe1

+



B0015  
(TT)

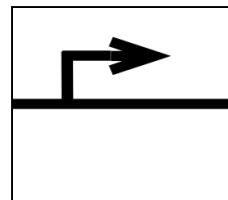
Cut w/ EcoR1/XbaI

# Device 1

## First Round of Cloning

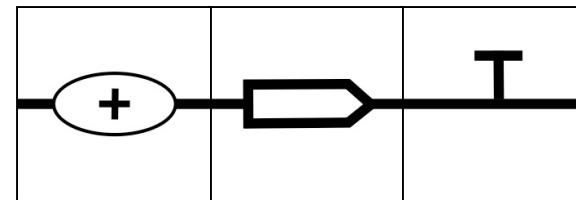
1.1d Promoter + Blh gene w/ Terminator

(# 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

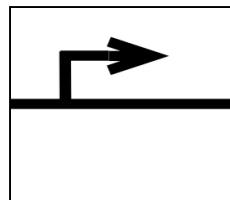
## First Round of Cloning

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

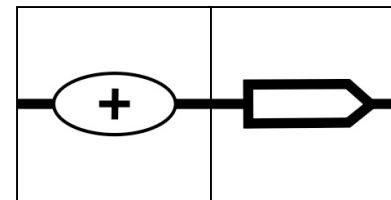
(# J23100)

+

(Synthesized Part on way )



+



J23100

Promoter)

B0034 + (?)

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

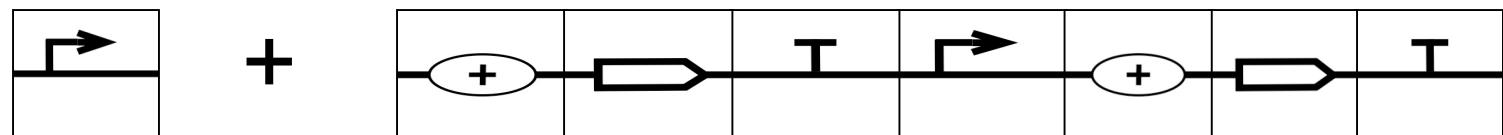
Cut w/ Spe1/Pst1

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 B0034 + k223000 + B0015 + k2230001+ B0034 +E0040 + B0015  
 Promoter) (RBS) (SoxR gene) (TT) (SoxS Promoter) (RBS) (GFP) (TT)

Cut w/ Spe1/Pst1

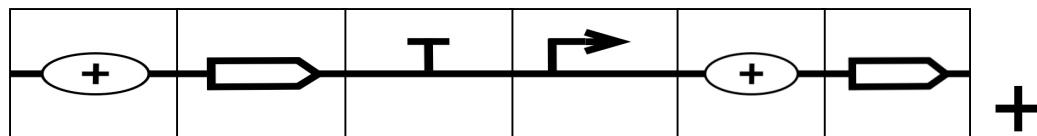
## Cut w/ Xba1/Pst1

# Device 1

## Second Round of Cloning

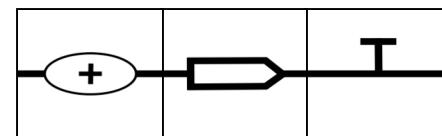
1.2b      SoxR/S w/ Blh      +      RALDH II group

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



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

Cut w/ Spe1/Pst1



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

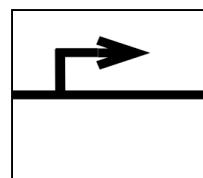
Cut w/ Xba1/Pst1

# Device 1

## Second Round of Cloning

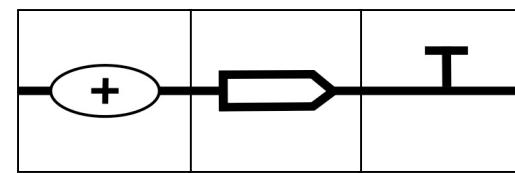
1.2c Promoter + RALDH II group

(Part # J23100) + (Synthesized part # 52)



J23100  
Promoter

+



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

Cut w/ Spe1/Pst1

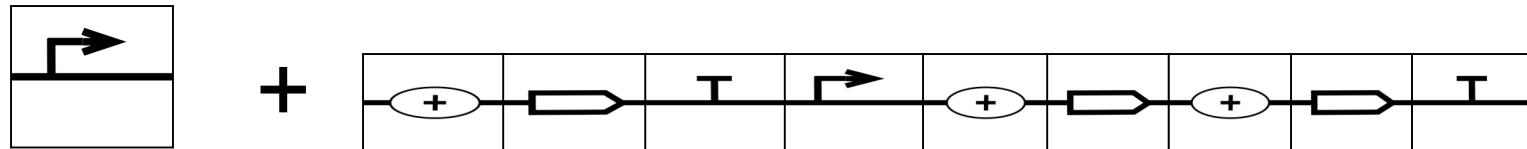
Cut w/ Xba1/Pst1

# 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

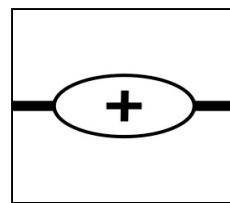


# Device 2

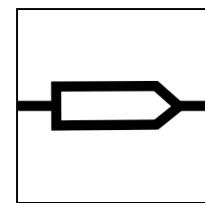
## First Round of Cloning

2.1a    RBS    +    II-6 and HlyAs linker

( # B0034)    +    (Synthesized part # 53)



+



B0034

(RBS)

K223022

(II-6 + HlyAs linker)

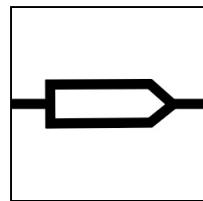
Cut w/ Spe1/Pst1

Cut w/ Xba1/Pst1

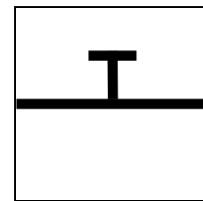
# Device 2

## First Round of Cloning

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



+



K223022

(II-6 + HlyAs linker)

B0015

(TT)

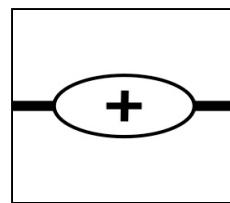
Cut w/ EcoR1/Spe1

Cut w/ EcoR1/Xba1

# Device 2

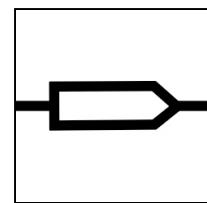
## First Round of Cloning

2.1c    RBS    +    TolC gene  
( # B0034)    +    (Synthesized part)



B0034  
(RBS)

+



K223024  
(TolC gene)

Cut w/ Spe1/Pst1

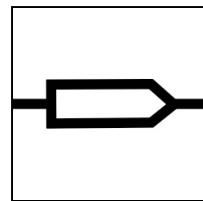
Cut w/ Xba1/Pst1

# Device 2

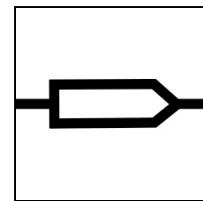
## First Round of Cloning

2.1d HlyA signal + YFP

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



+



#

(HlyA signal0

I75700

(YFP)

Cut w/ NgoMIV/AgeI

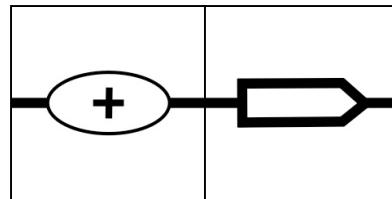
Cut w/ NgoMIV/AgeI

# Device 2

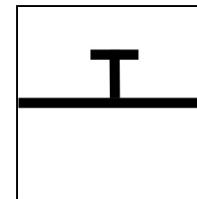
## Second Round of Cloning

2.2a RBS w/ II-6 and HlyA + Terminator

(Cloned from step 2.1a) + (#B0015)



+



B0034 + k223022

(RBS) (II-6 and HlyAs linker)

Cut w/ EcoR1/Spe1

B0015

(TT)

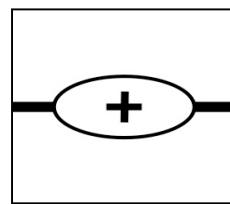
Cut w/ EcoR1/Xba1

# Device 2

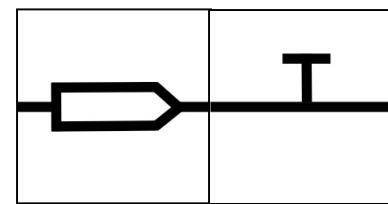
## Second Round of Cloning

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

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



+



B0034

(RBS)

K223022 + B0015

(II-6 + HlyAs linker) (TT)

Cut w/ Spe1/Pst1

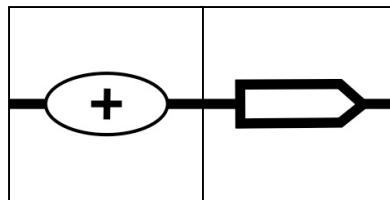
Cut w/ Xba1/Pst1

# Device 2

## Second Round of Cloning

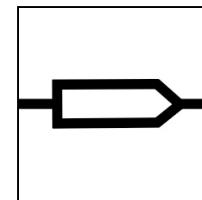
2.2c RBS w/ TolC + Hly R,B,D,C cluster

(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

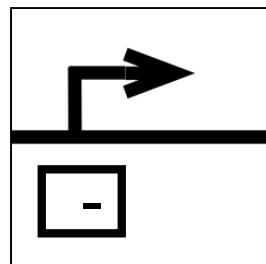
Cut w/ EcoR1/Xba1

# Device 2

## Third Round of Cloning

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

(?) +

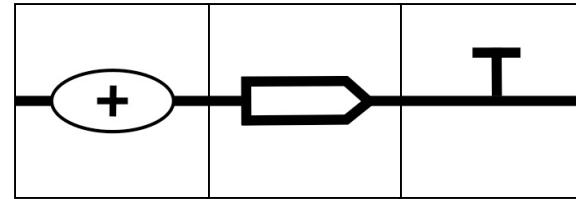


k223023  
(Trp Promoter)

Cut w/ EcoR1/Spe1

(Cloned from step 2.2a)

+



B0034 + k223022 + B0015  
(RBS) (II-6 and HlyAs linker) (TT)

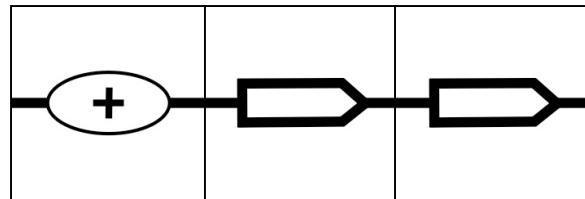
Cut w/ EcoR1/Xba1

# Device 2

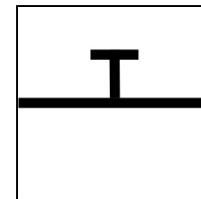
## Third Round of Cloning

2.3b RBS w/ TolC and HlyR,B,D,C + Terminator

(Cloned from step 2.2c)



+



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

B0015  
(TT)

Cut w/ EcoR1/Spe1

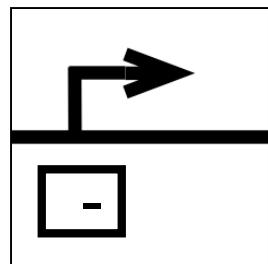
Cut w/ EcoR1/Xba1

# Device 2

## Fourth Round of Cloning

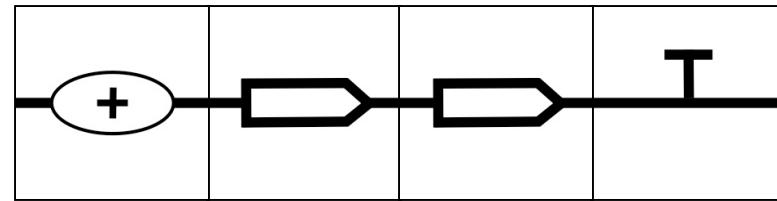
2.4a Trp Promoter + Rbs, TolC and HlyR,B,D,C, TT

(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/Xba1

