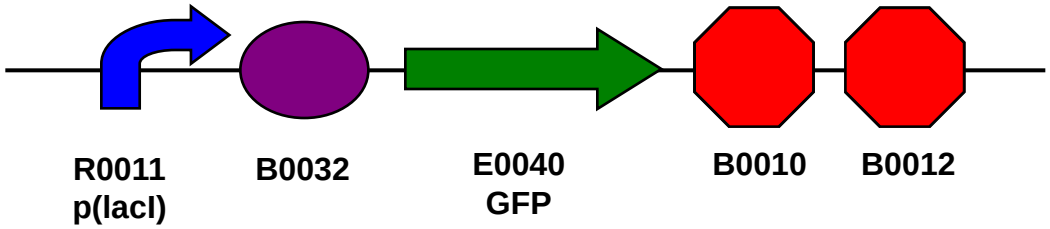
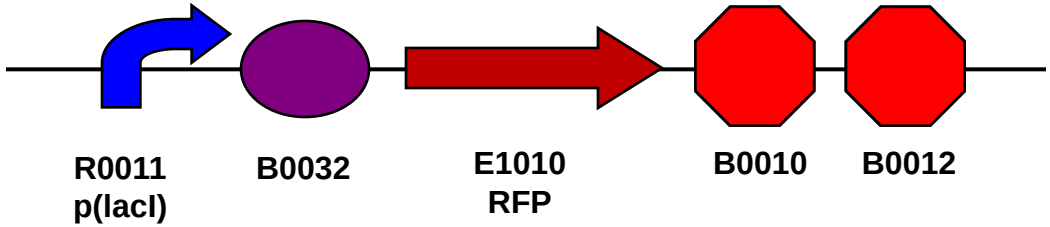
Circuits to engineer
Using In-Fusion BioBrick Assembly

GFP Circuit



RFP Circuit



Lab course outline

Lab 1: Safety, pipetting, PCR, make gels

Lab 2: Run gel on PCR products, purify PCR products, gel

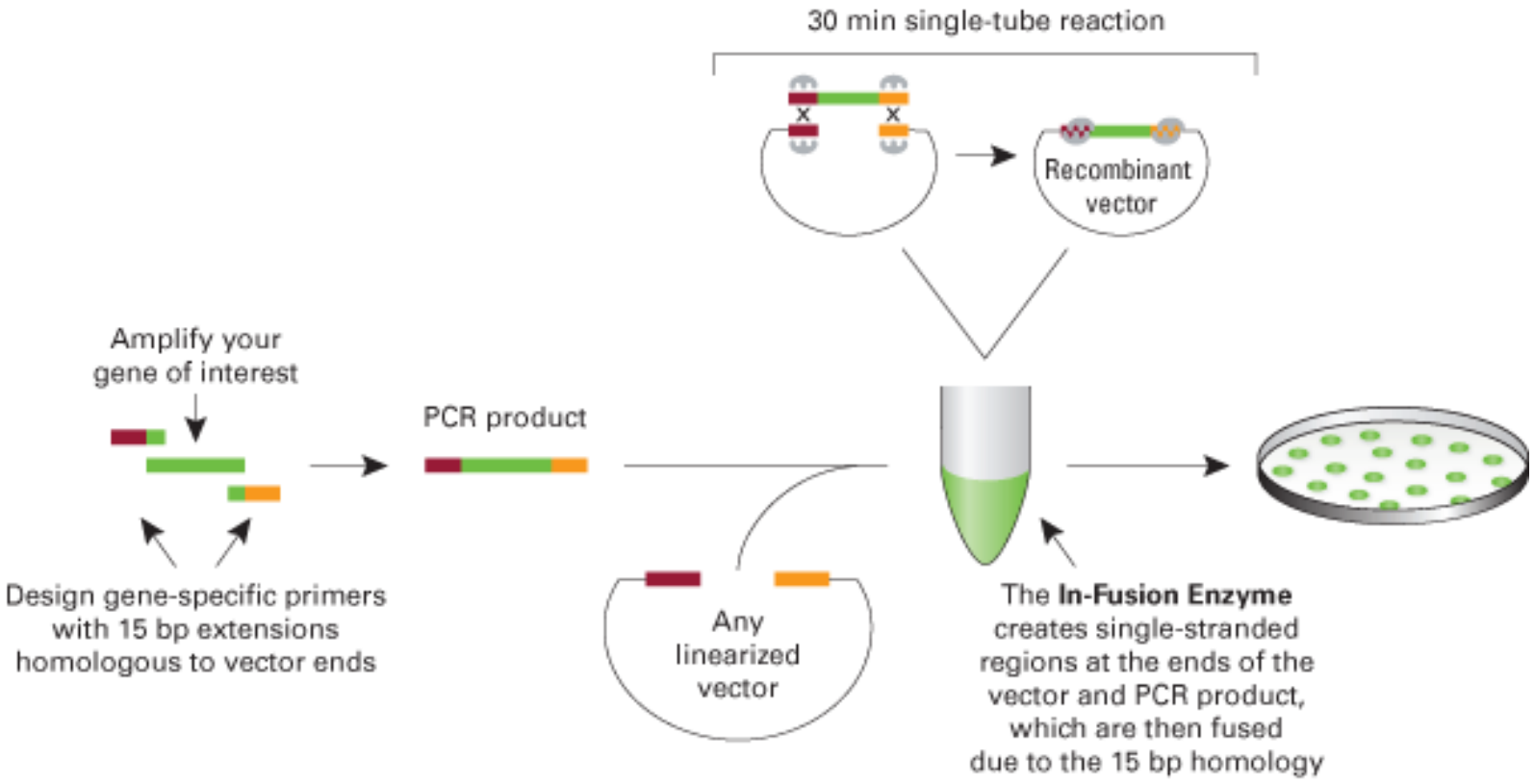
Lab 3: In-Fusion reactions, transformations

Lab 4: Colony PCR, grow transformants overnight

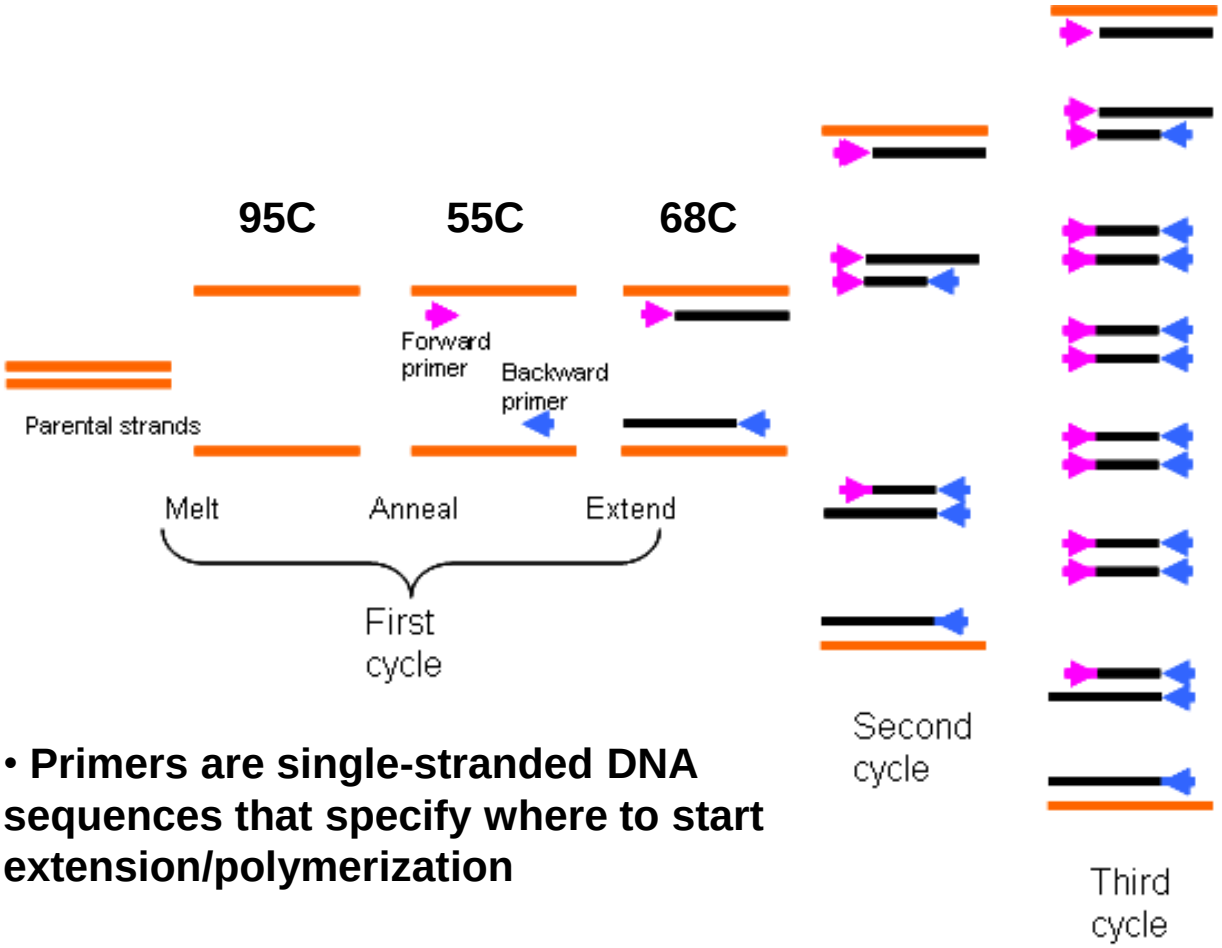
Lab 5: Minipreps, submit for sequencing

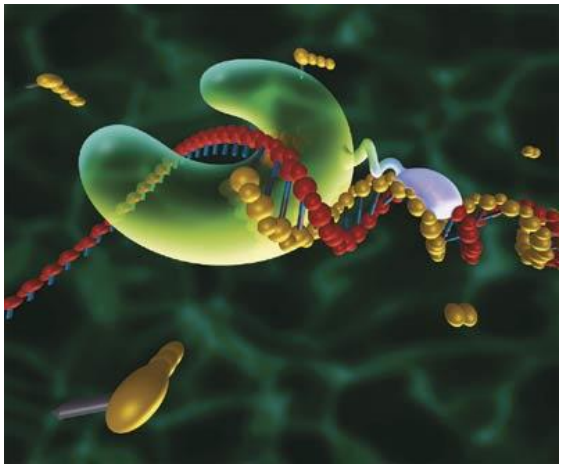
Lab 6: Circuit characterization with plate reader and fluorescence microscope

In-Fusion PCR Cloning Kit



PCR (Polymerase Chain Reaction)



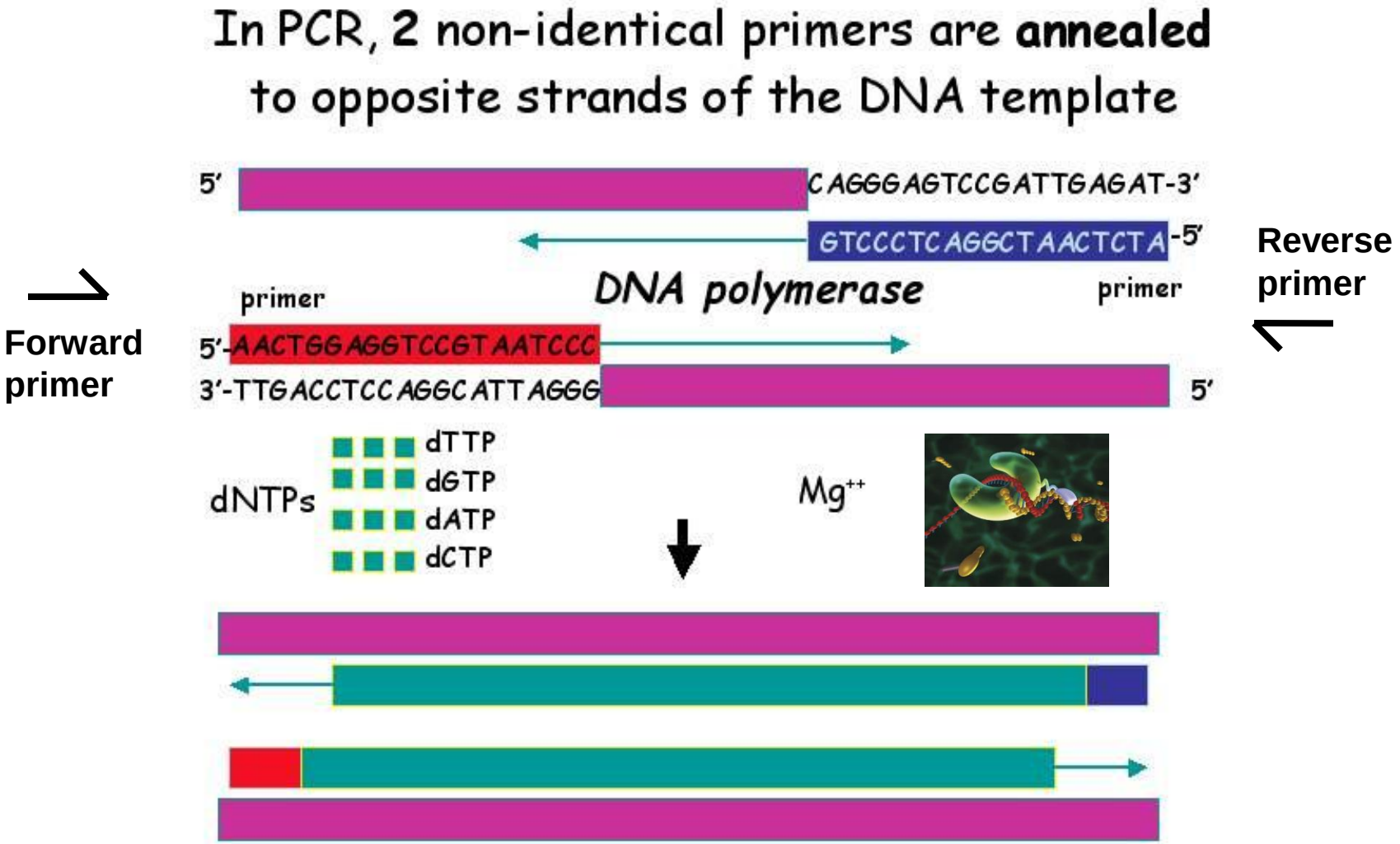


Taq Polymerase

- **Primers are single-stranded DNA sequences that specify where to start extension/polymerization**

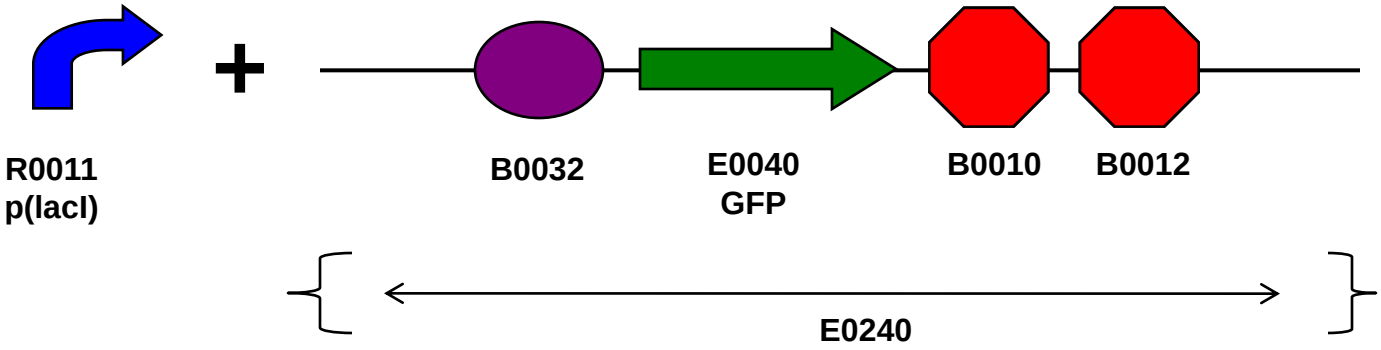
- **The primer sequence will be built into the PCR product**

PCR Primers


In PCR, 2 non-identical primers are annealed to opposite strands of the DNA template
5′
CAGGGAGTCCGATTGAGAT-3′
GTCCCTCAGGCTAACTCTA-5′
Forward primer
primer
DNA polymerase
primer
Reverse primer
5′-AACTGGAGGTCCGTAATCCC
3′-TTGACCTCCAGGCATTAGGG
5′
dNTPs
dTTP
dGTP
dATP
dCTP
Mg++

GFP circuit assembly

BioBricks



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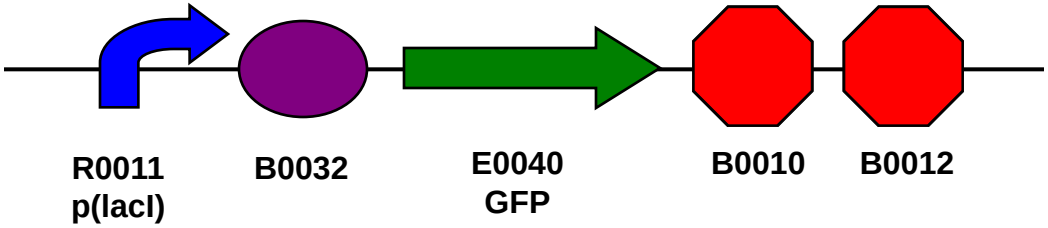
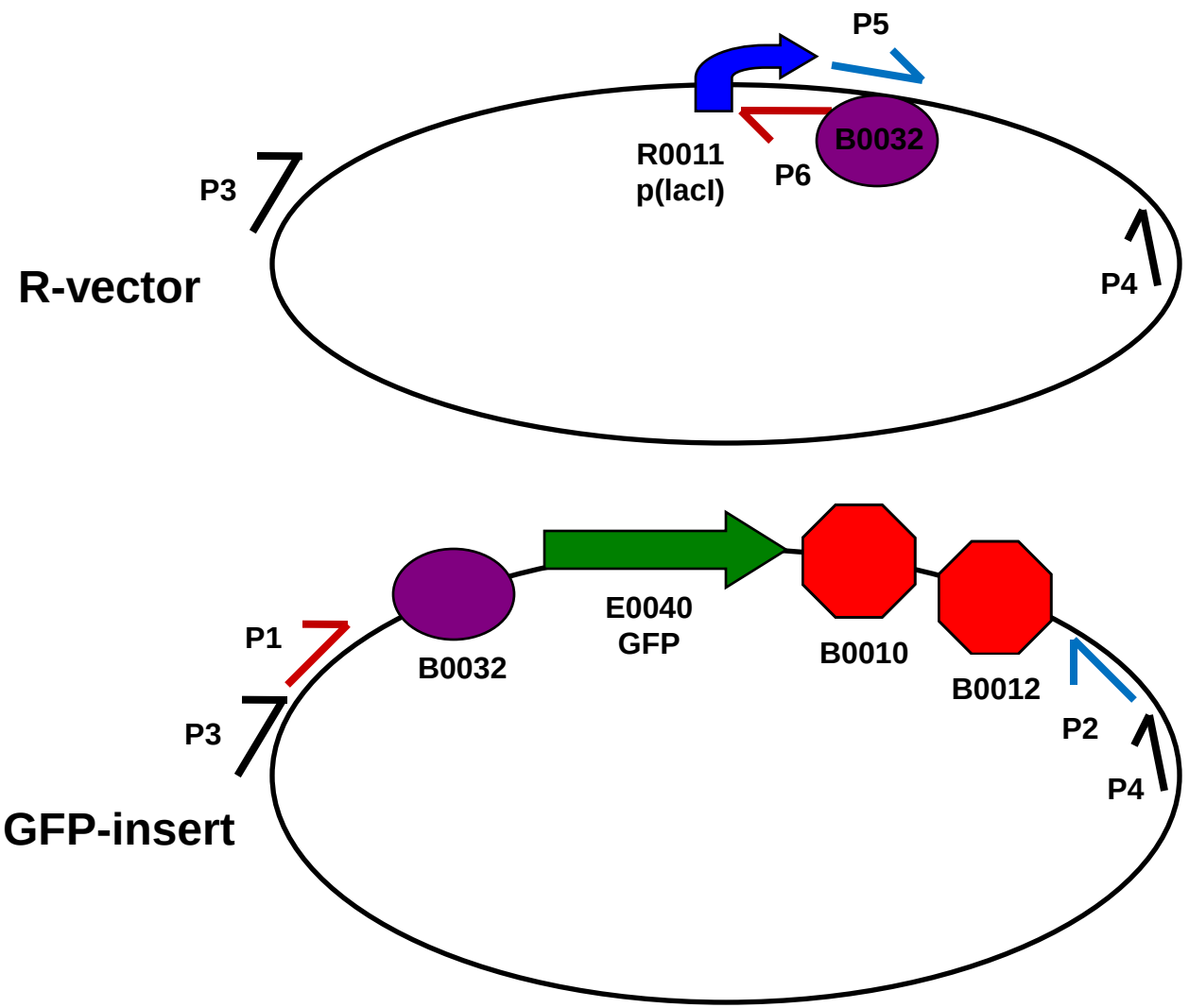
GFP Circuit



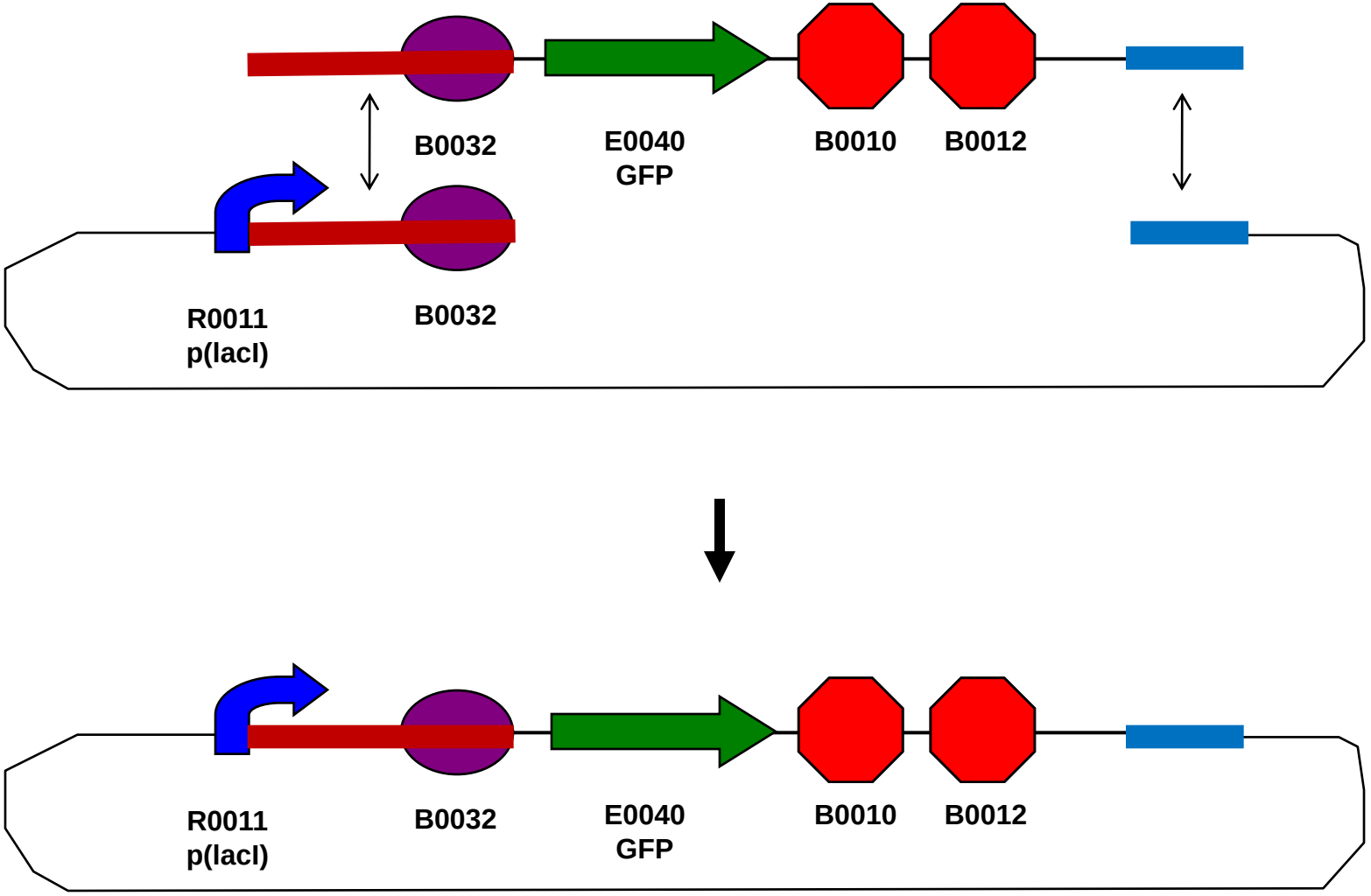
Primer design for PCR-amplifying the R-vector and GFP-insert



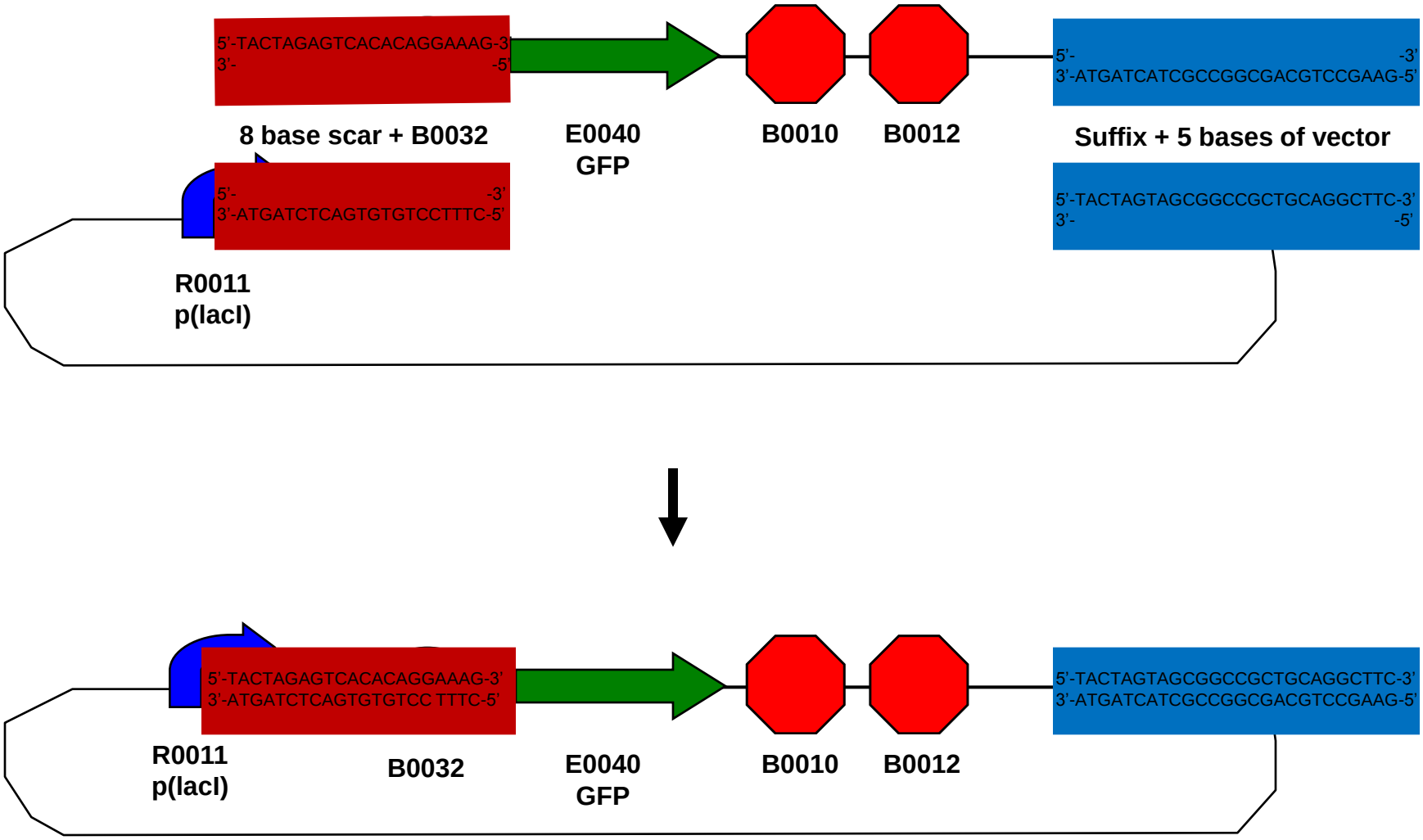
Forward (P5) = suffix + 5 bases vector
Reverse (P6) = last 20 bases R0011 + scar + B0032 (RC)

Forward (P1) = scar + B0032 + scar + first 2 bases E0040
Reverse (P2) = suffix + 5 bases vector (RC)

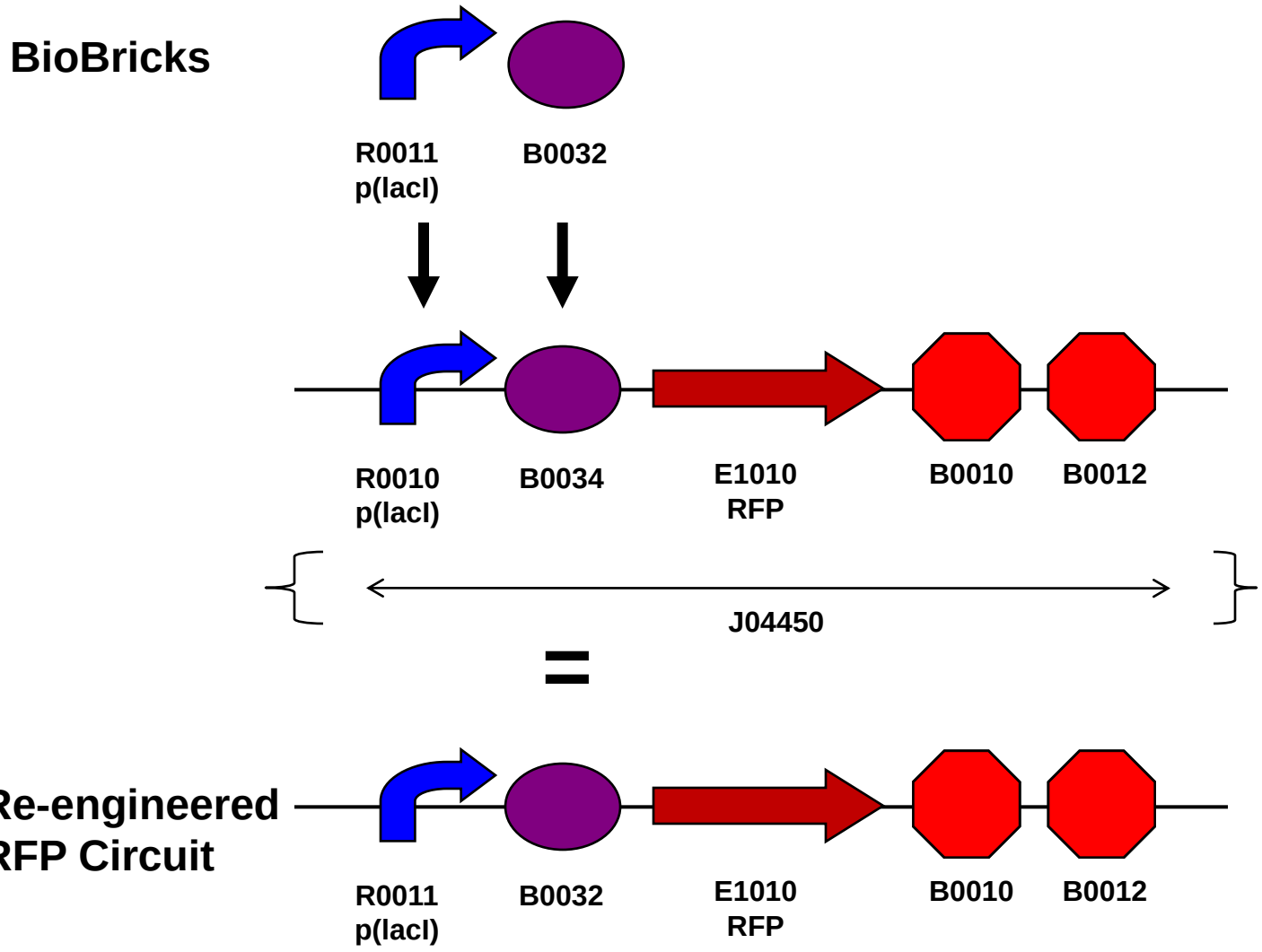
In-Fusion GFP circuit construction



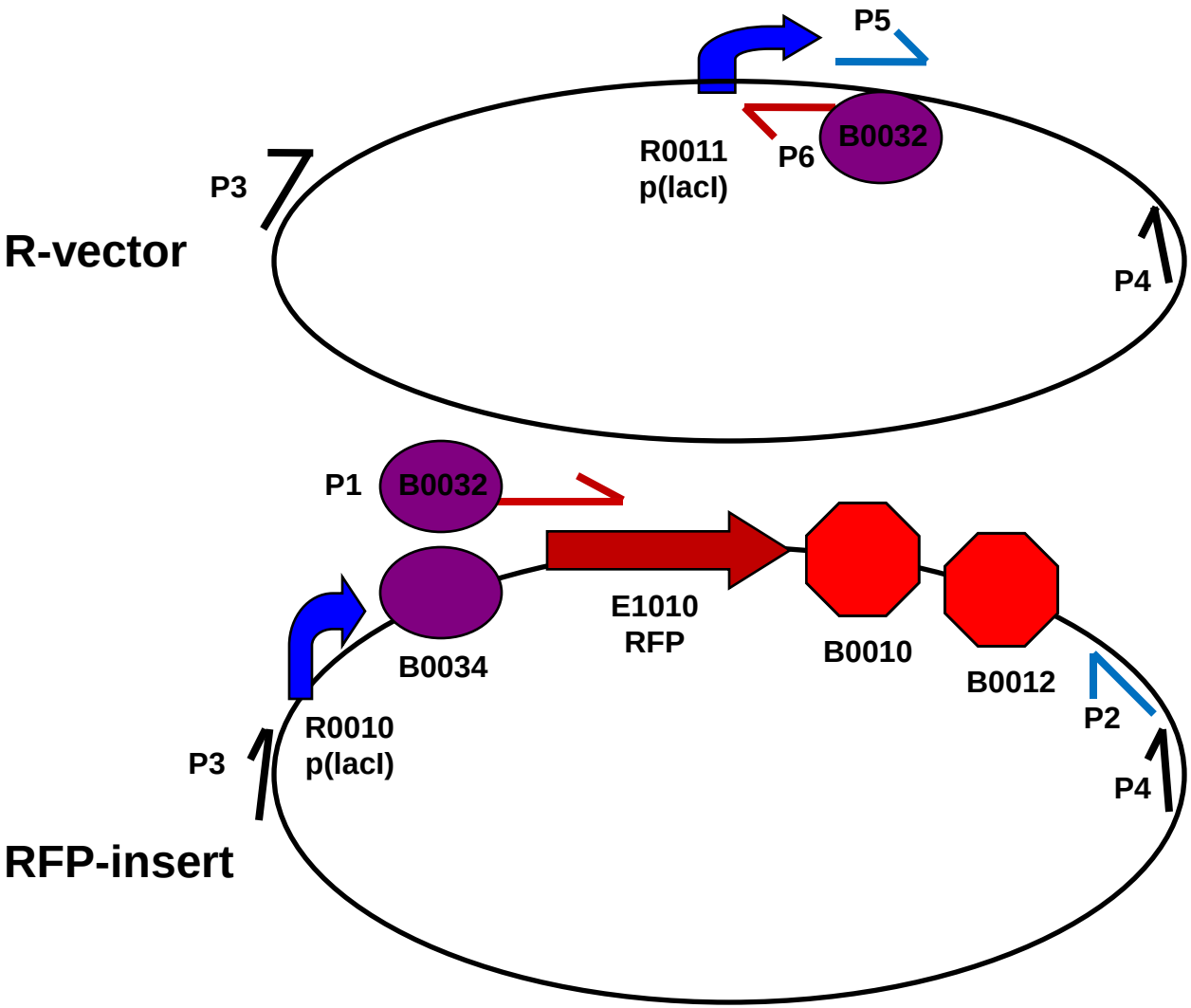
In-Fusion GFP circuit construction



RFP circuit re-engineering



Primer design for PCR-amplifying the R-vector and RFP-insert



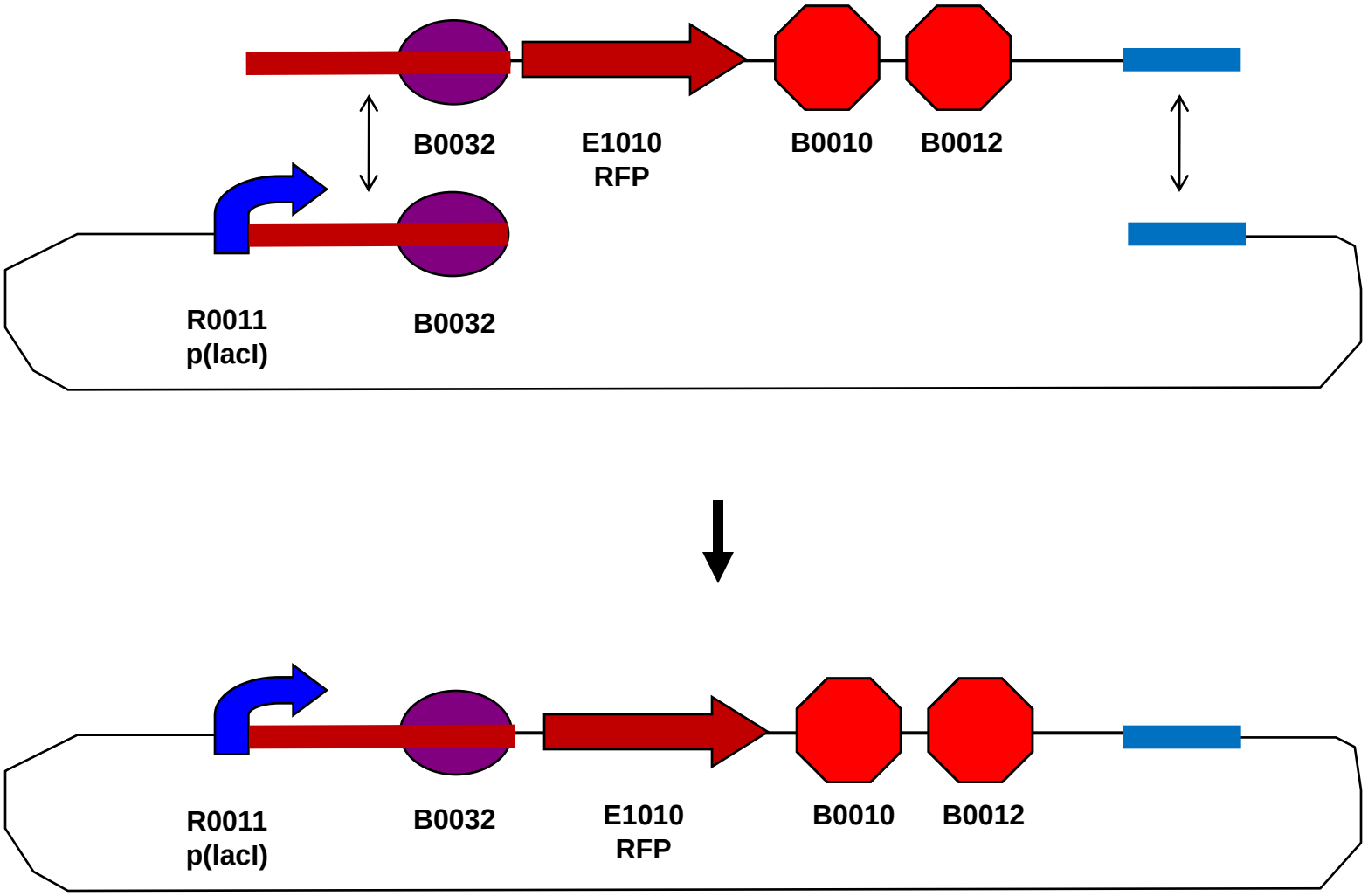
Forward (P5) = suffix + 5 bases vector

Reverse (P6) = last 20 bases R0011 + scar + B0032 (RC)

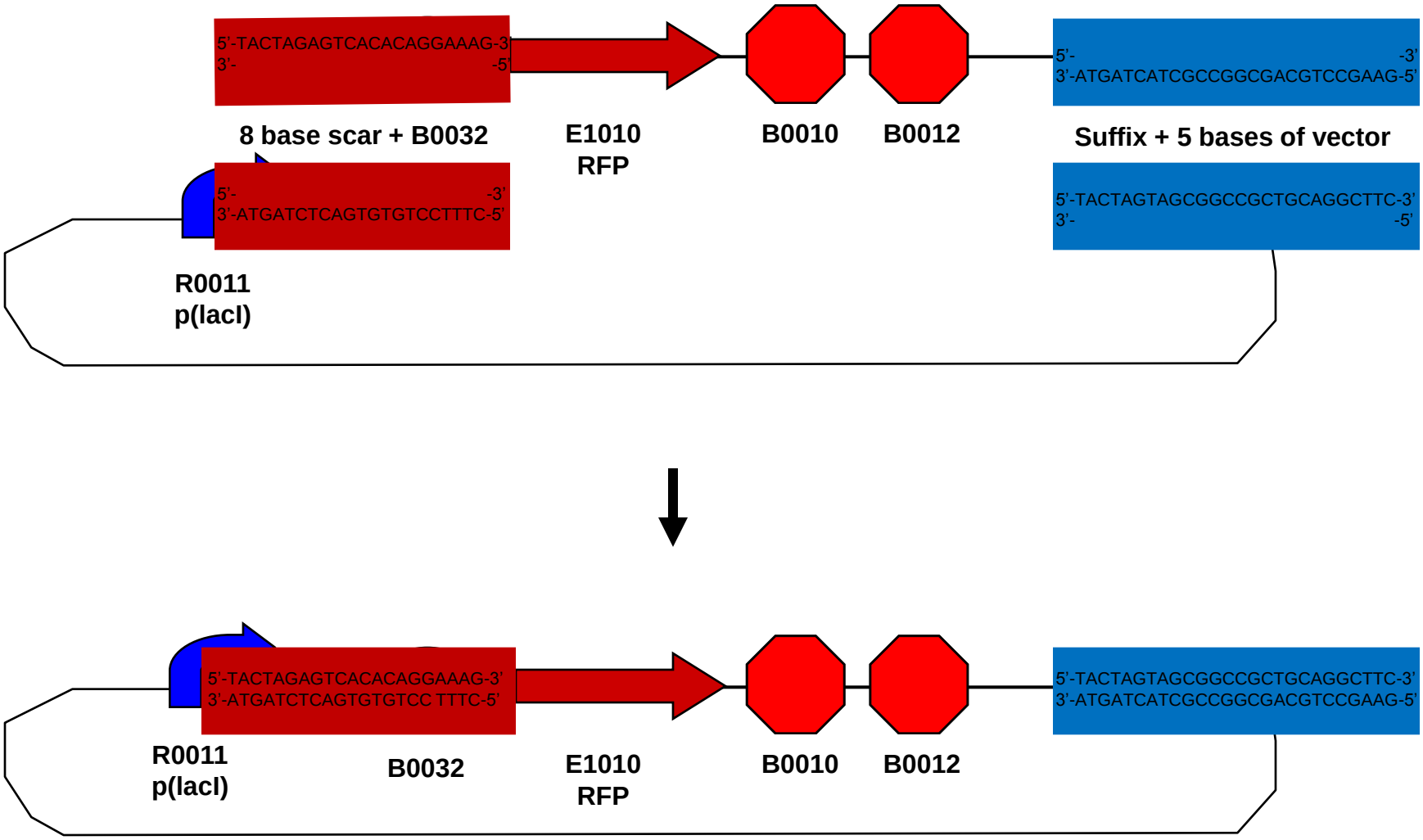
Forward (P1) = B0032 + scar + first 25 bases E1010

Reverse (P2) = suffix + 5 bases vector (RC)

In-Fusion RFP circuit construction



In-Fusion GFP circuit construction



PCR in Practice

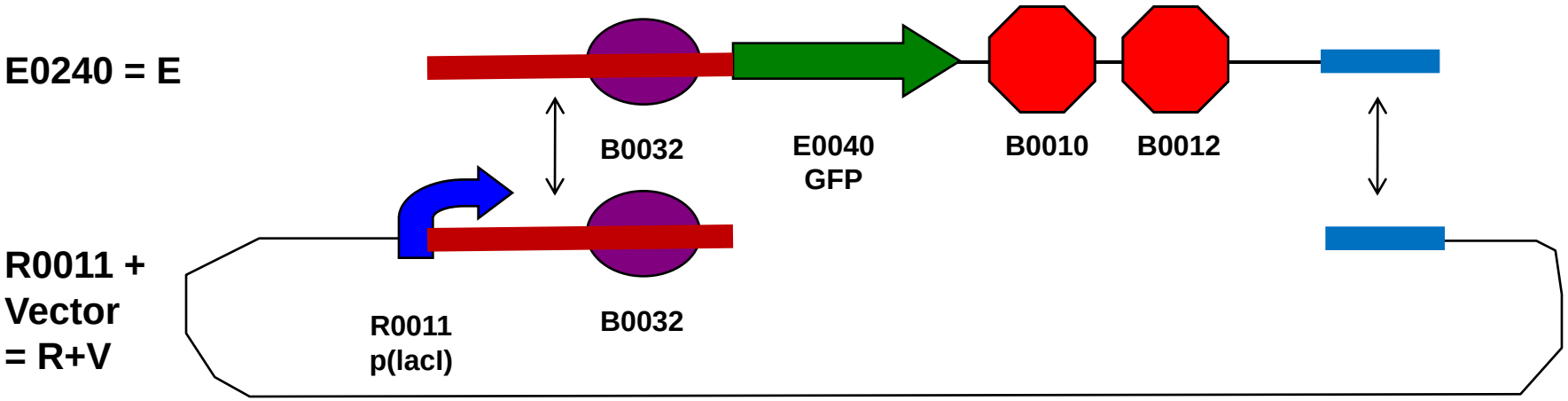
Combine:

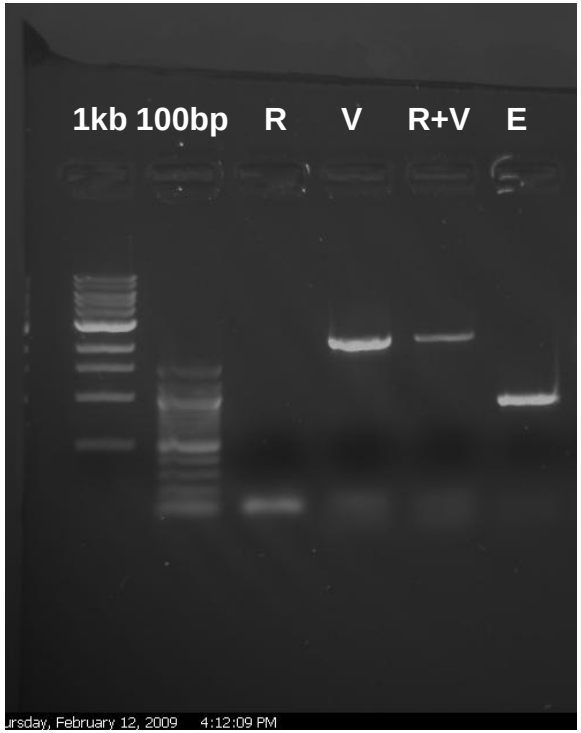
Supermix
- **Taq**
- **Proof reading enzyme**
- **dNTPs**
- **Buffer**
- **Water**

Forward primer
Reverse primer
Template DNA
(1:1000 plasmid)

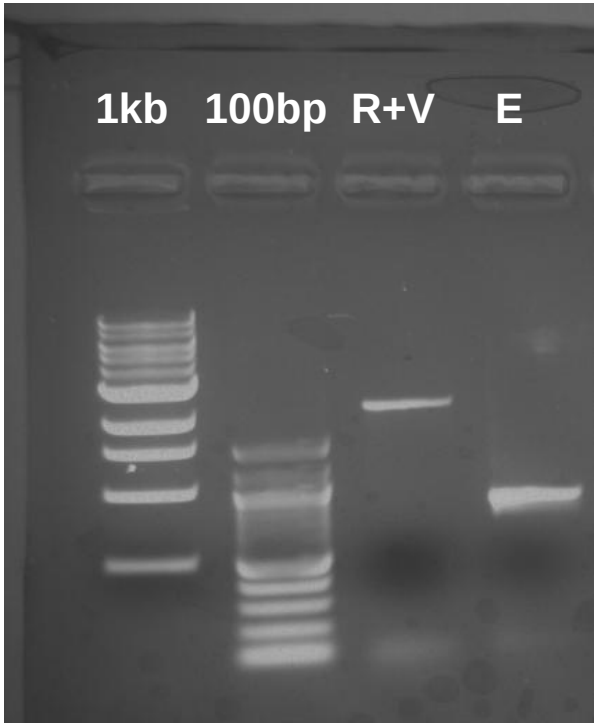
Thermocycler

PCR Products





Purified PCR Products



In-Fusion PCR Cloning Kit Procedure

R+V PCR Product (~2000 bp) 33.79 ng/ul
E PCR Product (~1000 bp) 64.12 ng/ul

Want a 2:1 insert:vector molar ratio and 100 ng vector.
ng E = 2 X (1000/2000) X 100 = 100 ng E

100 ng R+V = 3 ul
100 ng E = 1.5 ul
Water = 5.5 ul
Total = 10 ul

In-Fusion PCR Cloning Kit Procedure (continued)

10 ul reaction

15 min 37C, 15 min 50C

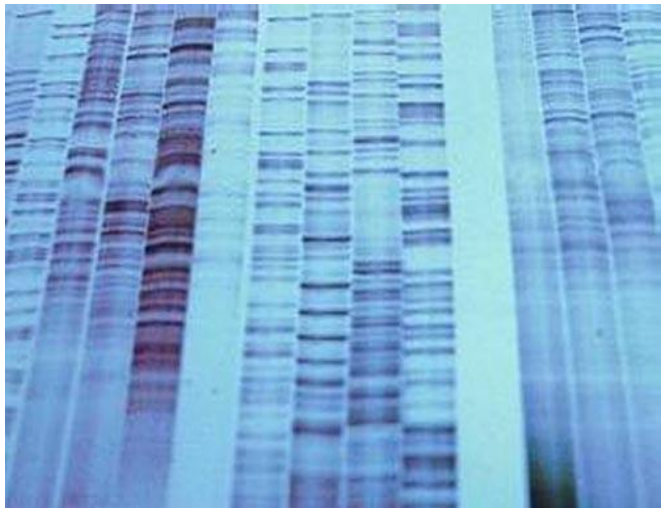
Dilute in TE Buffer

Transformation

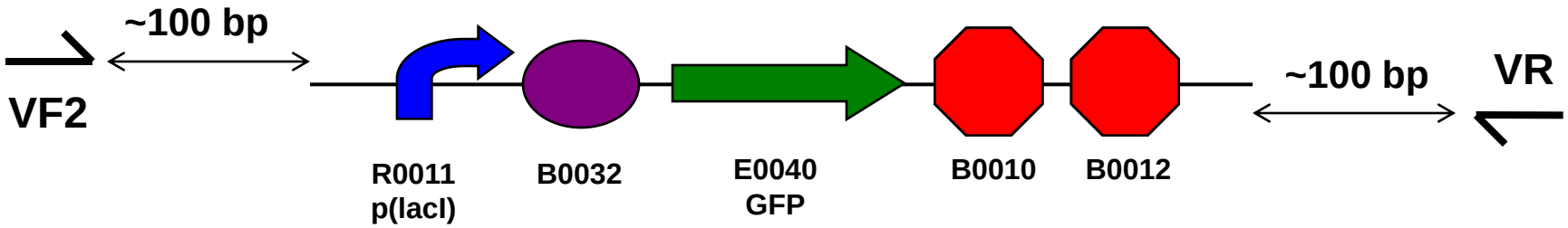
Colony PCR

Overnight culture of successful transformants, minipreps, sequence

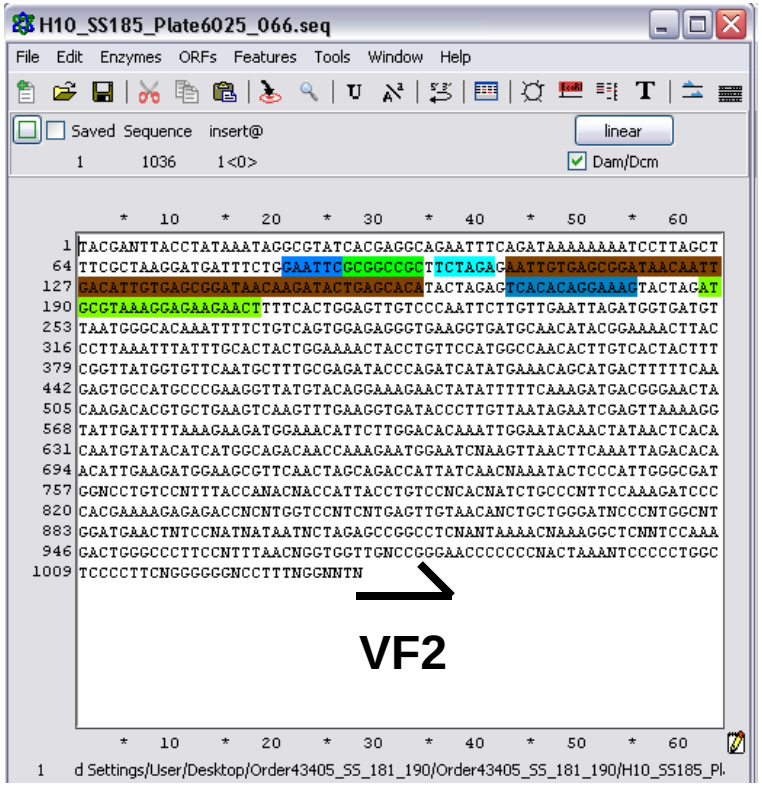
DNA sequencing

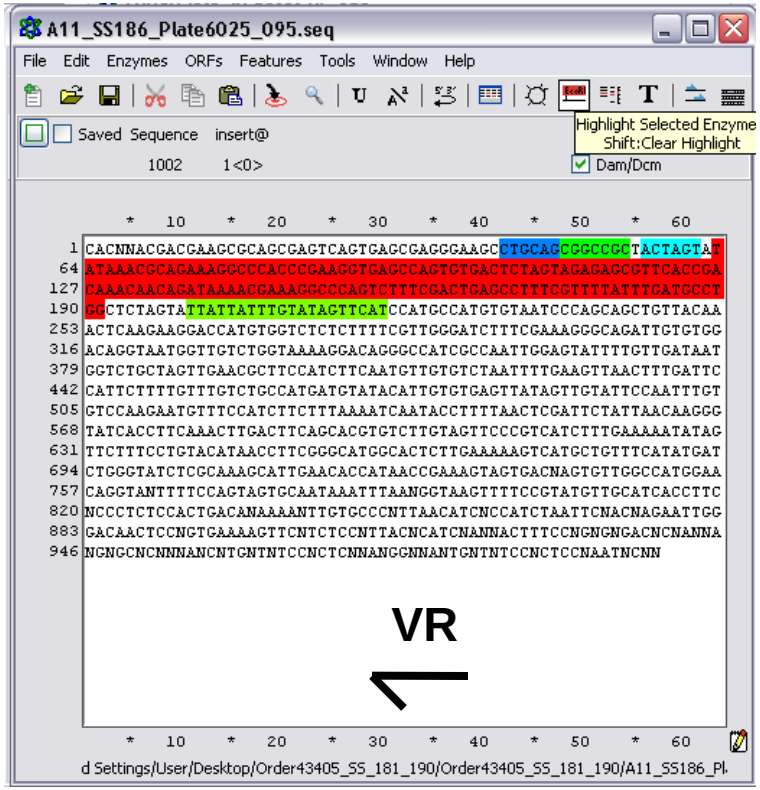


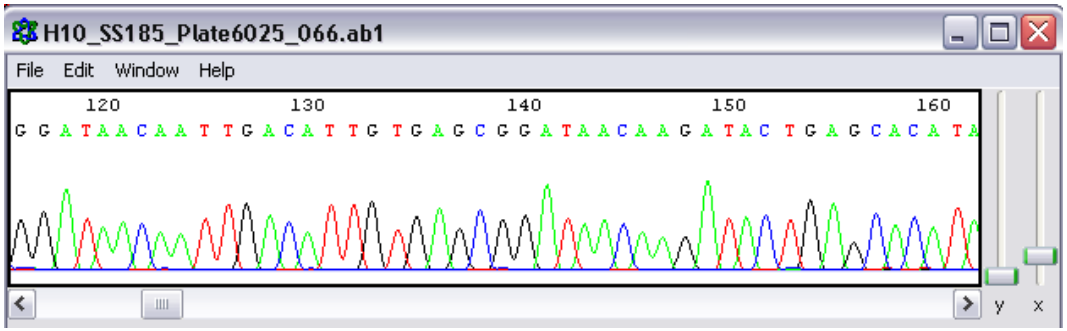
- UW Sequencing Facility requires 1200 +/- 300 ng plasmid DNA per reaction
- Normally a good miniprep gives about 300 ng/ul
- Add 4 ul miniprep, 1 ul primer, and 7 ul water for 12 total ul into eppy tube
- Submit labeled tube to sequencing facility with filled out form



Analyzing sequence data



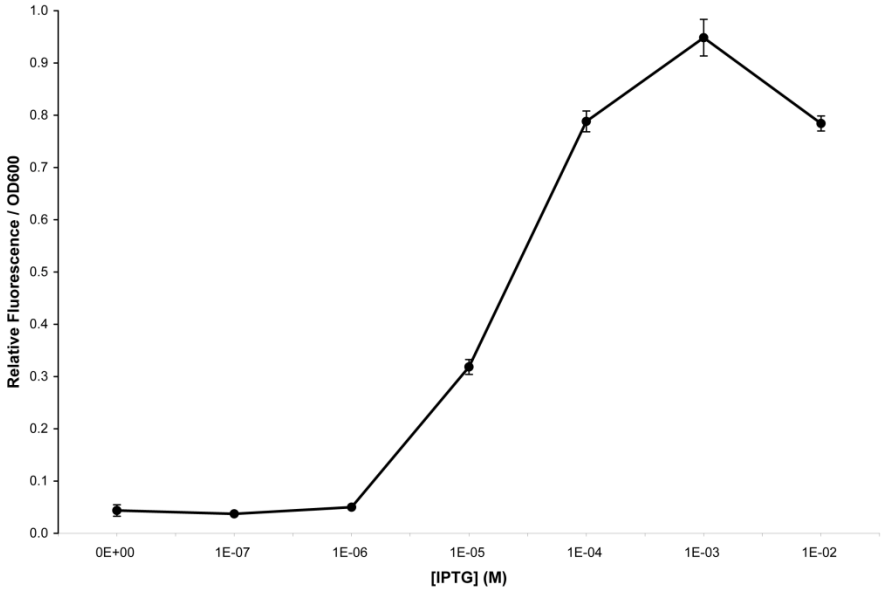




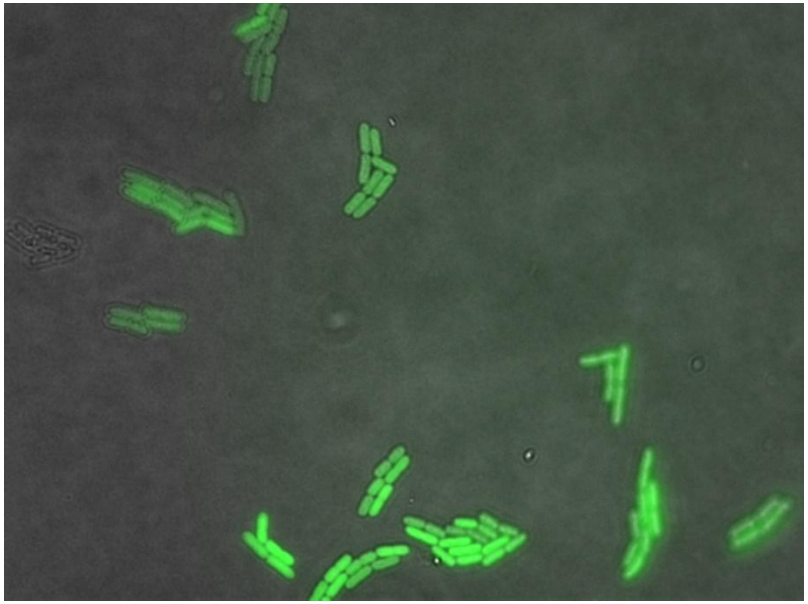
A Plasmid Editor (APE): http://www.biology.utah.edu/jorgensen/wayned/ape/

Circuit characterization

Population level



Single cell level



Transfer curve:
Population level circuit characterization

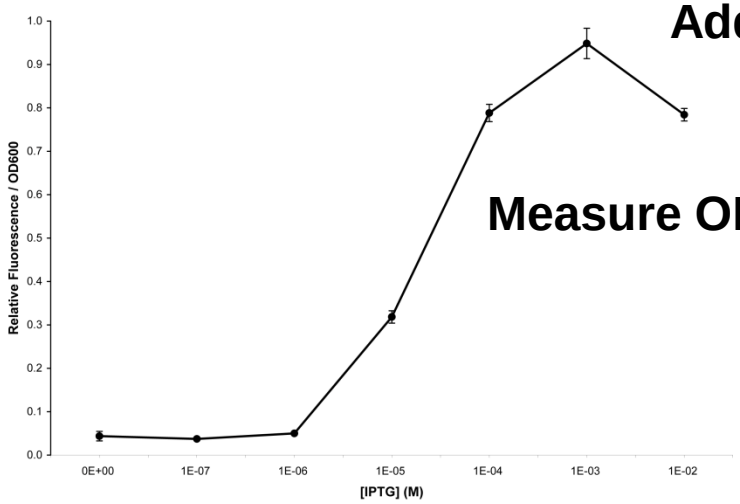
Grow culture overnight

Dilute 1:100 and grow to OD 0.2

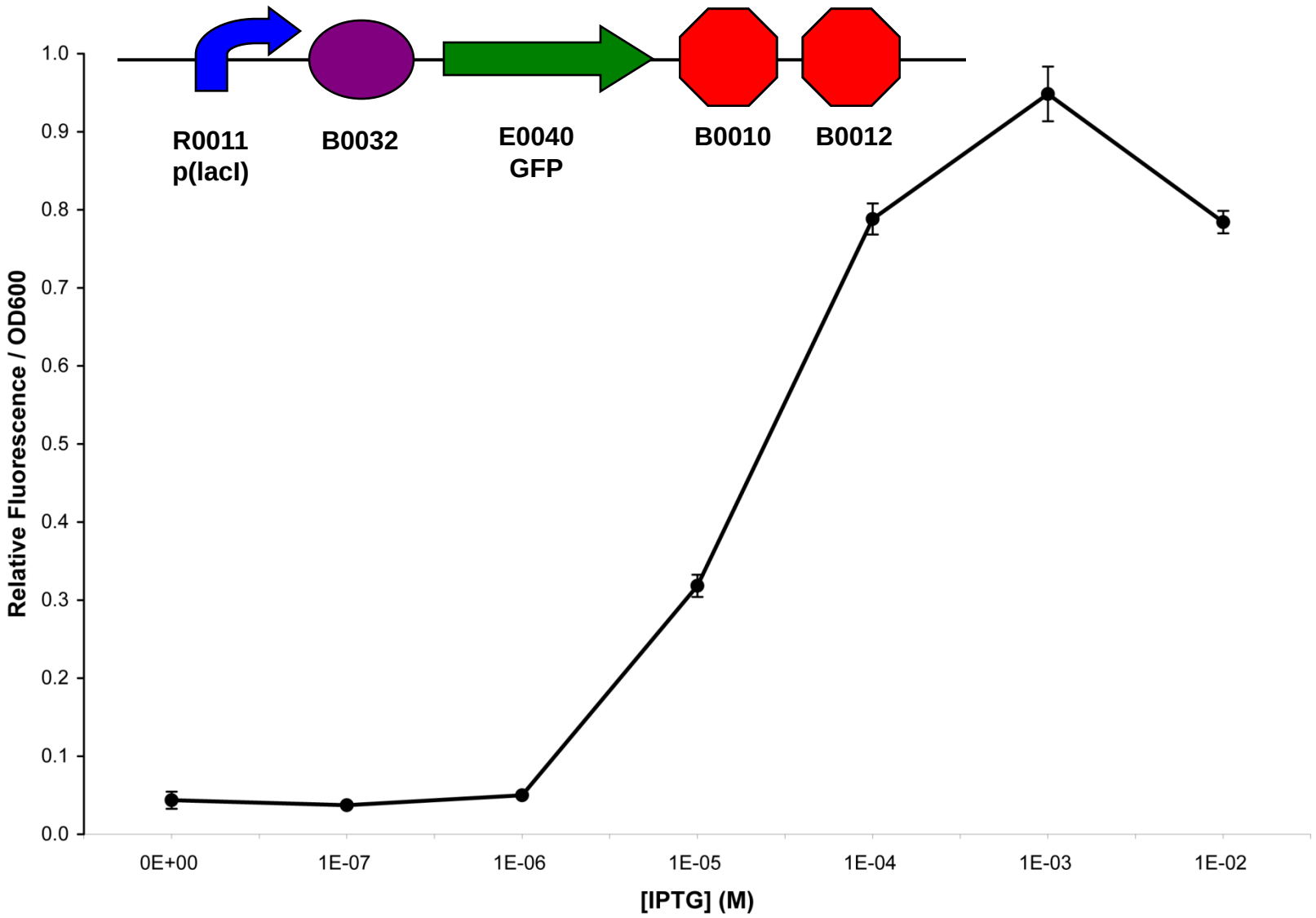
Dilute 1:10 and add IPTG

Add to 96-well plate

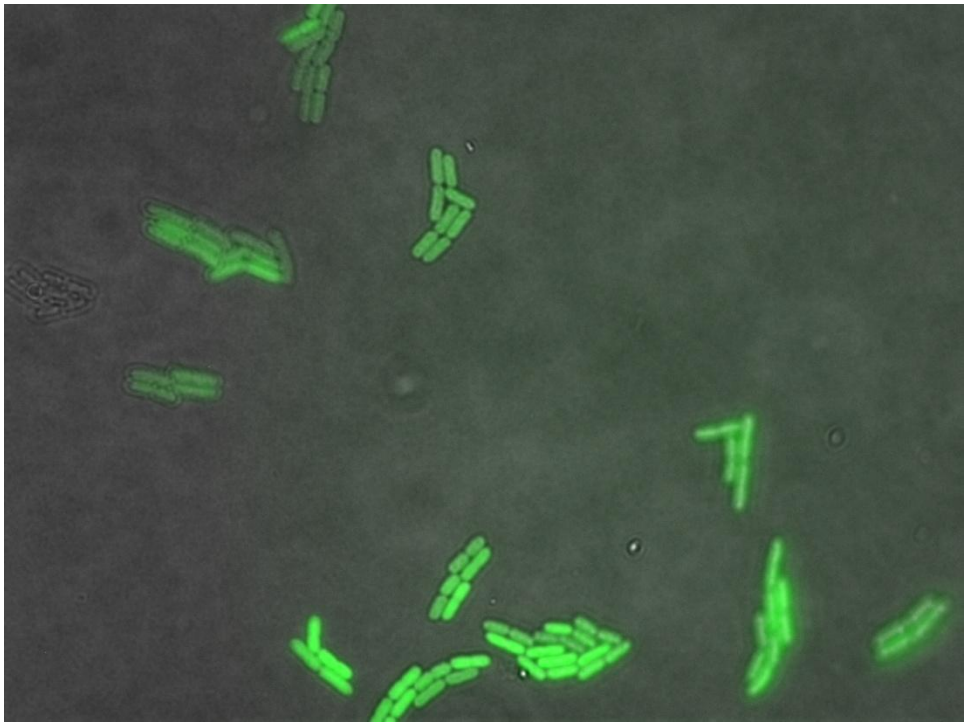
Measure OD and fluorescence over time



Transfer curve



Fluorescence microscope movies: Single cell level circuit characterization



- Dilute overnight culture to a single cell via serial dilution
- Setup correct filter on microscope for GFP excitation/emission
- Set temperature
- Place single cell on agar pad with IPTG
- Hit the record button!
- Track fluorescence of every cell in microcolony