Name: Tishi Chowdhury

Date: 06/16/14

WEEKLY ONE SENTENCER:

Finish ~~testing~~ assembling the promoters & RBS with CMV.

DAILY ONE SENTENCER:

Complete plasmid digest, digestion of gel extracted and purified DNA then do a ligation. (Promoter L1 & all RBS) ✓ Also, transform. ✓ Wow!

METHOD(S) OF CHOICE:

General digestion & ligation

*Mention enzyme pairs: X, P

P L1 & RBS

EXPECTED RESULT:

Proper bands on 1kb ladder of 2.8kb. ☺ - Ligation

~2 kb pSB1C3 vector

~700bp mCherry

(☑ check if APE file exists on Drive)

TEMPLATE(S): mCherry (use stock name)

OLIGO(S): N/A ✓

THERMOCYCLER SETTINGS: 1'-37°, 20"-80°, 4° forever. ✓

CONTROLS: ↓ Heat inactivate enzymes.

POSITIVE: pSB-1c3-mCherry → 2kb band & 700bp band! ✓

NEGATIVE: Without enzymes. ✓

CONFIRMATION?

Gel electrophoresis ✓

mCherry gel = good!

★ GEL LANE ORDER:

mCherry Vector: L 1 2 3 4 5 6

RBS+mCherry: ~~1kb~~ MC1P MC2P MC3P MC4P MC5P MC6P MC1N MC2N MC3N

RBS neg: MC4N MC5N MC6N [negatives had no polymerase]

GEL PICTURE / OTHER SPACE:

on google drive 140616 RBS + mCherry Negatives and Positives

★Second PCR of Promoters & 363 with new mCherry templates attempting to amplify @ 65°C

★ Did RBS primer PCR at 65°C
↳ Used Template 1 (conc. ~~207.5~~) (mCherry)

★ Miniprep mCherry cultures.

6/16 : PCR

template: Miniprep mCherry 1 (207.5)

Oligos: RBS primers (VF) 074-079

0363

Settings: 65°C Program

Negative: No polymerase.

Name: Kathryn Fitton

Date: 6/16/19

CONFIRMATION?
thin gel

WEEKLY ONE SENTENCER:
continue testing RBS primers/promoter

GEL LANE ORDER:

DAILY ONE SENTENCER: ~~D w/ which enzymes~~ (tbd) → ok: fill out during day (pm) ✓
digest plasmid 5' RBS primer/promoter 3'
ligate together, miniprep mcherry cultures

GEL PICTURE / OTHER SPACE:

METHOD(S) OF CHOICE:
digestion w/ x 2 p
ligation
gel extraction TRANSFORM!

EXPECTED RESULT:
✓ Plasmid digest → ~~[illegible]~~ 2 Kb; 700 bp
✓ insert digest → ~~[illegible]~~ 700-800 ish
ligation → 2.8 Kb ✓

(☒ check if APE file exists on Drive)

TEMPLATE(S): mcherry ✓

OLIGO(S): — ✓

THERMOCYCLER SETTINGS: 1 hr @ 37° C ; 20 min @ 80° C ; 4° C forever

CONTROLS:

POSITIVE:
~~[illegible]~~ digested mcherry
700 & 2 Kb band ✓

NEGATIVE:
w/o enzymes ✓

Name: Jennifer Zhang

Date: 6/16/14

WEEKLY ONE SENTENCER: continue testing the RBS primer / promoter pairs

DAILY ONE SENTENCER: digest plasmid and our RBS primer/promoter insert and ligate

which?

METHOD(S) OF CHOICE:
plasmid / insert digestion (w/ xhoI and ~~PstI~~ PstI) ✓
gel extraction
ligation
transformation

EXPECTED RESULT:
plasmid digest → ~~4.5~~ 2 kb
insert digest → ~~?~~ 700-800 kb
ligation → ~2.5 kb

(☒ check if APE file exists on Drive)

TEMPLATE(S): [mcherry / RBS primer/promoter w/ mcherry] which template

OLIGO(S): —

THERMOCYCLER SETTINGS: 1hr - 37°C ; 20min - 80°C ; 4°C - forever

CONTROLS:

POSITIVE:

psB1c3-mCherry → 700 bp. ? 2kb band

NEGATIVE:

ΦEnzymes

CONFIRMATION?

GEL LANE ORDER:

mCherry Vector: L 1 2 3 4 5 6

GEL PICTURE / OTHER SPACE:

- miniprep mcherry cultures ok!
- 6/16 PCR of
  template: miniprepped mcherry 1 (207.5 ng/mL)
  oligos: RBS primers (VF) 074 - 079
  0803
  settings: ~~RBS prim~~ RBS605 program
  ↳ 60sec

negative: no polymerase

→ gel extracted all lanes except RL2

→ reran RBS promoter PCR w/ the gel extracted products from previous RBS promoter PCR as templates
↳ 65°C

Name: Coleen Tran

Date: 6/16/14

WEEKLY ONE SENTENCER: redo RBS/promoter combinations

DAILY ONE SENTENCER: ~~xxxx~~ digest + ligate RBS/promoters into mCherry vector

which? P_{Hz} RBS ...

METHOD(S) OF CHOICE:

digest w/ X and P

EXPECTED RESULT:

plasmid ⇒ 2.8 kb ✓ undigested; 2kb digested

700 bp digested → mCherry

(☐ check if APE file exists on Drive) ✓

TEMPLATE(S): mCherry + RBS/promoter which??

OLIGO(S):

THERMOCYCLER SETTINGS: 1 hr 37°; ~~30~~ 10' - 80°C ; 4°C

CONTROLS:

POSITIVE:

mCherry template w/ X+P

(mCherry 1)

NEGATIVE:

no enzyme

CONFIRMATION?

gel

GEL LANE ORDER: RBS + mCherry

L	MCP 1	MCP 2	MCP 3	MCP 4	MCP 5	MCP 6

GEL PICTURE / OTHER SPACE:

On google drive

140616 RBS + mCherry Gel Purify

Today: PCR w/ new mCherry template (gel confirmation on Google Drive) + CBS primer

Name: Stefan Tassoulas

Date: 06/16/2014

WEEKLY ONE SENTENCER:
Characterize Promoter-RBS pairs w/ mCherry PL2 ✓
Submit Funding Proposals to Companies ✓ really keep writing this

DAILY ONE SENTENCER:
- Miniprep ~~DNA~~ mCherry clone
- PCR clone ~~RBS~~ into ~~new DNA~~ mCherry
(which?)

METHOD(S) OF CHOICE:
- Rxn PCR
- QiaCube
- Gel extract

EXPECTED RESULT:
~900 bp RBS_mCherry ✓ ??which
~ clean DNA ✓ ~2.7 ng/µL

(☐ check if APE file exists on Drive) ✓

TEMPLATE(S): pSB1C3_mCherry ✓ ← each of these w/ 0363?

OLIGO(S): 073-078; 0363

THERMOCYCLER SETTINGS: 94-2' { 94'-15", ~~65~~ 60?-15"; 68°C-1.5' } x31 ✓ -68°C-4'; 4°C ✓

CONTROLS:

POSITIVE:
w/ polymerase

NEGATIVE:
w/o polymerase ✓

CONFIRMATION?
Bright band at ~900 bp ✓

GEL LANE ORDER: 1 2
Lbb RH1 RH2 RM1 RB1 RM2 RL1 RL2 ✓

GEL PICTURE / OTHER SPACE:

RBS PCR

1	2	3	4	5	6
074	75	76	77	78	79