Name: Stefan Tsesoulas

Date: 06/18/2014

WEEKLY ONE SENTENCER: Characterize promoter-rbs-RFPcherry Funding, comp cells, clones

DAILY ONE SENTENCER: prep-Comp cells Funding / world cup

METHOD(S) OF CHOICE:

- ~~Receive~~ prepare Reagents (SOB, agar plates)
- TV, ~~pub~~
- ~~Phone for calling~~ ok...

EXPECTED RESULT:

- success
- Cells prepared - culture started

(☐ check if APE file exists on Drive)

TEMPLATE(S):

OLIGO(S):

THERMOCYCLER SETTINGS:

CONTROLS:

POSITIVE:

NEGATIVE:

CONFIRMATION?

GEL LANE ORDER:

GEL PICTURE / OTHER SPACE:

Name: Tishi Chowdhury

Date: 6/18/14

WEEKLY ONE SENTENCER:
- Complete testing for RBS primers & promoters.
- Make competent cells.

DAILY ONE SENTENCER:
~~- Digesting two of Tay's sample~~
~~- Make competent cells~~
- Do cell cultures & make frozen stocks.
- Colony PCR

METHOD(S) OF CHOICE:
~~- Digestion & ligation, per, gel extract of plasmid & mCherry products.~~
- Cell cultures.
- PCR

EXPECTED RESULT:
- ~ 1000 bp bands on gel ✓
~~- OD between 0.05 - 0.07 for comp. cells~~

(☑ check if APE file exists on Drive)

TEMPLATE(S): RBS mCherry clones ✓

OLIGO(S): ~~0362~~ RBS primer (H1, H2, M1, M2, L1) ~~& 0363~~ / RBS primer.

THERMOCYCLER SETTINGS: 94 -2', {94'-15", 72-15", 68°C - 1.5'}x31, 68°C-4', 4C ✓

CONTROLS:

POSITIVE: ~~RBS~~ Amplified mCherry. ✓
~7006p

NEGATIVE: No polymerase ✓

CONFIRMATION?
Gel ✓

GEL LANE ORDER: Colony PCR
1kb RL1 RL1 RL1C RL1C RH2 RH2 RH2C RH2C RH1 RH1C

GEL PICTURE / OTHER SPACE:
On drive named as ~~Colony~~ 140618 Colony PCR RBS+mCherry

*Also set up cell cultures of the selected A & B colonies! (in 37°C incubator

T4 Ligase → 1uL
ATP → 2uL (1uL)
Ligase Buff → (2uL)

Name: Coleen Tran

Date: 6/18/14

WEEKLY ONE SENTENCER:

Working on RBS + promoter combinations in mCherry

DAILY ONE SENTENCER:

Colony PCR + culture RBS+mCherry ✓

METHOD(S) OF CHOICE:

colony PCR , cell culture

EXPECTED RESULT:

~1000 bp ✓

(☐ check if APE file exists on Drive)

TEMPLATE(S): ~~RBS primer , 363~~ RBS+mCherry clones ✓

OLIGO(S): RBS primer , 363 ✓

THERMOCYCLER SETTINGS: 94 - 2' ; {94' - 15" ; 60 - 15" ; 68 - 1.5' } x31 - 68 - 4' ; 4C ✓

CONTROLS:

POSITIVE:

NEGATIVE:

no polymerase ✓

CONFIRMATION?

gel ✓

GEL LANE ORDER:

L RH1 RH1N RM1 RM1 RM1N RM1N _ RM2 RM2 _

GEL PICTURE / OTHER SPACE:

RM2N RM2N

140618 Colony PCR RBS + mCherry

Name: Jennifer Zhang

Date: 6/18/14

WEEKLY ONE SENTENCER: continue RBS primer/promoter pairs testing

DAILY ONE SENTENCER: ~~make cultures and perform colony PCR~~ ~~with promoters~~ ligation transformation ~~with~~ ~~primers~~ from RBS primer

→ digest Troy's promoter w/ mCherry at xbaI and pst I ←

METHOD(S) OF CHOICE: ~~PCR~~ PCR purification + digest mCherry X/P

~~PCR~~ gel

digest at x/p

Ligate + Transform

PSB1C3

EXPECTED RESULT:

vector band ~ 2kb ✓

insert band ~ 808

(☒ check if APE file exists on Drive)

TEMPLATE(S): promoter RBS mCherry | PH1 RM1 | PH2 RL1

OLIGO(S):

THERMOCYCLER SETTINGS: 60'-37°C; 80°C-20'; 4C-4eva

digest

CONTROLS:

POSITIVE:

insert: 808 bp

vector: 2kb

NEGATIVE:

no enzymes

CONFIRMATION?

gel

GEL LANE ORDER:

1kb negative mCherry vector

GEL PICTURE / OTHER SPACE: 140618_PSB1C3_x/p_ends.jpg

digest:

insert:

- 12.5 ~~NB~~ mL DNA
- 0.5 mL xbaI
- 0.5 mL pst I
- 2 mL buffer
- 4.5 mL H_2O

vector:

- 5 mL DNA
- 0.5 mL XbaI
- 0.5 mL pst I
- 2 mL ~~dddd~~ buffer
- 12 mL H_2O

ligation:

PH1 : 3.09 (insert)
RM1 : 1.91 (vector)

PH2 : 2.96 (insert)
RL1 : 2.04 (vector)

T4 ligase → 1 mL
ATP → 2 mL
ligase buffer → 2 mL

- funding
- checklist of items (verify)
- think about outreach

Name: Kathryn Fitton

Date: 6/18/14

WEEKLY ONE SENTENCER:

Keep testing RBS primers/promoters ✓ → which (PH1 RM1) & (PH2 RL1)

DAILY ONE SENTENCER:

digest mcherry plasmids; 2 promoter RBS mcherry's; look at colonies from plates; grow up cultures from colonies; make competent cells

METHOD(S) OF CHOICE:

Digestion @ 37°; run gel for mcherry's extracts; purify; purify PCR product digest of promoter RBS mcherry's; ligate; transform

ligation ✓

transformation ✓

gel extraction ✓

gel purification

PCR purification

EXPECTED RESULT:

colonies on LB/cm plates ✓

band @ 2kb on 8 mcherry plasmid gel ✓ → how large is the promoter-RBS-mCherry

(☒ check if APE file exists on Drive)

TEMPLATE(S): mcherry / Prom-RBS-mcherry

OLIGO(S):

THERMOCYCLER SETTINGS: 60'-37°C; 20'-80°C; 4°C 4ever

CONTROLS:

POSITIVE:

NEGATIVE:

w/o enzymes ✓

CONFIRMATION?

gels

GEL LANE ORDER:

Ladder Negative mcherry

GEL PICTURE / OTHER SPACE:

140618_PSB1C3_x/p_ends.jpg

Two promoter RBS pairs testing:
PH1 RM1 & PH2 RL1
Two primer promoter pairs

T4 ligase → 4 µL

ATP → 8 µL

Ligase buffer → 8 µL