

Name: fishw.C + Colleen

Date: 06/20/14

WEEKLY ONE SENTENCER:

Finally complete testing the RBS primers & promoters with mCherry ✓

DAILY ONE SENTENCER:

- Test Promoters H1, M1, M2 & L2 with RBS mCherry. possibly help make competent cells. Min prep to cell cultures. ✓
- PCR with promoters, gel extract & purify ✓
- find 5' & 3' location of P.L. to sequencing. ✓
- METHOD(S) OF CHOICE:
 - General PCR @ 65°C ✓
 - Gel extraction ✓
 - Purification by hand ✓
 - Nanodrop ✓
 - Alu probe of nanodrop ✓
- EXPECTED RESULT: ✓
- Bands @ 1kb ✓
- Good concentration on nanodrop. ✓

(✓ check if APE file exists on Drive)

TEMPLATE(S): RBS primer + mCherry

OLIGO(S): 0363 + 0362 - 80/82/85 ✓

THERMOCYCLER SETTINGS: 94-2', 94-15", 65-15", 68-1.5' x31 - 68C-4' M.C. ✓

CONTROLS:

POSITIVE: ~~vector + promoter~~ X

NEGATIVE: No polymerase

CONFIRMATION? Gel extraction. ✓

GEL LANE ORDER:

For PH1: 1kb RH1 RH2 RNL RY2 RL1 RLIN

P2: 1kb RH1 RH2 RM1 RM2 RL1 RLIN

GEL PICTURE / OTHER SPACE:

PH2: 1kb RH1 RH2 RM1 RM2 RLIN
PH4: 1kb RH1 RH2 RM1 RM2 RL1,N

*Gel extracted for P2 & PH1

-Purified the ten extracts.

File 140620PromoterM1_RBS_mCherry

140620PromoterP2_RBS_mCherry.

140620PromoterM2_RBS_mCherry

140620PromoterH1_RBS_mCherry.

Name: Kaitlyn Fitton

CONFIRMATION?

Date: 6/26/14

221

WEEKLY ONE SENTENCER:

GEL LANE ORDER:

(continue testing promoters RBS primer) ✓

DAILY ONE SENTENCER:

GEL PICTURE / OTHER SPACE:

~~good~~ ~~idea~~ make frozen stock for PH1RM1? ✓
PH1ZPL1's mini-prep & send for sequencing/digest
METHOD(S) OF CHOICE: 5' ligase PL1 w/ RBS primers except
mini-prep w/ QACUBE PL2
digestion w/ XAP
ligation ✓

transformation
EXPECTED RESULT:

band @ 800 bp

vector w/ 2 kb

(check if APE file exists on Drive)

TEMPLATE(S): ~~vector~~ promoter RBS primer machinery
PL1 w/ all RBS except PL2
machinery (p5B1C3)

OLIGO(S):

THERMOCYCLER SETTINGS: ~~vector~~ ~~vector~~ DIGEST program

CONTROLS:

✓

POSITIVE:

NEGATIVE:

no enzymes ✓

Name:

Stefan Tassodes

CONFIRMATION?

Date:

06/20/14

WEEKLY ONE SENTENCER:

~~Control Cells~~

GEL LANE ORDER:

DAILY ONE SENTENCER:

Finish Prep. of Comp Cells
Finish Prep. of Comp cells

GEL PICTURE / OTHER SPACE:

METHOD(S) OF CHOICE:

EXPECTED RESULT:

(check if APE file exists on Drive)

TEMPLATE(S):

OLIGO(S):

THERMOCYCLER SETTINGS:

CONTROLS:

POSITIVE:

NEGATIVE:

Name: Jennifer Zhang

Date: 4/20/14

WEEKLY ONE SENTENCE: continues testing ZRS primer / promoter pairs ✓

DAILY ONE SENTENCE: -miniprep cultures grown overnight and make frozen stock and send for sequencing ✓
-digest PL1 with X/P and vector then transfer to ligate insert and vector transform

METHOD(S) OF CHOICE: Qiagen
digest w/ X/P ✓
gel purification ✓

EXPECTED RESULT:
band (vector) ~2kb
insert ~800bp ✓

check if APE file exists on Drive)

TEMPLATE(S): PL1 with all RBS primers except R-LZ
insert (PS1C3) ✓

OLIGO(S): ✓

THERMOCYCLER SETTINGS: digest ✓

CONTROLS:

POSITIVE:

NEGATIVE:

no enzymes ✓

CONFIRMATION? ✓

gel ✓

GEL LANE ORDER:

GEL PICTURE / OTHER SPACE:

digest: 12 mL DDA (insert)	5 mL DDA (vector)
0.5 mL Xba1 (3)	0.5 mL Xba1
0.5 mL PS1 1 (3)	0.5 mL PS1
2 mL buffer (12)	2 mL buffer
5 mL H2O (15)	12 mL H2O

T4 ligase - 0.1 mL (7)
 ATP → 2 mL (14)
 ligase buffer → 2 mL (14)