Name: Jennifer Zhang

Date: 6/10/14

WEEKLY ONE SENTENCER: test our RBS primers and RBS promoter M, with mcherry

DAILY ONE SENTENCER: run PCR with all RBS primers and mcherry, then purify product

METHOD(S) OF CHOICE:
PCR
gel extraction

EXPECTED RESULT:
purified products with RBS sites inserted ~~[illegible]~~ in front of mcherry

~900-1000

(☒ check if APE file exists on Drive) mcherry exists
don't think mcherry w/ RBS sites exist > MAKE!

TEMPLATE(S): mcherry in plasmid (miniprepped from culture grown from frozen stocks) (159.7 ng/μL)

OLIGO(S): RBS primers (forward) (7.5 μL of each 2 mM)
VR → 0363

THERMOCYCLER SETTINGS: [94-2'; {94-15", 60-15", 68-1.5'} ×31
68-4', 4°C forever ]

CONTROLS:

*POSITIVE:*

*NEGATIVE:*
PCR: No Polymerase control (H2)
no template control (H1)

do reverse PCR with 55°C not 60°C

CONFIRMATION?
gel!

GEL LANE ORDER:

gel #1: 1kb ladder NP *RM2 *RL1 RL2

gel #2: 1kb ladder NT RH1 RH2 RM1

GEL PICTURE / OTHER SPACE:

* no mcherry so more mcherry cultures need to be grown overnight (140611)
* only gel extracting RM2 and RL1
- too many bands for H1 and H2
- L2 didn't show anything

→ is the temp. too low?

* re-ran RBS primers PCR

061214 RBS mCherry.jpg
061214 RBS mCherry2.jpg

Name: Coleen Tran

Date: 6/11

WEEKLY ONE SENTENCER: Express CMV + mCherry in E. coli + ~~characteristic~~ insert promoter + RBS into vector

DAILY ONE SENTENCER: start promoter + RBS, finish mCherry + CMV ✓

METHOD(S) OF CHOICE: PCR, ~~digest~~, gel ✓

EXPECTED RESULT: RBS ⇒ ~ 900 - 1000 bp ✓

CONFIRMATION? gel electrophoresis

GEL LANE ORDER:

GEL PICTURE / OTHER SPACE:

0614 RBS mCherry.jpg

061214 RBS mCherry2.jpg

(☐ check if APE file exists on Drive)

TEMPLATE(S): RBS primer, VR0863 ✓, mCherry ✓

OLIGO(S):

THERMOCYCLER SETTINGS: ~~95-60-68~~ 94 - 2' ; {94' - 15"; 60 - 15"; 68°C - 1.5" }x31 ✓

68°C - 4' ; 4°C

CONTROLS:

*POSITIVE:*

*NEGATIVE:*

no polymerase ✓

Name: Stefan Tassoulas

Date: 06/11/2014

WEEKLY ONE SENTENCER: talk tomorrow
Finish crowdfunding, get as much done on characterizing promoter RBS pairs

DAILY ONE SENTENCER:
Start characterizing promoter RBS pairs

METHOD(S) OF CHOICE:
PCR clone Pro-RBS pairs in mCherry plasmid
{ Transform in Comp cells
{ Test Expression
Probably ∅ today

EXPECTED RESULT:
{ High Promoters High RBS - High Expression Low Quality
{ Med - Promoter Mod - RBS - High Expression High Quality
{ Low Promoter Low RBS - Low Expression High Quality
Size pls

(☐ check if APE file exists on Drive)

TEMPLATE(S): ~~PSB~~ pSB1C3_mCherry (great notation)
pls make

OLIGO(S): Forward ~~RBS~~ primer VR primer (0363) ✓

THERMOCYCLER SETTINGS: 94-2' { 94-15", 60-15", 68°C-1.5' } x 31 - 68°C-4'; 4°C ✓

CONTROLS:

*POSITIVE:*
with psB1C3 mcherry with polymerase 0362/0363

*NEGATIVE:*
~~w/o psB1C3 mcherry~~
w/o polymerase
w psB1C3 mcherry ✓

CONFIRMATION? ✓ Band around 920 bp - mCherry w/RBS

GEL LANE ORDER:

GEL PICTURE / OTHER SPACE:

061214 RBS mCherry.jpg
061214 RBS mCherry2.jpg

Name: Katie Fitton

Date: 6/11/14

WEEKLY ONE SENTENCER:

Insert RBS primer & promoter into plasmid in biobrick format; ~~analyze expression w/ diff.~~ primers & promoters

DAILY ONE SENTENCER:

DO PCR w/ AGS & RBS primer ~~assay~~ & then gel extract & purify; meet w/ Lambert

METHOD(S) OF CHOICE:

✓ PCR, gel extract, gel purify

EXPECTED RESULT:

✓ RBS mcherry piece

~900-1000 bp band in thin gel

(☑ check if APE file exists on Drive)

TEMPLATE(S): AGS (mcherry) ✓

OLIGO(S): RBS primers & VR (0363) ✓

THERMOCYCLER SETTINGS: 94-2', {94'-15", 60-15", 68C-1.5'}$_{x31}$, 68C-4', 4°C

CONTROLS:

*POSITIVE:*

*NEGATIVE:*

PCR - no polymerase ✓

CONFIRMATION? ✓

thin gel

GEL LANE ORDER:

GEL PICTURE / OTHER SPACE:

054-59

~~06/12/14~~

061214 RBS mCherry.jpg

061214 RBS mCherry2.jpg