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PCR Screening Protocol

Materials/Notes

- This protocol is for screening transformation colonies for the proper insert in a standard BioBrick backbone
- Thaw all PCR components on ice, particularly dNTPs. This may take awhile (30-60 min), so pull them out well in advance (except enzyme!!).
- Always keep enzymes on ice or in a cold block, only remove them from the freezer right when you need them, and return them immediately afterwards.
- This protocol is written for Phusion polymerase. Tm's and extension times will vary with different polymerases.
- You will need to ensure that you have all PCR reagents before starting procedure (see PCR Mix below)

Procedure

1. Open a clean microplate by the flame using sterile technique. Cut strips of parafilm (half a box high, one box across) and cover the microplate in strips covering the short width of the plate (8 well rows, numbered. Not the lettered 12 well columns) Each strip should of parafilm should cover 2-3 rows of wells.
    a. Skip this step if a microplate is already available.
2. Add 50 µL of sterile DI water to each well in a row for each screening you would like to do.
3. Resuspend a colony from each plate into a well on the row (8 total)
4. Label a strip of PCR tubes, and add 1 drop of mineral oil (by PCR machine) to each tube with a transfer pipette (in the large beaker on the shelf in 1239 ERB).
5. Add 1 µL of respective template to each tube (eg. 1 µL from well A into tube A, and so on).
6. Cover microplate with parafilm/tinfoil, and store in 4 ^{o}C. It should be good for a few days.
7. Make a master mix for your PCR, making 1-2 extra in your master mix (eg. 9x master mix for 8 reactions). Also, make this mix on ice, add water first, and enzyme last (see next step).

    PCR Mix (1x)
    - 10 µL 5x Phusion Buffer
    - 1 µL 10 mM dNTPs
    - 0.5 µL of VF2 (0.5 uM concentration from 50 µM stock)
    - 0.5 µL of VR (0.5 uM concentration from 50 µM stock)
    - 0.5 µL Phusion polymerase
8. Briefly vortex (2 sec) master mix **BEFORE** enzyme has been added. Take a pipette to 1239 ERB, and use chilled pipette tips in the freezer to add enzyme to your master mix, and return enzyme to freezer.
    a. Do this in 1239 to minimize time the enzyme is out of the freezer

   b. Enzymes are stored in 50% glycerol, so after pipetting up your enzyme, wait for a second to make sure the pipette gets the complete volume.
   c. Pipette enzyme directly into master mix solution (eg. your tip should be in the liquid), and pipette up and down a few times to make sure it's all out of your pipette.
9. Invert mix a couple times to ensure it is properly mixed. Never vortex enzymes or DNA.
10. Ensure all liquid is at the bottom of the tube, and then put it on ice and return to 1230.
11. Add 49 µL of master mix to each PCR tube and cap the strip. Flick to mix, and then ensure all liquid is at the bottom of the tube, and fully covered by mineral oil.
12. Immediately place tubes from ice into PCR machine and start "VF2VRPCR" program.

PCR Program

1. 95 ^{o}C for 10 min
2. 95 ^{o}C for 30 sec
3. 62 ^{o}C for 30 sec
4. 72 ^{o}C for X min (30 sec/expected kb)
5. Return to step two 34 times
6. 72 ^{o}C for 10 min
7. 4 ^{o}C store

13. Run a gel of PCR products.
14. Choose a sample with the proper insert size and use the remaining 49 µL in the microplate to streak the bacteria onto a plate with the appropriate antibiotic resistance. Place the plate in the 37 ^{o}C overnight.

References

http://openwetware.org/wiki/Engineering_BioBrick_vectors_from_BioBrick_parts/Colony_PCR_protocol

http://openwetware.org/wiki/Endy:Colony_PCR

http://www.finnzymes.com/pdf/f530sl_phusion_datasheet_2_2_low.pdf