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TrpB passaging protocol

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is Sparrow[1]

[1]UIUC

burgess-lab-uiuc

is Sparrow
UIUC

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We use this protocol and it's working

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Abstract

Instructions for passaging the evolving TrpB populations using the OrthoRep system for continuous directed evolution. Protocol developed from Rix et al., 2020 and optimized in Burgess lab.
Special thanks to Vincent Hu for his aid in troubleshooting.

Growth conditions

1 TrpB evolution-ready strains should be grown in SC -UL with shaking at 220 RPM at 30 °C

The first growth should be done at [M] 100 micromolar (µM) Trp, then move on to [M] 37 micromolar (µM) Trp. It may be a good idea to passage the cells in the same condition for a few times regardless of final OD to help prevent crashes.

Passages

2 After ~2 days of growth, the cultures will be saturated, either by excessive growth or tryptophan limitation. If cultures are visibly saturated **by excessive growth** before the 2 days (typically on the afternoon of the day after a previous passage when tryptophan is not limiting), passaging can be done then.

3 Aliquot 1 mL of culture into:

- a spectrophotometer cuvette
- a clear 1.5 mL tube
- a screw-cap centrifuge tube.

4 Add 1 mL 50% glycerol to the screw cap tube containing culture, label it, and store at -80C.

Note: for strains with poor growth (OD < 0.5), instead centrifuge 1 mL of culture and resuspend in 0.5 mL YPD, add to the screw cap tube, and then add 0.5 mL 50% glycerol as above. This helps with recovery by concentrating the cells and putting them in friendlier media.

5 Centrifuge the 1.5 mL tubes for 1 minute at 16000 x g.

Inspect the pellet visually for contamination. Yeast will be a white or off-whitish color, sometimes a little yellowish. A huge, brown/yellow/green pellet indicates contamination and means you should go back 2 or 3 passages in the evolution.

You can discard the tubes.

6 Measure the OD600 of each strain and record it.

If the strain is above 1.0, passage to a slightly lower tryptophan concentration.

37 > 25 > 20 > 15 > 10 > 7.5 > 5 > 0 works well.

Note down the Trp concentration used to graph out evolution trajectories.

Note: at [M] 10 micromolar (µM) Trp, strains will saturate at around 0.5-0.6 OD 600. If high growth is suddenly observed, it can be an indication of an evolved variant which successfully produces Trp. You may want to consider starting 2 cultures from this one, one at 0 uM as a "test", and one at the slightly lower Trp concentration above as a precaution.

7 To passage, prepare a 50 mL Falcon tube containing 10 mL of SC -UL media for each population.

Don't forget to add His and Glucose to the stock of media you made to avoid having to do that every time you passage.

Add the following:

- Filter-sterilized Indole (final concentration = [M] 400 micromolar (µM) . Indole's maximum solubility in water is 16 mM (250 uL for a 10 mL tube)
- Filter-sterilized Tryptophan to the final concentration specified above (if needed). I keep a 10 mM stock to make things easier: for a final [M] 5 micromolar (µM) concentration, you only need to add 5 uL.
- For the wild-type control, add filter-sterilized 100X Uracil (as this strain is URA-). **The wildtype control is always grown at** [M] 100 micromolar (µM) **to gage the saturation levels of a would-be evolved culture.**

After adding the required additives, add 100 µL of the previous passage to the tube (1:100) and gently invert to mix.

Label the tubes as appropriate.

8 Place tubes to grow in the conditions specified above.

End of evolution

9 Once a culture grows in 0 uM Trp, passage it in 0 uM Trp (not forgetting to add the indole) 6 additional times to saturate the variant.

After the 6th passage, make 2 glycerol stocks (one for the evolution box and one for the main yeast box)

Proceed to gDNA isolation, PCR amplification of p1, and Sanger Sequencing.