Chemostat-Based Experimental Evolutions

Alex Ward and David Gresham June 2008

Aim:
The aim of these experiments is to subject yeast to long-term selections in chemostats. A steady-state chemostat culture is established under some defined nutrient limitation and maintained for hundreds of generations. During the selection samples of the population are taken and archived at -80C. Upon completion of the selection experiment clones are isolated from the population for further analysis.

To begin:
- Determine which strain you wish to use and the desired limiting nutrient (see chemostat manual)
- Autoclave vessels with the rotors set for 200mL (place the rotor at the bottom of the rod)
- Make 10L of media per vessel…be aware that you will have to change out the media bottle once every 2 weeks
- Inoculate vessel with 1mL ON culture
- Set dilution rate to obtain the desired growth rate for evolution. We use D=0.12 by setting pumps to 20/2 for 200mL vessel (i.e. generation time ~6 hours)

Sampling and maintenance:
- Determine that steady-state has been attained
- Sample the chemostat at defined intervals. We sample on Monday, Wednesday and Friday.
    - Passively sample into a sterile Klett tube
    - Take a Klett measurement, coulter count and freeze 1mL of sample in 500uL 50% glycerol at -80C
- Take any notes throughout the evolution regarding any mishaps
- Change the media bottle as necessary ~once every 2 weeks

Ending Experiment:
- At the end of the evolution (after 250 generations)
    - Sample chemostat as per usual and take relevant measurements
    - Sonicate cells using sterile technique (i.e. sterilize sonication probe with ethanol)
    - Determine cell count using coulter counter and make relevant dilution in sterile water
    - Plate enough cells on YPD and minimal media plates to get ~250 colonies x 3 plates
    - Allow cells to grow for 2 days
    - Photograph plates

Clone preservation:

- Aliquot 100uL YPD into sterile 96 well plate
    - Pick an unbiased sampling of colonies using toothpick and inoculate individual wells
    - Place ancestor (we used FY4 (MAT**a** prototroph)) in position A4
    - Do not innoculate H96 as it is used as a contamination control
- Allow three days growth in a plastic bag in 30C room
- Make two replica plates
    - Fill 2 x 96well plates with 95uL YPD (one is a copy plate and one is a working plate)
    - Take 5uL from master plate – inoculate copy plate by pipetting up and down, take 5uL from that plate and inoculate working plate – allow them to grow 2 days at 30C
- Add ~75uL 30%glycerol to master plate (this reduced volume accounts for evaporation) and freeze at -80C.
    - The next day cover this plate with foil tape.
- After two days add 95uL 30% glycerol to copy plate and freeze at -80C. Foil the next day.
- Use working plate for initial clone characterization

Clone characterization:

- Add 95uL of relevant growth media to a 96 well plate and inoculate with 5uL of working plate – grow in Tecan using GreshamGrowthRate.mth -> Name file e.g. CDG21_400uMAmmoniumSulfate (make sure you save the excel spreadsheet at the end of the run)
- Use 96 well frogger to frog working plate onto rectangular YPD plate. Allow two days growth, take a photo and keep at 4C. Working plate can be kept at 4C.