

Pichia Induction Protocol for Initial Screening of PCR Positive Clones

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Before you begin the induction screening, you must have the following:

1. sufficient amounts of BMGY, BMMY media and Methanol prepared.
2. selected Mut^s and Mut⁺ phenotypes and PCR screened these clones for presence of recombinant DNA.
3. an orbital shaker at a stable 30°C. Temperatures >30°C will kill the yeast.
4. Sterile 50ml conical tubes and sterile toothpicks

Induction Protocol:

Step 1. Inoculate 10ml of BMGY media with either a single colony from MD screening plate (toothpick) or with a 10µl of a single colony cell suspension from a earlier date.

NOTE: yeast colonies are still viable after 3-4 weeks on the benchtop at RT and even longer the colony have been stored at 4°C providing that they have not been contaminated by other yeast or bacteria. Streak out a colony is you suspect contamination and select a new colony from the streak plate.

Step 2. Incubate the 10ml BMGY culture for 48 hrs in 30°C shaker.

Step 3. After 48hrs, remove culture from shaker. If the yeast have not produce a sizable biomass, continue BMGY growth. If yeast culture is thick then proceed. Spin 50ml sample tubes in Beckman Benchtop centrifuge at 3000rpm, 3-5min.

Step 4. Remove BMGY supernate, leaving whitish cell pellet in tube.

Step 5. (OPTIONAL) Wash yeast cell pellet with 30ml of sterile PBS. Repeat centrifugation and remove PBS supernatant.

Step 6. Resuspend yeast cell pellet in 2ml of BMMY media.

Step 7. Incubate BMMY culture for in 30°C shaker. Every 24hrs, add 10µl 100% methanol to BMMY culture (final conc. = 0.05% MeOH) to replace methanol lost to evaporation. Continue induction growth on BMMY + methanol for a minimum of two days, preferable 5-6 days.

If time points are to be taken over course of induction, remove 100µl of the induction culture every 24 hrs beginning on day 2 of induction growth. IN ADDITION, you must add 90µl of fresh BMMY media when you add the 10µl of MeOH to replace the missing culture volume. The 100µl aliquots should be immediately centrifuged at 14K rpm for 5min in microfuge. The supernatant containing the media and secreted protein (?) should be removed and placed in another 1.5ml tube. Store the sample pellet at 4°C and the sample supernate at -20°C until you are ready for SDS-PAGE analysis.