

Large Plate Protocol

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This protocol enables to harvest linearly growing hyphae and hyphal mass with defined age. In order to circumvent problems associated with the diauxic shift and heterogeneity of cell types, this method is a good alternative to liquid culture.

Large Plate: Fisher scientific Cat#12-565-224

Cellophane: VWR 100357-652

1. Inoculate silica or conidial stock to a Vogel's minimal medium slant (Vogel 1956), incubate at 30C for 2-3 days, then transfer to a 25C incubator, and leave for 3-5 days under constant light.
2. Cast 150 ml Bird's medium (Metzenberg 2004) to a large square plate (22 cm x 22 cm), let it solidify and overlay a sheet of cellophane which has been autoclaved in ddH₂O.
3. Harvest conidia in the slant with 1ml ddH₂O, vortex.
4. Make 100x dilution and count conidia by hemacytometer or by spectrophotometer at λ 420nm.
1 OD = 5×10^6 /mL according to the electroporation protocol (Robert L. Metzenberg and Kristin Black, personal communication) or
1 OD = 1.2×10^7 /mL for FGSC 2489 (our observation).
Probably, you'll get up to 6×10^8 /mL concentration of the original suspension.
5. Inoculate 100 μ l (5×10^6 to 5×10^7 conidia are good, be consistent) to the large plate with cellophane on Bird's medium. Use glass beads to evenly distribute conidia. Incubate at 25 C, constant light for 24 hours.
6. Cut out narrow cellophane strips (~0.5 cm x 22 cm), and use one to inoculate a fresh large plate with cellophane on Bird's medium and incubate at 25 C, constant light, for 24 hours.
7. To harvest, cut 1 cm x 22 cm strips from desired age regions (we usually cut the youngest 1cm (1-3 hours old) and another 1 cm of 12-15 hours old region. Divide each strip (about 20 cm) into three 7cm segments, peel off mycelia and put each into 1.5 ml tube and freeze in liquid nitrogen. Keep samples at -70C.
8. Total RNA can be harvested using a trizol method (Microarray Protocols: <http://www.yale.edu/townsend/Links/ffdatabase/downloads.html>). Add 1 ml

trizol to each sample (1 cm x 7 cm). Yield will be between 50 to 100 μ g RNA per strip.

- Metzenberg, R.L. 2004. Bird Medium: An alternative to Vogel Medium. *Fungal Genet. Newslett.* **51**: 19-20.
- Vogel, H.J. 1956. A convenient growth medium for *Neurospora* (Medium N). *Microbial. Genet. Bull.* **13**: 42-43.