# *A. niger* Protoplastation

## Introduction

The purpose of this protocol is to strip away the cell wall of fungi to let the naked DNA enter the cells.

## Materials

- Fungal plates (either streaked or 3-point)
- Drigalski spatula
- 500 mL shake flasks
- Counting chamber
- Solutions (APB, ATP, PCT, milli-Q, TM)
- 30 °C incubator with shaking
- Sterile teaspoon
- Mira cloth in funnel (sterile)
- Glucanex
- Magnet stirrer
- Magnets
- 50 mL sterile Falcon tubes
- 0.45 $\mu$m filters
- 50 mL syringe
- Centrifuge for Falcon tubes

Media

- Aspergillus transformation buffer (ATB)
- Aspergillus protoplastation buffer (APB)
- YPD media

## Procedure

### Initiation

1. Streak spore suspension of host strain on an agar plate with corresponding media and supplements and let it grow for a week (in order to obtain a high amount of spores).
2. All solutions should be sterile.

### Day 1 (inoculation)

1. Add 95 ml of liquid media with supplements to a shake flask and add 5 ml of the liquid media to a plate with *A. niger*. Collect conidia and spores from the plate by carefully scraping off the conidia using a drigalski spatula. This should give a concentration of around $10^8$ spores/100 ml. It is a good idea to make more than one shake flask at a time.
2. Incubate shake flasks at 30 °C and 150 rpm for 48 hrs.

**Day 3 (mycelial harvest)**

1. Place the sterile funnel with a mira cloth into a sterile blue cap bottle and transfer the contents of the shake flasks to the mira cloth (content should be brown and thick).
2. Wash the mycelia using *Aspergillus* protoplastation buffer (APB) to remove residual glucose from the mycelia (this can inhibit protoplastation). You need to use quite a bit of APB. squeeze out remaining liquid using a sterile spoon.
3. Then, transfer the mycelium to falcon tubes (≈2 g per tube => 1 shake flask ≈ 2 falcon tubes).

**Protoplastation**

1. Add glucanex to APB to get a final concentration of 40 mg glucanex per ml APB and dissolve glucanex via gentle magnetic stirring and no heat.
2. Sterile filter 20 mL of APB+glucanex to each falcon tube using a 0.45 $\mu$m filter and a 50 mL syringe (the filter gives a bit of resistance)
3. Shake/incubate enzyme-mycelium mix at 30 °C, 150 rpm for 2-3 hrs.
4. From now on, whenever you pipette anything with cells, cut off the edge of the pipette tip and carefully pipette the cells. If not, they break as they don't have a cell wall to keep them stable.
5. Evaluate the number and quality of protoplasts in a microscope and discard a batch that is too diluted (i.e. < $10^5$ protoplasts/ml). Approved protoplast solutions are then diluted by pouring APB up to 40 ml. and the tubes are balanced. Dilute *Aspergillus* transformation buffer (ATB) to 1/2x with sterile milli-Q $H_2O$ and carefully place 5 mL of this on top of the APB, creating an overlay. Centrifuge samples on rotor settings rotor code Sla-600TC; time: 13 minutes; Speed: 3000g; Temperature: 16 °C, Acc: 2, Brake: 2.
6. In the interphase between the two liquids, a halo of white slurry consisting of concentrated protoplasts should be observed. Withdraw the protoplasts with a pipette and wash them in a new falcon tube. Add ATB up to 40 mL and pellet the protoplasts at 3000 g for 13 minutes (acc. 2, brake 2). Discard supernatant by decanting.
7. Count protoplasts in a microscope by diluting a small sample 1:100.
8. Resuspend protoplasts in 4 mL ATB to obtain concentrated solution.