

Agrobacterium* transformation of *Aspergillus fumigatus

***Basic Protocol adapted from Sugui et al. 2005, Appl. Environ. Micro Vol 71. 1798-1802 and Mullins et al. 1999, Phytopathology.**

I. *Agrobacterium* Preparation

-Start with your *Agro* strain of choice and streak out onto LB plates, grow at 28 C for 2 days. Take a swipe of colonies, at least 6 or 7 and start a culture in Luria Broth (LB) ~25 ml. We and others have gotten superior results with strain EHA105.

-Grow culture up overnight 28 C shaking 200 RPM. In the morning take 1 ml aliquots of culture and spin down 5 minutes 13,000 RPM. Remove supernatant. Wash Pellet 3X in ICE COLD 10% glycerol. Resuspend pellet in 50 μ l of 10% ICE COLD glycerol and keep on ice.

-Add 0.5 μ l of your *Agro* plasmid of choice and electroporate using the Agr setting on the Biorad micropulser-electroporator. Immediately add 250 μ l of SOC or LB media and shake at 28C for 2 hours.

-Plate out on LB + antibiotic plates (our *Agro* Ti plasmids are typically Kanamycin resistant 50 μ g/ml) and incubate at 28 C for 1-2 days until colonies appear.

II. Transformation of *Aspergillus fumigatus* via ATMT

-Swipe at least 6-7 colonies off *Agrobacterium* electroporation plates and inoculate into 25 ml of LB + 50 μ g/ml of kanamycin. Grow for 12-14 hours at 28 C 200 RPM.

-In the morning, take 1 ml of the starter culture and dilute into 9 ml of ***IM+AS*** media (total volume = 10 ml) (NO KAN is added). Grow for 6 hours at 28 C, 200 RPM OR UNTIL O.D.₆₀₀ reaches 0.8. **This is important, O.D. must be 0.8 for successful transformation! Monitor the culture EVERY HOUR.**

-After 6 hours or O.D. of 0.8 add 100 μ l of AT culture in *IM+AS* to 100 μ l of Fresh (3-4 day old) *Aspergillus fumigatus* spores. The idea ratio of bacteria to spores is 10:1. Thus, 10^7 *Aspergillus* conidia + 10^8 *Agro* cells (if O.D.₆₀₀ is 0.8 this is your *Agro* concentration approximately). **This 200 μ l mixture is your co-cultivation mixture.**

-Place Hybond N (Amersham/GE Life Sciences) Nylon membranes (82 mm) onto *IM+AS* co-cultivation agar plates.

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-Take the 200 ul co-cultivation mixture and plate out onto nylon membranes (Hybond N) which are now on IM+AS agar plates. Be careful when spreading not to push all the mixture to the edge of the filters. Try and keep in the middle but get a good spread.

Incubate plates at 24 C for 48 hours if doing random insertion, or 36-40 hrs if doing homologous recombination, in the dark.

-After co-cultivation period is over, transfer filters using sterile forceps to SM plates (Aspergillus minimal media + YOUR SELECTABLE MARKER and 200 ug/ml Cefotaxime). Incubate plates at 37C for 2 days.

-Colonies should start to appear after 24 hours.

-Colonies can be picked to new SM plates and single spored etc. etc.

Materials:

Acetosyringone, Sigma Aldrich, ca# D134406-56

Media prep:

-For media preparation I typically make 150 ml stock solutions of all the salts and filter sterilize. Add these sterilized stocks to appropriate amount of ROOM TEMPERATURE sterile (autoclaved) water. Again if there is a substantial temperature difference, the salts will fall out of solution.

-For IM add acetosyringone (which is dissolved in ethanol) fresh at room temp, and FILTER sterilize the MES and add fresh as well.

-For CM plates, add agar to water and autoclave. Cool agar to 55 C in a water bath, then add stock components . . .mix thoroughly and pour. I always pour FRESH CM agar plates the day of transformation.

One Liter Minimal Media for *Agrobacterium* growth

Add the following to 941.5 ml sterilized water

- 10 ml K-Buffer (pH 7.0)
 - 200 g/l K_2HPO_4
 - 145 g/l KH_2PO_4
- 20 ml M-N Buffer
 - 30 g/l $MgSO_4$
 - 15 g/l NaCl
- 1 ml 1% $CaCl_2 \cdot 2H_2O$
- 10 ml 20% glucose (w/v)
- 10 ml 0.01% $FeSO_4$ (w/v)
- 5 ml Spore Elements
 - 100 mg/l $ZnSO_4 \cdot 7H_2O$
 - 100 mg/l $CuSO_4 \cdot 5H_2O$
 - 100 mg/l H_3BO_3
 - 100 mg/l $MnSO_4 \cdot H_2O$
 - 100 mg/l $Na_2MoO_4 \cdot 2 H_2O$
- 2.5 ml 20% NH_4NO_3 (w/v)

One Liter Induction Medium (IM)

Add the following to 898.7 ml of sterilized water

- 10 ml K Buffer
- 20 ml M-N buffer
- 1 ml 1% $CaCl_2$
- 10 ml 0.01% $FeSO_4$
- 5 ml Spore Elements
- 2.5 ml 20% NH_4NO_3
- 10 ml 50% glycerol
- 40 ml 1 M MES (2-N-Morpholino ethanesulfonic acid, pH 5.3) Use NaOH to adjust pH and filter sterilize
- 5 ml 2M Glucose
- 2 ml 100 mM Acetosyringone (3' 5' Dimethoxy-3'hydroxyacetophenone) stored at -20C and prepared with 100% ethanol
- Add 15g/l of agar for solid medium

Co-cultivation medium – same as IM medium except add 1.5% agar

SM Plates = *Aspergillus Glucose Minima Media Plates* + your selectable marker at appropriate concentration and CEFOTAXIME to kill Agro at 200 ug/ml.