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Transformation Protocol ATMT Trichoderma atroviride V2

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ATMT

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Protocol status: Working
We use this protocol and it's working

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Abstract

This is the protocol for the transformation of conidia by *Agrobacterium tumefaciens* to perform the BarSeq technique in *Trichoderma atroviride*, which is reported by Villalobos-Escobedo et al., 2025.

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1 DAY 1

1.1

Inoculate 3 plates of Trichoderma @ Temperature27 °C Grow them for 4 days
Sterilize flasks and bottles
5 bottles of 500 ml
2 100 mL
2 250 ml

1.2 Prepare LB + Kan (500 mL)

50 ug/mL is the working concentration for Kanamycin; theamount added depends on the stock concentration
Ex: 50 mg/mL stock concentration
1 L of LB would require 1 mL of 50 mg/ml Kan
  -Usually, we put 1 ml in 500 ml of LB

2 DAY 3

2.1 Make liquid agro induction media (ABI)

For 100 transformations, 5 mL of ABI is needed, water is autoclaved, and all others are filtered and sterilized
200 mL to wash, 200 mL to resuspend (grow)

MIX AND FILTER INSIDE HOOD

-- CASamino acids (10% CASamino acids) (Heat up and stir)
-- Glucose (36% Glucose) (Heat up and stir)
--Thiamine (10% Thiamine)

Sterilized bottles

For AB Buffer, check pH, then filter. The pH of the AB needs to be 5.5.

	A	B	C
	ATMT.		
	ABI plates(25ml x# of plates)		
		1L	liquid ABI
	ddH2O(ml)	437	0.2
	Bacto Agar(g)	20	87.4
	AB salts (ml)	50	10
	500mM phosphate solution (ml)	2.4	0.48
	2x AB buffer (ml)	500	100
	10% thiamine (ul)	100	20
	36% glucose stock (ml)	5.5	1.1
	10% CASamino acids (ml)	5	1
	acetosyringone (ml)	1	0.2

Preparation of buffers (ABI)

2.2 Prepare: 1 mL 3,5-dimethoxy-4-hydroxy-acetophenone (acetosyringone) dissolved in dimethyl sulfoxide (DMSO). (make this fresh every time)

-- Acetosyringone is filtered through syringe and 0.22 um filter top in DMSO (0.098 g/mL)

2.3
1. Autoclave 437ml ddH2O with 20g Bacto Agar
2. Add MIXED induction liquid to agar.
3. Mix together inside hood for a few minutes
4. Pour plates

Pour solid agro induction media plates and leave on the bench to dry for 2 days.

2.4 To prepare medium for Agrobacterium LB + Kan.

2.5 Inoculate A. tumefaciens strain harboring the plasmid for Trichoderma transformation onto LB + Kan.

250 µL in 50 mL

2.6 Shake at 250 rpm at 30C overnight
(Place around 7 pm- 8 pm)

3 Day 4

3.1 Measure the OD600 of the agro cell culture. (It will likely be around OD600 1.)
(Around 9 am)

3.2 To obtain an accurate measurement, dilute the sample 1:10 in H2O in the cuvette.
(100 uL agro cell culture, 900 uL H2O)

3.3 Back dilute the agro culture to OD600 0.5 in LB+Kan with twice the number of ml of media to the number of transformations you plan to do (i.e., if you are doing 100 transformations, back dilute into 200ml LB).

100 mL from stock and 100 mL new LB+Kan

3.4 Shake at 250rpm at 30C for 2h

3.5 Measure the OD600 of the agro cell culture after 2 hrs

3.6 Centrifuge the agrobacterium culture at 4000 rpm, 00:15:00

3.7 Pour supernatant into waste container.

3.8 Wash cells in the Agrobacterium induction media.

-- The number of mL should correspond to twice the number of transformations that you are doing
ex: 20 ml for 10 transformations
New flasks are used

3.9 Centrifuge the agro cells at 4000 rpm, 00:15:00

3.10 Pour supernatant into waste container.

3.11 Resuspend cells in *Agrobacterium* induction media:

The number of mL should correspond to twice the number of transformations that you are doing
For 100 transformations, 200 ml of induction media, and each transformation has 4∗10^7 conidia.

It's **2∗10^7 per ml—concentration of *Agrobacterium*.**
We can use tubes with caps for roller.

3.12 Put cultures on shaker @ 25 °C and rpm 250, for 24 hrs

To prepare materials for the next day:
- Filter top
- 0.45 um Filter
- Vacum

4 DAY 5 6d

4.1 (using plates of *T. atroviride* that are 4 days old)
NOTE: Make sure the big square plates are at room temperature.

Approximately 23h after putting agro cells into induction media, collect Trichoderma conidia from plates into 5-10ml of H2O.

4.2 Shake and vortex vigorously to suspend conidia.

4.3 Measure conidial concentration

Count four corner squares, and middle square
Avg spores: Sum of both sides / 10 squares
Math: (Avg. spores)(1×10^4)(25)(100)

4.4 Aliquot $2e^7$ conidia into 2ml Eppendorf tubes

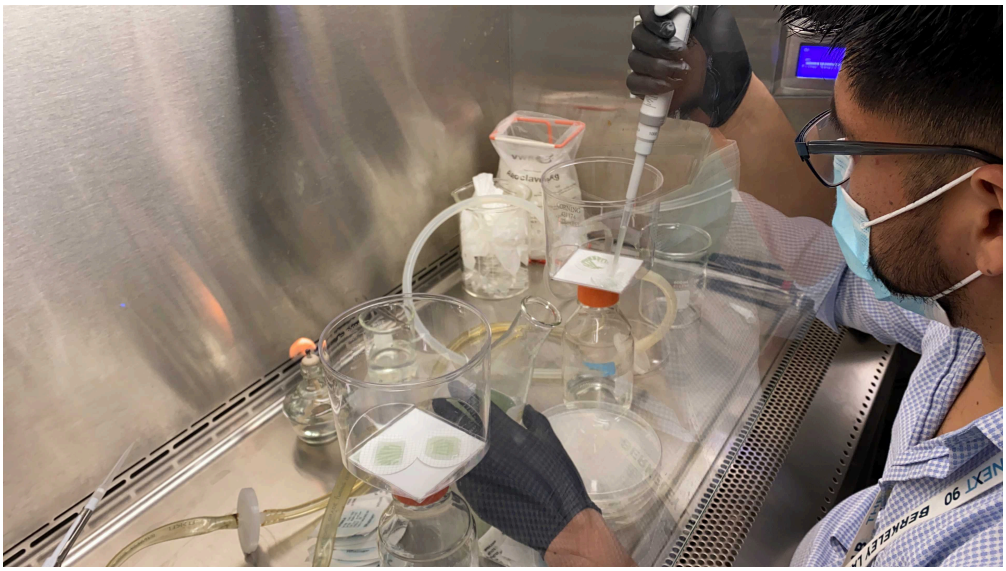
4.5 Spin cells at 10000 rpm, 00:05:00
or 4000 rpm for 20 min

4.6 Pipet off supernatant

5m

4.7 Incubate at room temperature for at least 5 min

4.8 Put a 0.2 um sterile PES bottle-top filter on a bottle and set up the vacuum.

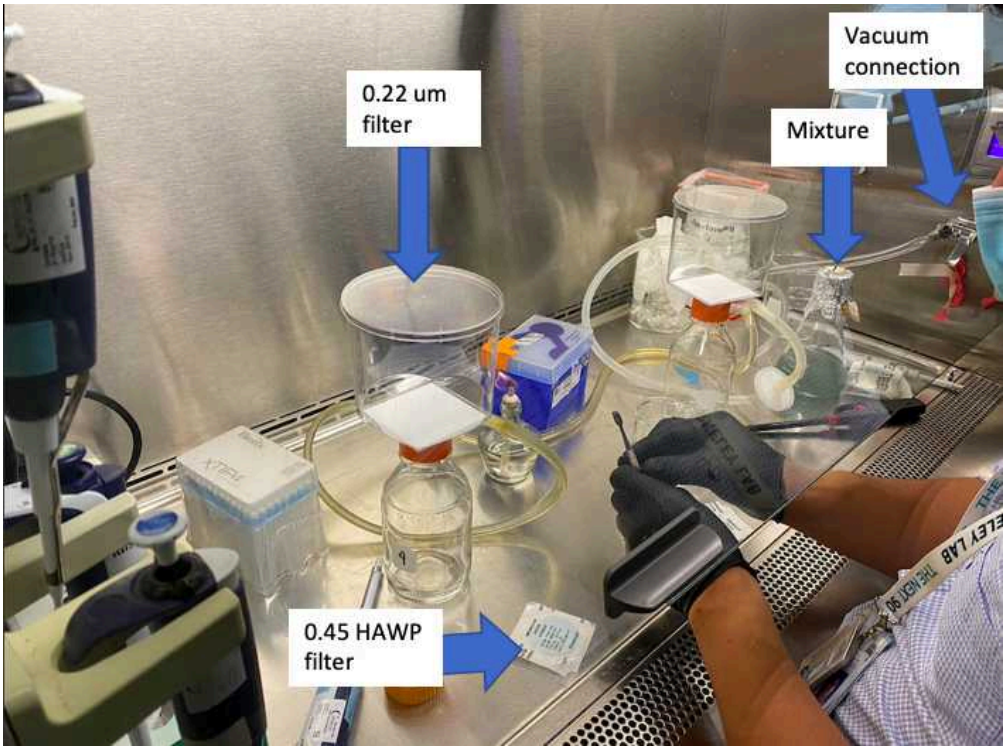


Transformation in the hood.

Equipment:
Thermo Scientific™ Nalgene™ Rapid-Flow™ Sterile Disposable Bottle Top Filters with PES Membrane
NAME
0.2 µm Bottle Top Filter or equivalent
TYPE
Nalgene
BRAND
09-741-07
SKU
https://www.fishersci.com/shop/products/nalgene-rapid-flow-sterile-disposable-bottle-top-filters-pes-membrane/0974107
LINK
500 mL, 0.2 µm PES Bottle Top Filter
SPECIFICATIONS

‰ Nalgene™ Rapid-Flow™ Sterile Disposable Bottle Top Filters with PES Membrane" title="Bottletopfilter.Jpg">

4.9 Place a 0.45 µm HAWP filter onto the bottle top filter using sterile forceps:



Equipment
NAME

HAWP MF-Millipore Membrane Filter, 0.45 µm pore size
TYPE
Membrane filter
BRAND
Millipore
SKU
HAWP04700
LINK
https://www.sigmaaldrich.com/catalog/product/mm/hawp04700?lang=en&region=US&gclid=CjwKCAjw8pH3BRAXEiwA1pvMsdoaQbbYstapLy8iGgQMuPbpUIubisFSK9v3zg7Ab-Uv1HEHZmOhSBoCPx8QAvD_BwE
SPECIFICATIONS
0.45 um 47 mm

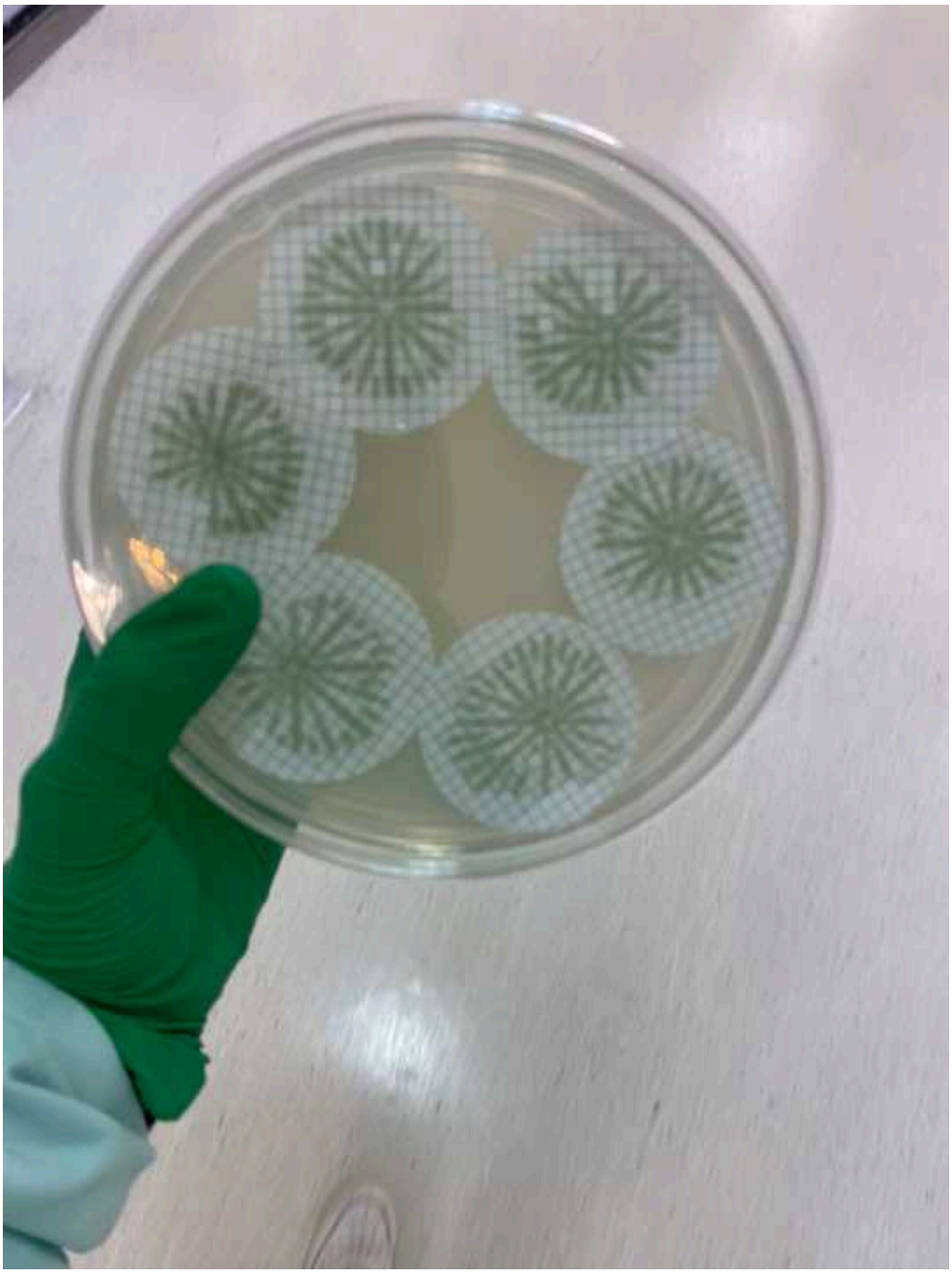
4.10 Pipet the 2ml of Trichoderma/agrobacterium cell mixture onto 0.45um HAWP filter

-- Being careful to only get cell mixture on the HAWP filter.

4.11 Wait for the liquid induction media (ABI) to filter through the bottle top filter

4.12 Using sterile forceps, carefully transfer the filter with cells on it to the solid induction media petri dish, making sure to keep the side with the cells on it facing up!

4.13 Incubate induction media plates with filters at 21 °C in the dark for 6 days.



Dried filters ready for incubation

4.14 Materials for next part:. - 50 mL or 500 mL tubes with sterile beads and sterile water. - Layered PDA plates. - MM plates for quantifying. - Sterile water.
Arrow
Materials for next part:
- 50 mL or 500 mL tubes with sterile beads and sterile water

- Layered PDA plates
- MM plates for quantifying
- Sterile water

5 Day 11

5.1 Label 2 Falcon tubes for each HAWP filter
-- 25 - 50 filters fit into 500 mL tubes

5.2 Add 4ml sterile distilled H2O to a 50ml conical tube
-- 500 mL conical tube

5.3 Using sterile forceps, carefully transfer the HAWP filter with cells on it to the 50 mL conical
-- Use beads and VORTEX until it has all fallen off

5.4 Vortex vigorously to get the rest of the cells off of the HAWP filter

5.5 Transfer 2 mL of cell suspension to a sterile 2 mL Eppendorf tube.

5.6 Spin at 10000 rpm, 00:05:00 .

5.7 Pipet off supernatant into a waste bottle.

5.8 Transfer the rest of the cell suspension to the 2 mL Eppendorf tube and Spin at 10000 rpm, 00:05:00 .

5.9 Pipet off supernatant into a waste bottle

5.10 Resuspend cell mixture into 100 µL sterile distilled H2O

5.11 To spread in large plates (150 mm) PDA.

1.- PDA with Hygromycin B and Carbenicillin (4 ml Hygromycin B and 1 ml Carbenicillin per L): dispense 50 ml.
2.- PDA with Carbenicillin (1 ml per Liter): dispense 13.5 ml.
3.- Conidia transformants: 1 ml
4.- Low-melt PDA with Carbenicillin (1 ml per L). 13.5 ml. 37 C. We can mix with conidia.
5.- PDA layer Carbenicillin and Hygromycin B. 13.5 ml, kept at 55 C.

5.12 1 plate with filter goes to 3 plates of top/bottom media

5.13 Plate cells on
5mL Top: MM + Carb
25 mL bottom: MM + Carb + Hyg

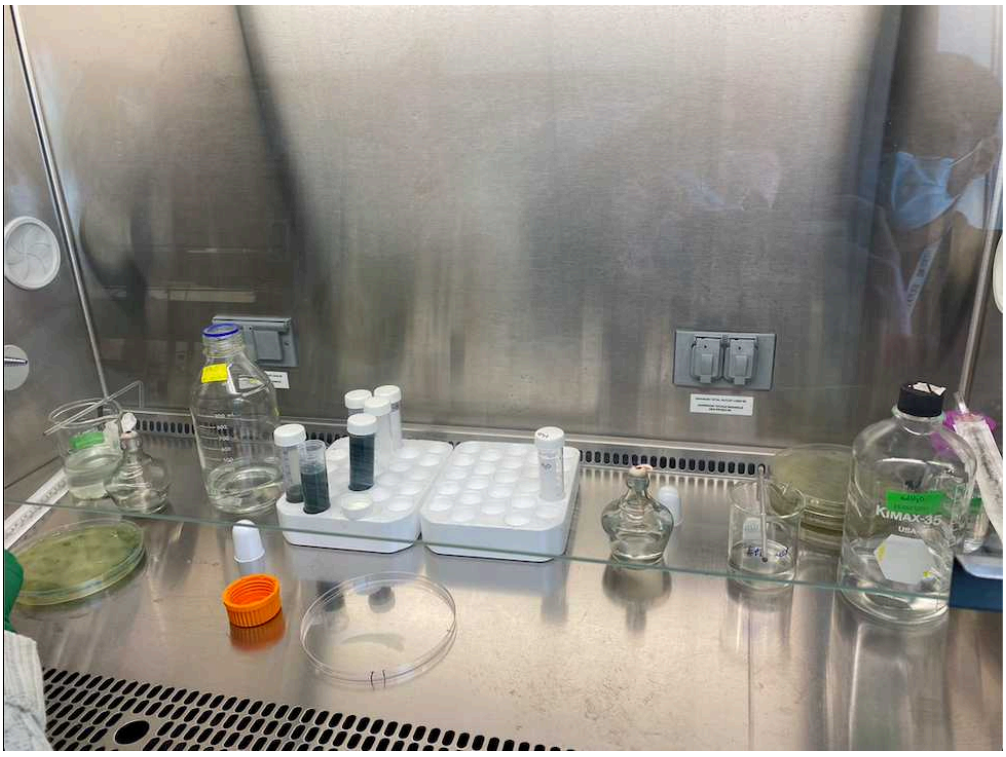
Note: You don't need to dilute the control plate, but for the experimental ones:
1. 100%:
2. 1:25 **4 ul of cells in 96 ul of water**

Note: Make up media with half the amount of water, autoclave thewater separately, then combine.

5.14 Place at 25C in the light for 7 days (cheking efficiency with Triton)

Final Harvest (Day 18)

6



Washing of plates with water

Materials needed:

- 50% glycerol
- Sterile water
- Glass spreaders
- 1 ml Pipettes
- 25 ml pipettors and pipet tip
- Tips 100-1000 ul
- 15 mL Falcon tubes

Dispense 10 mL of sterile water onto the plate

7 Use the sterile glass spreader to detach as many as possible.

8 Transfer into a 50 mLFalcon tubes.

8.1 Add another 10 mL of sterile water to plate and transfer into tube.

9 Spin down 50 mL Falcon tubes at 4,000 RPM for 20 minutes 20m

10 Resuspend pellets in 10 ml of 50% glycerol

10.1 Store in 1.5 mL tubes that are no more than half full, stored at an angle to avoid rupturing the tube.

--Store to -80 °C in Cryotubes.

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