Transformation of Neurospora crassa conidia by electroporation

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Circadian Clock Control of tRNA synthetases

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ABSTRACT

Here, we describe the transformation of *Neurospora crassa* conidia by electroporation. This process introduces DNA constructs such as reporter tags or genes conferring resistance to antibiotics for selection.

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Here, we describe the transformation of *Neurospora crassa* conidia by electroporation. This process introduces DNA constructs such as reporter tags or genes conferring resistance to antibiotics for selection.

NEUROSPORA CRASSA TRANSFORMATION by ELECTROPORATION PROTOCOL

Grow strain on 50-100ml large agar slant in Erlenmeyer flask at 30^{O}C.It will take about a week to get a good amount of conidial growth.

Materials to prepare prior to transformatio (TFM):

To be autoclaved:
Adjust total volumes as needed. Use milliQ H_2O for preparing media.
Prepare in beakers first, then transfer to bottles before autoclaving.

1M Sorbitol (FW: 187.17 g/mol)- 182.2 g, H_2O to 1000ml H_2O

10x FGS – 100g L-sorbose, 2.5g fructose, 2.5g glucose, H_2O to 500 ml

1X Recovery Solution – 10 ml 50x Vogel's add 400 mL H_2O à pH 6, then fill H_2O to 500 ml

Bottom TFM Agar Plates
with Hyg selectionwith Basta selection
1x Vogel's - 20 ml 50x Vogel's 1x N-free Vogel's - 20 ml N-Vogel's
pH 6, then H_2O to 900 mL0.5% proline - 5g proline

1.5% agar -15 g agarpH 6, then H_2O to 900 mL

1.5% agar -15 g agar

After autoclaving:After autoclaving:
100 ml 10x FGS100 ml 10x FGS
4 ml of 50 mg/ml hygromycin800 ul of 250 mg/ml Basta

Top TFM Agar (make fresh or just a few days before TFM; alternatively, can be stored at 4^{O}C without FGS. If stored – melt agar by putting tubes in boiling water bath, then add 2 mL 10X FGS to each tube.)

Recipe for 200 mL. Sorbitol takes so much volume, so in a 400/600 mL beaker, put only around 100 mL to get the sorbitol to dissolve. Follow the recipe below and pH, then add water to 160 mL.

with Hyg selectionwith Basta selection
1x Vogel's - 4 ml 50x Vogel's 1x N-free Vogel's - 4 ml 50X N-Vogel's
2M sorbitol - 72.9 g sorbitol 2M sorbitol - 72.9 g sorbitol
pH to 6. Add H_2O to 160 ml.2g proline

Add agar.pH to 6. Add H_2O to 160 ml.

2% agar – 4gAdd agar.
Add 800 uL of 50 mg/mL Hyg2% agar – 4g
Boil until agar dissolves.Add 250 uL of 250 mg/mL Basta
Dispense ~ 8mL into long tubes. Boil until agar dissolves.

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Dispense ~ 8mL into long tubes.

Setup in the hood for TFM. UV-treat the following for ~5-10 mins. This is for when you only have one strain to collect conidia from. Use different beakers/cheesecloth/conical tubes for multiple strains.

Materials: Sterile and baked beaker, autoclaved cheesecloth, 4 50-ml conical tubes labeled with strain name, and put on ice, 50-ml conical tubes (add in tubes for controls) placed in a rack, 1M sorbitol, 1x recovery solution (1x Vogel's for Hyg TFM, 1x N-free Vogel's for Basta TFM).

1. In 1-50 ml conical tube on ice, fill with 50 mL 1M sorbitol. Fill the rest (3 tubes) on ice with 20 mL 1M sorbitol.
2. Put the sterile cheesecloth over the receiving beaker. Avoid touching the center of the cheesecloth. Just hold on the sides to avoid contamination.
3. Pour the 50 mL 1M sorbitol GENTLY into your flask with growing conidia. Swirl/mix/vortex to dislodge conidia.
4. Filter big chunks of mycelia/hyphae by pouring the conidial suspension onto cheesecloth. Discard cheesecloth (put back into foil àbiohazard bag). Pour back the conidial suspension into the 50 mL conical tube. You will just recover around 40mL.
5. Spin down at max speed for 5 mins using the old room temperature centrifuge. Make sure there's a balance tube.
6. Pour out supernatant into waste beaker. From another tube with 20 mL 1M sorbitol on ice, take a 1mL, transfer into pelleted conidia. Pipette up and down to take as much conidia as you can. Then transfer into the fresh 1M soribitol.
7. Repeat steps 5-6 three more times to wash the conidia well.
8. With the last wash, discard supernatant, but leave around 200ul of sorbitol. Resuspend the conidia.
9. For each TFM, you need ~60ul of conidia in 1.5 mL tubes. Don't forget the control tubes. Leave on ice.
10. Add the transforming DNA into your conidia (~10ul total volume, target between 500ng-1000ng of DNA, for co-TFM, add in appropriate plasmid, 200ng-500ng).
11. Leave on ice for ~30 mins. Meanwhile, put in the labeled electroporator cuvettes on ice. Put ~9-10mL of recover solution on 50 mL conical tubes on rack. This goes into the 30C incubator to pre-warm.
12. A few minutes before the 30-min incubation ends, transfer the transformed conidia into the cuvettes.
13. Setup the electroporator (voltage – 1.5kv for 1mm cuvettes), cold 1M sorbitol – will pipette 960ul of this so you need your pipettor and sterile pipette tips as well, recovery solution tubes.
14. Wipe sides of the cuvette with Kimwipe, put cuvette into chamber. Press the two red buttons until you hear a beeping sound.
15. Quickly add 960 ul of cold 1M sorbitol. Pipette up and down to get most of the mix. Then transfer into the recovery solution. Do the same for the rest.
16. Incubate the tubes with constant shaking ~100 rpm at 30C for 1.5h-2h.
17. Meanwhile, prepare the appropriate top and bottom agar plates. Label and UV-treat the bottom agar plates if nobody's using the hood. Add in 10x FGS bottle, a conical tube to transfer some of the FGS into, sterile 1000ul pipettor and tips.

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18. 20 mins before the incubation is over, boil the top agar to melt.
19. Under the hood, pour the needed amount of 10x FGS into the conical tube. Avoid dipping your pipettor into the 10x FGS bottle. Then from the transferred FGS, add 2 mL of this into each top agar tube. Vortex.
20. Then add the molten top agar into the transformation mix. Gently mix. Then pour into the bottom agar plates.
21. Wait for these to solidify, before transferring into 30C incubator.

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