Natural Transformation of Campylobacter jejuni Version 2

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Abstract

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Protocol

Day 1
Step 1.

Thaw cells, grow 24-48 hours.

Day 2
Step 2.

Restreak, grow 18-24 h.

Day 3
Step 3.
Early in the day: Streak cells in a thin layer onto BHI + 2% yeast (BHI+Y) (yeast is optional but is helpful). Grow 6 h.

Day 3
Step 4.
Pre-warm 1-2 BHI+Y plates.

Day 3
Step 5.
Harvest 6-h plate of cells in 2 mL PBS, wash 1x in PBS, resuspend in 250 uL PBS.

Day 3
Step 6.
Spot entirety of cell suspension onto pre-warmed BHI+Y plates by filling a pipette tip and spotting discrete 20-uL drops across the plate(s).

Day 3
Step 7.
Pipette plasmid DNA (20 ng/uL in water) atop each spot (10-20 uL drops). Let sit to dry a few mins. Incubate O/N (right-side-up).

Day 4
Step 8.

Pre-warm antibiotic-containing BHI plates (will need 6)

Day 4

Step 9.

Streak growth from cell+DNA spots onto BHI + antibiotic plates in **a thin layer** (streak out as much of the growth as you can - use several plates). Incubate 2-5 days, checking each day for colonies.

Day 6

Step 10.

Patch colonies onto a new selective plate and grow overnight.

Day 7

Step 11.

Perform colony PCR using gene-specific or vector-specific primers to check for insert DNA.

Day 7

Step 12.

Streak out full plates of growth from at least 3 different positive colonies in order to make frozen stocks.

NOTES

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Tip: It can be easier to transform 81-176 than 11168, so if you're having trouble getting DNA into 11168, try putting pDNA into 81-176 first, extracting pDNA from those cells, and transforming that into 11168.