Natural Transformation of Campylobacter jejuni

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Abstract

Day 1

1. Thaw cells, grow 24-48 h.

Day 2

1. Restreak, grow 18-24 h.

Day 3

1. 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.
2. Pre-warm 1-2 BHI+Y plates.
3. Harvest 6-h plate of cells in 2 mL PBS, wash 1x in PBS, resuspend in 250 uL PBS.
4. 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).
5. 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

1. Pre-warm antibiotic-containing BHI plates (will need ~6)
2. 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 (or whenever colonies are observed)

1. Patch colonies onto a new selective plate and grow overnight.

Day 7

1. Perform colony PCR using gene-specific or vector-specific primers to check for insert DNA.
2. Streak out full plates of growth from at least 3 different positive colonies in order to make frozen stocks.

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.

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