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Curing *Wolbachia* infections in *Nasonia* (Parasitoid Wasp)

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This protocol describes curing a *Nasonia* strain of its *Wolbachia* symbionts (Breeuwer and Werren 1990; Breeuwer and Werren 1993). *Wolbachia* cause cytoplasmic incompatibility (CI) in *Nasonia*. Crosses between infected and uninfected wasps, or between infected wasps of different species, will fail because of *Wolbachia*. The sperm modification which induces CI appears to be established before the adult stage, so it is generally not possible to cure an infection and break the CI barrier in the same generation. A *Wolbachia* infection can be removed from a strain over a number of generations, however, by feeding antibiotics and selecting on females who show partial CI. The current genome sequenced strains of *Nasonia* (AsymCX, IV7(u), RV2X(u)) are all *Wolbachia*-free.

MATERIALS

REAGENTS

Sucrose
Tetracycline
Culture vials
Filter paper
Wasp Cultures

PROCEDURE

1. Create 1% tetracycline in 10% sugar water solution.
1ml ddH₂O
0.1g sucrose
0.01g tetracycline
2. Vortex solution for 20–30 sec or until all sugar crystals have dissolved.
3. Cut filter paper into small squares (1cm × 1cm)
4. Dip filter paper into antibiotic solution and place 1–2 squares into a small glass/plastic vial with 5 mated females of chosen strain. Repeat for about 5–10 replicates per strain.
5. Allow females to feed on filter paper overnight.
6. Remove filter paper and host females individually in 12mm culture vials with 2 hosts each. Re-host females 3–4 times in new vials in two day increments.

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7. Once offspring emerge, select mated female offspring from a family which shows a progressively reduced sex ratio (increasing proportion males over time). This is evidence of CI due to reduced *Wolbachia* levels in eggs produced after antibiotic treatment. Females from the later hostings with high proportion males are therefore more likely to have reduced *Wolbachia* loads. Select females from one of these later hostings.
8. Repeat curing procedure for at least 3 generations in order to completely eliminate the infection. Low levels of *Wolbachia* may not show CI but can recover over subsequent generations.
9. Test treated strain for *Wolbachia*. There are two options:
 - a. Phenotypically test by setting up crosses to test for cytoplasmic incompatibility. Set up replicate matings between uninfected virgin females and males from the supposedly cured strain.
 - b. Test for *Wolbachia* with PCR (e.g. Werren and Windsor 2000)

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References

- Breeuwer JAJ, Werren JH. Microorganisms Associated With Chromosome Destruction and Reproductive Isolation Between Two Insect Species. *Nature* 1990;346:558–560. [PubMed: 2377229]
- Breeuwer AJ, Werren JH. Cytoplasmic incompatibility and bacterial density in *Nasonia vitripennis*. *Genetics* 1993;135:565–574. [PubMed: 8244014]
- Werren JH, Windsor DM. *Wolbachia* infection frequencies in insects: evidence of a global equilibrium? *Proc R Soc Lond B Biol Sci* 2000;267:1277–1285.