Community Identification Fast Forward:
The use of High Resolution Melting (HRM) for
de novo characterization of *Wolbachia* diversity

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The *de novo* screening of a species for *Wolbachia* begins with a set of unknown parameters: Is the species infected at all? Which percentage of individuals is infected? How many different strains of *Wolbachia* are present? How many copies of *Wolbachia* exist per host cell? All of these parameters may be variable in a wide range. Traditionally, extensive cloning and sequencing in combination with rarefaction analysis are performed to survey the entire *Wolbachia* community of a host species [1]. High Resolution Melting (HRM) is a sensitive technique for detecting compositional differences in PCR amplicons without need for sequencing [2] and has recently been introduced for strain specific diagnosis in a predefined *Wolbachia* community [3]. Here, we propose a workflow that utilizes HRM for assessment of previously uncharacterized *Wolbachia* infections.

Box 1. Range of HRM curves of the *w*Cer strains generated with primer set A.

Box 2. HRM curve of *w*Cer1 (red) and curves of unknown strains from *Messor* ants. Four distinct shapes (insert) were selected for sequencing.

Collect a representative sample of the species of interest, extract DNA and do HRM-PCR

Setting up a HRM-PCR is straightforward, and results will be available after less then 3 hours. We have tested the method with 2 primer sets. Primer set A covers HRV3+4, primer set B only HRV3; due to the shorter product, primer set B will be more sensitive for single base polymorphisms.

Positive samples?

no → **Both primer sets used?**

no →

yes → **Wolbachia-free samples**

yes ↓

Congratulations! This gives a first impression on the infection rate and on the titre of the infection

Count the different shapes of HRM curves. Select 2-4 individuals per shape for sequencing

Unambigous sequences?

no → **At least one shape represents a mixed infection – cloning is required. Sequence a couple of plasmids.**

In theory, a homogenous amplicon will have a HRM curve with one inflection, while mixed amplicons will have multiple infelctions. Practically, sequence composition and strain titre in multiple infections will strongly affect the shape of HRM curves. Thus, also curves with a single infection have to be examined for the presence of multiple strains, often visible as double peaks in the sequence electropherogram.

yes ↓

One shape = one genotype?

no → **Very similar genotypes, HRM resolution insufficient. Try another primer set.**

HRM is very sensitive for the detection of DNA polymorphism, and even a single SNP may change the shape of a HRM curve. Sensitivity strongly depends on size and GC content of the amplicon. The short amplicon of primer set B will provide best resolution in closely related strains.

yes ↓

Wolbachia community characterized

References
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