How not to be seen: *Wolbachia* and low titer infections

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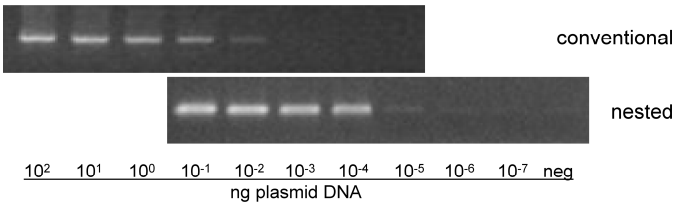
Wolbachia is usually diagnosed by conventional end-point PCR and visualization of the amplicon on agarose gels. PCR is considered as a highly sensitive detection tool, vastly outperforming methods like electron microscopy or antibody based assays. However, the variability of *Wolbachia* density covers ranges where conventional PCR will result in false negative results. We have developed two methods improving the detection limit by three to four orders of magnitude, and by this identifed infections in species and individuals considered *Wolbachia*-free before. Here we compare the benefits and constraints of the techniques.

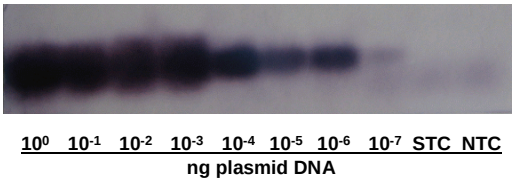
Nested PCR:

- 1st PCR with standard *wsp* primers, 15 cycles, 610 bp amplicon
- 0.5 µl of 1st PCR product as template in 2nd PCR with internal primers, 30 cycles, 362 bp amplicon
- Agarose – EtBr gel electrophoresis



Post PCR hybridization:

- PCR with standard *wsp* primers, 32 cycles
- Agarose gel electrophoresis
- Southern transfer of the separated PCR products to a nylon membrane
- Hybridization with a DIG labelled 201 bp conserved *wsp* probe
- Visualization by NBT/BCIP staining



- ☺ Sequencing is possible
- ☺ Adaptation of technique for MLST genes possible
- ☺ Good potential for automatization
- ☺ Fast: results within 5 hours
- ☹ Cleanroom conditions recommended
- ☹ Prone to carryover-contaminations

- ☺ Little risk of contamination
- ☺ Hybridization with specific probe provides additional evidence of correct amplicon
- ☺ More sensitive than nested PCR
- ☹ Strain identification requires specific primers
- ☹ Additional lab equipment needed
- ☹ Slower: results within 2 days

We have applied both techniques on DNA extracts from the bark beetle *Pityogenes chalcographus* and the fruit fly *Rhagoletis cerasi.* In both approaches, infected samples testing negative with conventional PCR were identified. Application of high sensitive diagnostic tools has the potential to provide new insights into *Wolbachia* abundance and distribution.

References:

Arthofer W., Riegler M., Schneider D., Krammer M., Miller W.J. and Stauffer C. (2009) Hidden *Wolbachia* diversity in field populations of the European cherry fruit fly, *Rhagoletis cerasi* (Diptera, Tephritidae). Molecular Ecology, 18, 3816–3830

Arthofer W., Riegler M., Avtzis D.N. and Stauffer C. (2009) Evidence for low titer infections in insect symbiosis: *Wolbachia* in the bark beetle *Pityogenes chalcographus* (Coleoptera, Scolytinae). Environmental Microbiology 11, 1923–1933.

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