Single tube nested-PCR (STN-PCR): A sensitive detection technique for *Wolbachia* that is less prone to contamination

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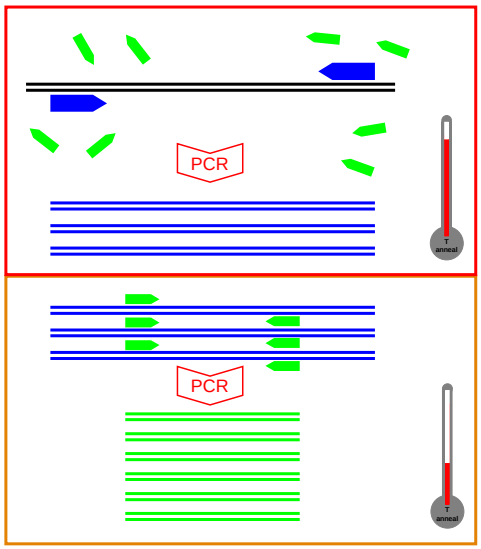
The low titer problem

Low titers of Wolbachia require improved methods of detection. Here we report preliminary findings obtained with use of single-tube nested PCR targeting different genes in different *Wolbachia* strains in research labs in Austria, the United States and Canada.

- Conventional end point PCR often fails to detect low titer infections.
- Nested PCR has excellent sensitivity but is prone to contamination.
- The most problematic step is the transfer of pre-amplified DNA from the first into the second reaction; even with filter tips and robotic equipment, cross contamination and false positives may happen.
- Single tube nested-PCR overcomes this issue as both reactions are performed in the same vessel. It allows high though-put analysis of low titer *Wolbachia* associations.

How does STN-PCR work?



1st amplification: Annealing temperature is high. The short inner primers (green) do not bind at this temperature and therefore do not affect the reaction. All amplicon originates from the long outer primers (blue), which are used to exhaustion in this step.

2nd amplification: Annealing temperature is low. Inner primers in huge molar excess (green) bind to the template amplified in the 1st round of PCR.

PCR conditions

Primers targeting *wsp*

wsp-L-F2	TGGTCCAATAAGTGATGAAGAAACTAGCTACTACGTTCG
wsp-L-R2	AAAAATTAAACGCTACTCCAGCTTCTGCACCAAC
*wsp*151F	TGGTTACAAAATGGACGACA
*wsp*599R	CACCAACAGTGCTGTAAAGAAC

Primers targeting IS5

IS5-outF1	ACTTCAGAGTATCATACAAGAAAGGAGGAAGG
IS5-outR3	GAAATTCTCAGTGGATGTTGTGAGTAAATCATACTCC
IS5-inF1	GCTATCGAAGACTGTGTATG
IS5-inR1:	TAGCAGCGCCTACGTAAC

PCR chemistry & cycling

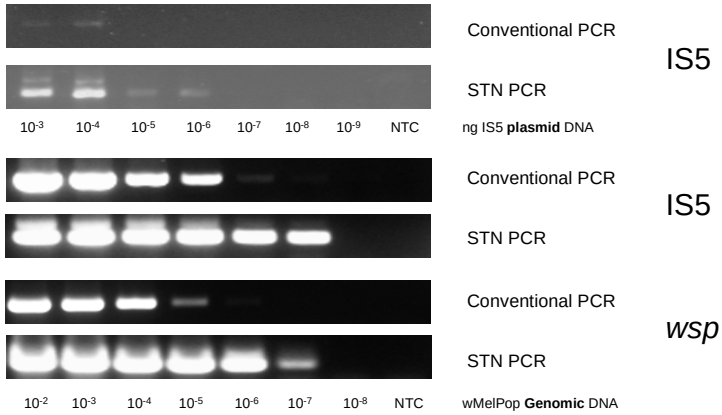
5 µl reaction scale, 1× MyTaq Buffer (bioline, UK), 200 nM (*wsp*) or 50 nM (IS5) outer primer, 1 µM inner primer, 1 µl template DNA, 0.5 U MyTaq (bioline)
95°C-2min / [95°-30s / Ta_1-30s / 72°-1min] × 18 / [95°-30s / Ta_2-30s / 72°-1min] × 35
wsp: Ta_1 = 68°C, Ta_2 = 50°C; IS5: Ta_1 = 67°C, Ta_2 = 55°C

Test strains

*w*Cer1, *w*Cer2, *w*MelPop

Results

IS5 and *wsp* amplification improved by two orders of magnitude compared to conventional PCR with standard primers using plasmid DNA as template. Using genomic DNA as a template, amplification of the IS5 gene lead to sensitivity increasing by an order of magnitude while an increase of two orders of magnitude was seen when amplifying the *wsp* gene.



Summary & Outlook

Our experiments show that STN is suitable for the detection of low titer *Wolbachia*. Additional work is needed to develop and test primers applicable with all supergroups. Portation of the STN principle to real-time PCR equipment will further reduce the risk of cross-contamination, as no amplicon will be released to the laboratory environment.

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