Selective whole genome amplification (SWGA) for *Toxoplasma gondii*: How will it help?

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8 million domestic cats in Britain

An estimated **80,000** cats shed the *toxoplasma* parasite at any one time

350,000 people in the UK are newly infected with the *toxoplasma* parasite every year…

…altogether, up to **20million** British people carry the *toxoplasma* parasite…

… of these, **80%** exhibit no symptoms

About SWGA

Selective whole genome amplification (SWGA) is recent approach which requires both **bioinformatics steps** and **laboratory steps** (Figure 1). It allows species-specific sequencing without culture of target organism, contamination by host DNA or extensive purification of target DNA. It specially amplifies the target genome using a set of selective primers and phi29 polymerase based multiple displacement amplification (MDA).

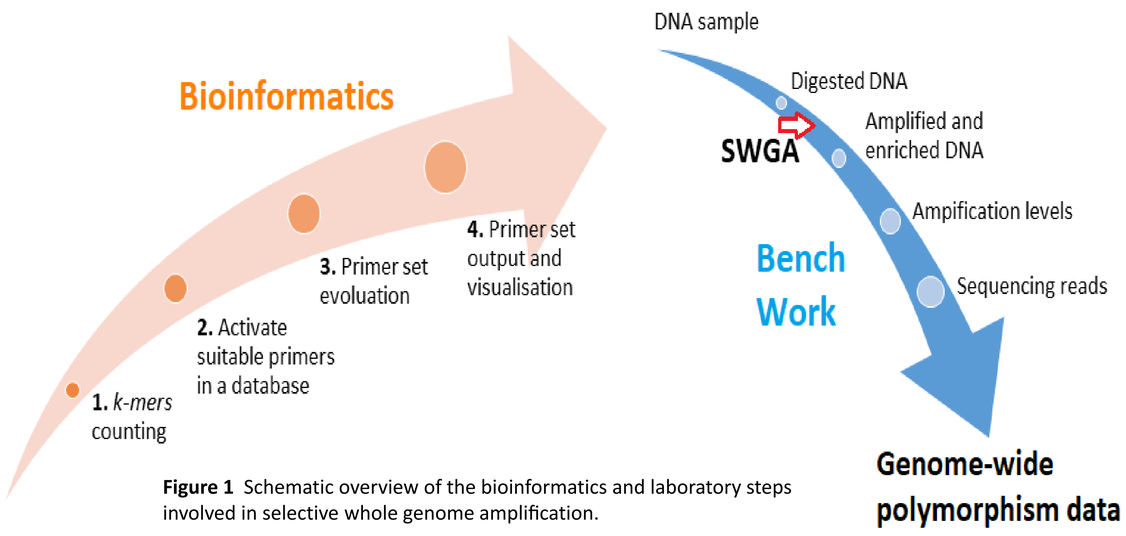


Figure 1 Schematic overview of the bioinformatics and laboratory steps involved in selective whole genome amplification.

Process of SWGA primer sets selection for *T. gondii*

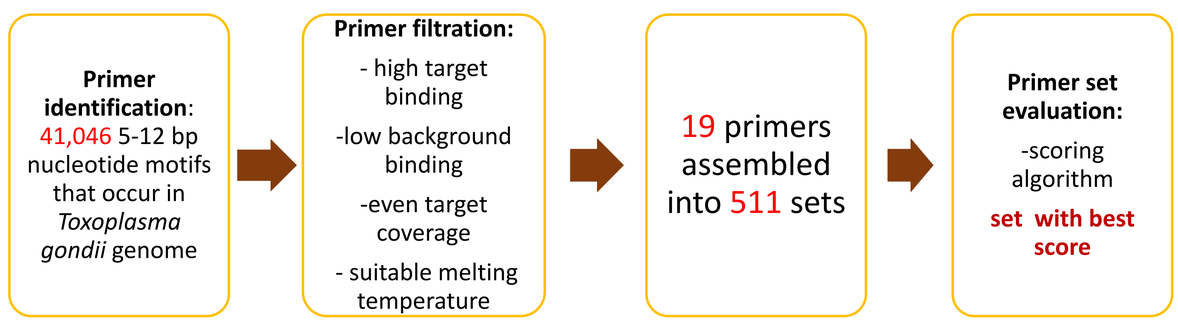
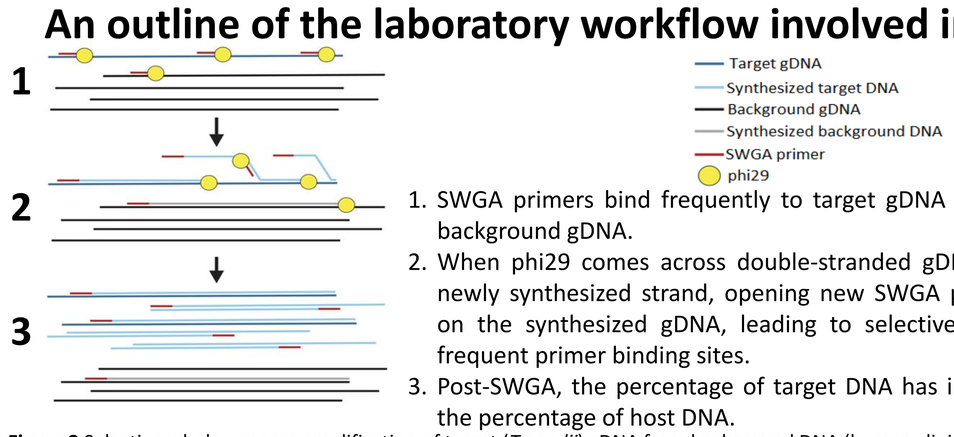


Figure 2 Flowchart illustrating the bioinformatics steps involved in process of SWGA primer set selection for *T. gondii.*

An outline of the laboratory workflow involved in SWGA



1. SWGA primers bind frequently to target gDNA and infrequently to background gDNA.
2. When phi29 comes across double-stranded gDNA, it displaces the newly synthesized strand, opening new SWGA primer binding sites on the synthesized gDNA, leading to selective amplification with frequent primer binding sites.
3. Post-SWGA, the percentage of target DNA has increased relative to the percentage of host DNA.

Figure 3 Selective whole genome amplification of target (*T.gondii*) gDNA from background DNA (human clinical sample) (Adopted from *Cowell et al.,* 2017).

Amplification levels

SWGA program was used to identify primer set for selective amplification of *T.gondii* genomic DNA from human DNA. Primer set was selected that successfully amplify *T.gondii* parasite (RH strain) from human backgrounds. Amplification ratio of *T.gondii* vs Human DNA was determined using qPCR. Targeted amplification of *T.gondii* mixed with human genome results in >10 fold amplification of the target genome with <4 fold for amplification of the background.

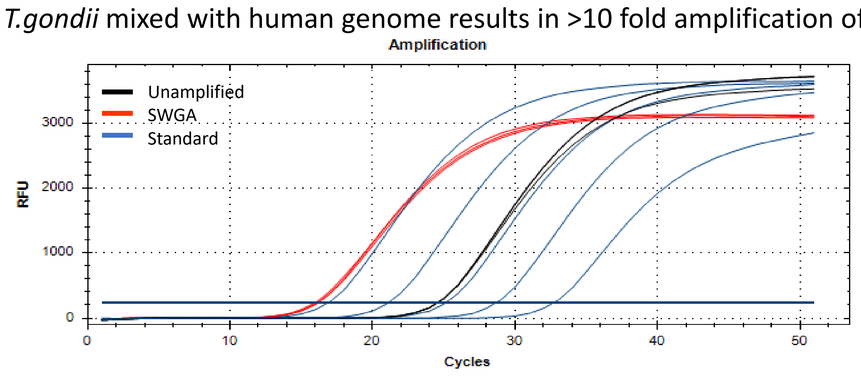


Figure 4 Q-PCR detection of the *T.gondii* RE gene in unamplified samples and after SWGA.

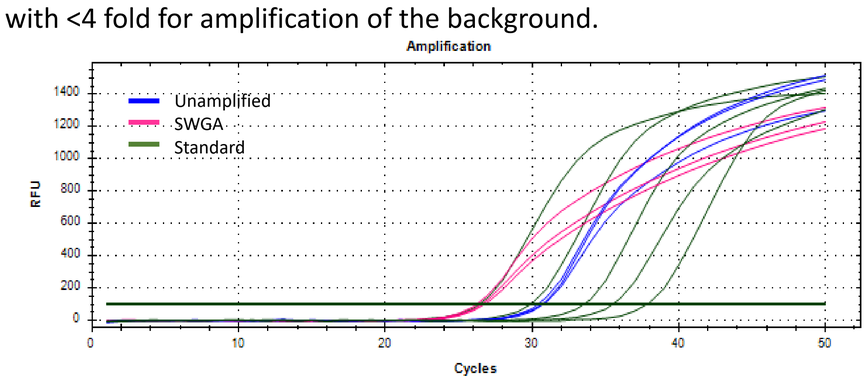


Figure 5 Q-PCR detection of the human RNase P gene in unamplified samples and after SWGA.

Agarose

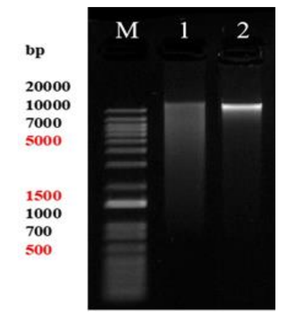


Figure 6 *T.gondii* genomic DNA was amplified by SWGA method. Product was visualized in 0.7% agarose gel. **M**) marker; **1**) SWGA; **2)** Unamplified

Amplification results on SWGA for *T.gondii* and Human from WGS

T.gondii and Human chromosomal coverage following SWGA using selected primer set. The base compositions of chromosomes were visualized in IGVTools using the *T.gondii* ME-49 reference genome and Human GRCh37. Shown in blue (unamplified) and red are coverage depth using designed primer set.

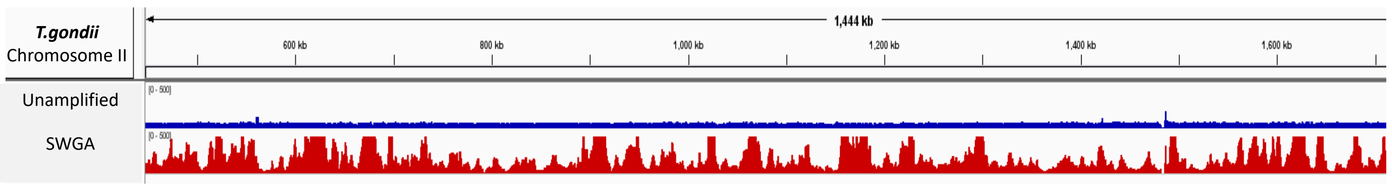


Figure 7 *T.gondii* chromosomal coverage. The SWGA method significantly increase the percentage of reads that mapped to *T.gondaii* reference genome, from 51% to 99%, and improve genome mean coverage obtained from 67x (blue) to 243x (red).

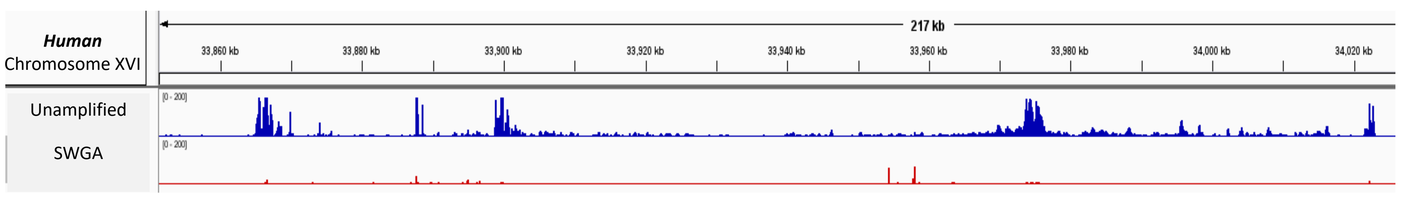


Figure 8 Human chromosomal coverage. The SWGA method decrease the percentage of reads that mapped to human reference genome, from 56% to 2.3%, and lower the mean genome coverage from 18x (red) to 0.07x (blue).

% reads that map to Human and *T.gondii* genome

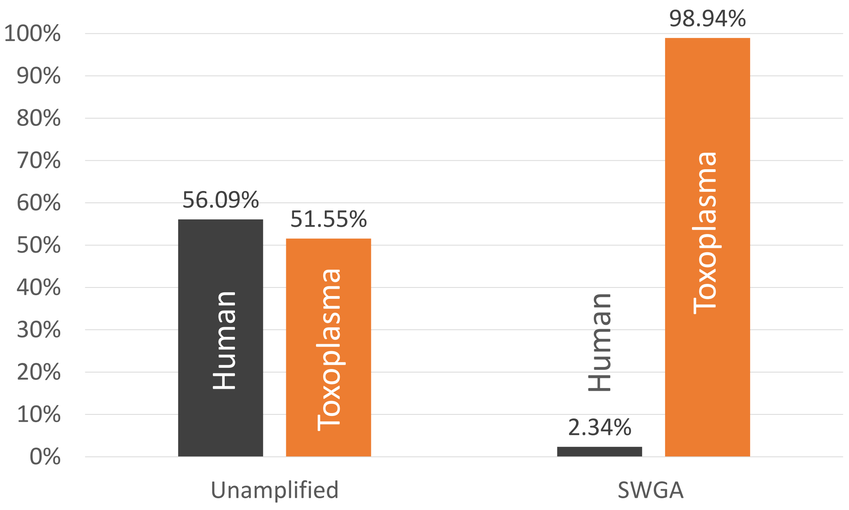


Figure 9 Testing of SWGA primer sets on DNA from an unprocessed, *T.gondii* cultured in vitro in human HFF cells. Unamplified DNA for *T.gondii* and DNA amplified with SWGA primer set 1 or set 2 was sequenced. The percentages of reads that mapped to the *T.gondii* and human reference genome in IGV were plotted for both unamplified and SWGA-amplified samples

How will it help? The potential outcomes of project will…

- ✓ allow analysis of *Toxoplasma* genome sequences directly from human clinical samples
- ✓ exploit a valuable collection of DNA extracts and original clinical samples with accompanying clinical data for analysis
- ✓ enable significantly more in-depth investigations, improving our understanding of the epidemiology, virulence and other traits of this important human pathogen, thus assisting in developing strategies for treatment, surveillance and infection prevention
- ✓ make an important contribution to the '3Rs' (Replacement, Refinement and Reduction in the use of animals in research) by precluding the need for *in vivo* isolation of *Toxoplasma* from clinical specimens prior to WGS

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Reference

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