Standard Operating Procedure (SOP)	
SOP Title	Selective Whole Genome Amplification (sWGA): Protocol for amplification of *Plasmodium falciparum*.
SOP number	DBS04
SOP Version	2

1. SCOPE

This protocol describes a method for selective whole genome amplification (sWGA) of Plasmodium falciparum from samples that contain a high proportion of human DNA (Oyola, S.O., Ariani, C.V., Hamilton, W.L. et al. Whole genome sequencing of Plasmodium falciparum from dried blood spots using selective whole genome amplification. Malar J 15, 597 (2016). This method has been designed for use on samples where the human contamination is too high to allow efficient or successful downstream processing of the parasite DNA, for example, where parasitaemias are very low or leuko-depletion methods are not sufficient to reduce the human contamination. The protocol is designed around the phi29 polymerase and therefore the reaction should produce long amplicons of several kb to 10's of kb in length and should be suitable for a range of procedures particularly whole-genome-sequencing.

This version of the protocol is called sWGA-HuPf (*Human, P. falciparum*). Modifications are necessary for sWGA of other Plasmodium species. Please note sWGA-HuPf cannot be used for *P. vivax*.

In essence, this method is equivalent to the commercially available multiple-displacement kits using Phi29 polymerase, except that we have replaced the universal primer set with a bespoke set that preferentially amplifies the genome of choice (*P. falciparum*). Do note that other plasmodium species will require a different set of primers.

As of this version of the protocol, the cut-off for the sWGA is about 200-300 parasites in the reaction itself. It is important not to confuse this with any parasitaemia metric. However, this current design aims to use 40ng input DNA that is approximately equivalent to 1µL of human blood.

This method can be used for tubes; just adjust the reagents and protocol accordingly

This method assumes that DNA has already been extracted.

2. MATERIALS REQUIRED:

2.1 – REAGENTS AND MATERIALS		
Reagents	***Supplier***	***Cat. No.***
Phi29 Enzyme kit	NEB	M0269L
dNTP mix	Bioline	BIO-39025
Nuclease free water (NFW) - Ambion	Fisher Scientific	AM9930
sWGA primer pool (250uM*) – see Appendix 1		
Low molarity Tris ETDA (Low-TE)	See section 2.3 – Low TE	
Materials	***Supplier***	***Cat. No.***
PCR machine		
96 well qPCR plate, skirted (AB2800)	fisher scientific	10032013
Microseal "A" seal	BIO-RAD	MSA 5001
Qiagen seal	Qiagen	19570
Centrifuge (requires 96 well plate adapter)		
Ice tray/cooling block		
Flacon tubes (15mL)		
Lo-bind Eppendorf tubes (2mL or 5mL)		
Tissue wipes (Azowipes)		
Repeater & tips (30µL)		
Pipettes (1000, 100 and 20 µL)		
Filtered tips of various volumes		

2.2 – dNTP MIX PREPARATION	
2.2.01	Combine dNTP Set (100 mM, 4 x 250 µL) in a 15 mL falcon.
2.2.02	Dilute to 10 mM by adding 11.5mL of NFW.
2.2.03	Dispense as 550 µL aliquots into Eppendorf tubes and store at -20°C.

2.3 – LOW TE BUFFER:

2.3.01 To make up Low TE buffer use the following recipe:

Components:	**Volume (mL)**
TRIS-HCl, pH 8.0 (1M)	10
EDTA (0.5M)	0.2
NFW	989.8
TOTAL	**1000**

3. METHODOLOGY

SUMMARY

- Add 40ng of DNA per well to a 96-well plate and make up to 30 µL with low-TE.

- Make up the master mix for sufficient number of wells required (containing, enzyme, buffer, dNTPs and primers).

- Total reaction volume of 50 µL.

    - Add 20 µL of mastermix to every well.

    - Add 30 µL of DNA.

- Make sure that the new plate has the same layout as the gDNA plate.

- Seal the plate and place on a PCR machine and run the special step-down iso-thermal temperature profile.

    - N.B. – This PCR will take approximately 18 hrs.

3.1 – sWGA METHOD

3.1.01	Clear out a sufficient work area and wipe down the lab bench surface and pipettes with tissue wipes (Azowipes) or equivalent. (to sterilise place pipettes, tubes, racks and any other equipment under UV light for 20 min).
3.1.02	Take a 96-well PCR plate and place onto an ice tray/cooling block.
3.1.03	Add 40ng of DNA per well and make each well up to 30 µL with low TE. N.B: minimum DNA concentration for use is 1.33ng/µL.
3.1.04	Place a plate seal over the plate and place in a fridge while you prepare the sWGA master-mix.
3.1.05	Make the sWGA mastermix for the number of plates being processed as shown below (Table 1) in a Lo-bind Eppendorf tubes (2mL or 5mL). The "x100" will be sufficient for a single 96 well plate.

		1 plate
sWGA mastermix: **Add in this order**	**Per well (µL)** **1 sample**	**x100 (µL)** **1 plate**
10x Phi29 buffer	5	500
100x BSA	0.5	50
250 uM primers	0.5	50
10 mM dNTP	5	500
Nuclease-free water	6	600
30units Phi29 Enzyme*	3	300
Total volume	**20.0**	**2000.0**

Table 1: Composition of the sWGA master-mix reaction per well and per 96-well plate.

* = To be added last in the master mix

3.1.06	Add 20µL of sWGA mastermix to each well of a plate and mix.
3.1.07	Seal the plate(s) using Microseal "A" seals and briefly centrifuge.

3.2 – PCR machine:

3.2.01	Turn on the PCR machine. Allow to initialise (this may take a few minutes).
3.2.02	Program the PCR machine using the following cycling conditions. Please select the heated lid option.

sWGA PCR protocol

STEP	Temp.	Duration	Comments
Step 1	**35°C**	05:00 min	
Step 2	**34°C**	10:00 min	
Step 3	**33°C**	15:00 min	
Step 4	**32°C**	20:00 min	
Step 5	**31°C**	30:00 min	
Step 6	**30°C**	16:00:00 hr	
Step 7	**65°C**	15:00 min	Heat-inactivation of the Phi29
Step 8	**10°C**	∞	

3.2.03	Place each plate onto the PCR platform then close and tighten the lids.
3.2.04	Once the machine is ready, start the SWGA PCR program. **<u>This PCR will take about 18 hrs to run</u>.**

3.3 – POST PCR

3.3.01	Remove the plates from the PCR machine, by firstly loosening the lid and gently removing the plates.
3.3.02	Centrifuge the plate briefly at 1000g for 30 seconds.
3.3.03	Carefully remove the Microseal 'A' film and replace with a Qiagen seal.
3.3.04	The plates can now be stored for future use.

4. STORAGE

Neat sWGA can be stored at 4°C for a month if necessary. However, it is best to seal and wrap the sWGA plate(s) with Parafilm and store at -20°C. The sWGA will keep for years in this state. The sWGA material will withstand freeze-thaw cycles although this should be minimised as much as possible.

Once the sWGA has been diluted for use in downstream PCR reactions it will start to degrade rapidly even if stored at 4°C.

APPENDIX 1 – sWGA PRIMERS

sWGA primers for *Plasmodium falciparum*			
Primer name	Primer sequence*	Primer quantity to order	Primer formulation
Pf1	ATATATATAT*A	250 nmole	STD
Pf2	TATATATATAT*T	250 nmole	STD
Pf3	TATATATATA*A	250 nmole	STD
Pf4	TAATATATA*T	250 nmole	STD
Pf5	TATATATATT*T	250 nmole	STD
Pf6	ATTATTATTA*T	250 nmole	STD
Pf7	TAATAATAAT*A	250 nmole	STD
Pf8	AAAAAAAAAAA*A	250 nmole	STD
Pf9	AATAATAATA*A	250 nmole	STD
Pf10	TATTATATA*T	250 nmole	STD

Table 2: Primers for the sWGA of *Plasmodium falciparum*.
*The asterix indicates that the 3' base is joined through a phosphorothioate bond to inhibit the exonuclease activity of Phi29.

Primer purchase:
Please purchase the primers lyophilized. IDT is the suggested supplier and the oligos above are formatted for IDT. Note that there is an extra charge for the phosphorothioate bond per primer.

Primer preparation:
Re-suspend each primer in water to a concentration of 250 µM

- Find the number of nmole of primer from the oligo label of datasheet.
- Microliters (µL) of water to add = nmole oligo/0.25
- To make the pooled mixture, take an equal volume of each oligo and mix them together.
  - i.e find the oligo with the smallest volume and that limits required volume.
- The final concentration of the pool is 250 µM, the final concentration of each oligo is 25 µM.

A 250 nmole quantity of each oligo should theoretically create a 10ml pool of primer mixture which is enough for 200 plates.