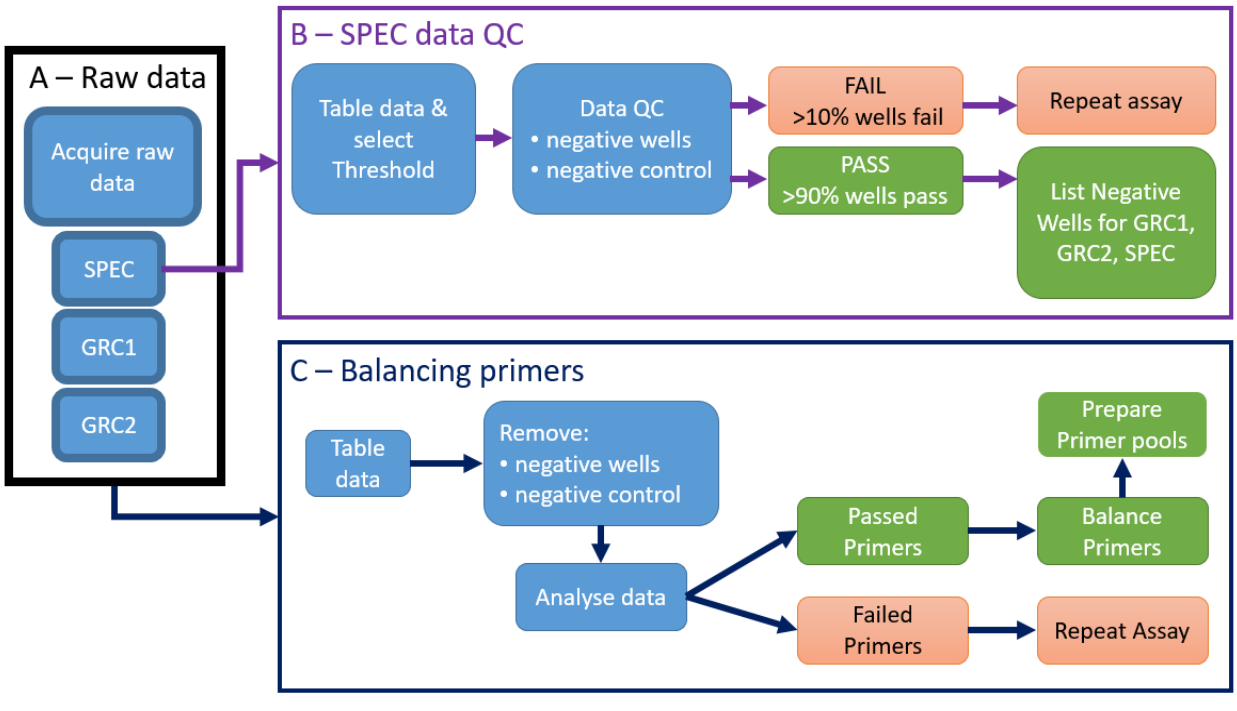
Standard Operating Procedure (SOP)	
SOP Title	Analysis of the testing and rebalancing data and aliquoting of the new panels
SOP number	GbS02b
SOP Version	2

1. SCOPE.

For the balancing of the primer panel, the negative control and any negative and/or failed wells will need removal from the dataset so that the reads from these wells do not skew the analysis (See Appendix 4).

To analyse the data (see flow diagram below):

A. Retrieve the raw data for SPEC, GRC1 and GRC2.
B. Organise SPEC wells and graph the reads. Mixed falciparum and vivax samples will be classified as passed (assuming positive reads). If plasmodium vivax samples are present these will be classified as failed. Calculate the number of passed and failed wells.
   - More than 10% of wells failed then repeat from start of protocol.
   - If the assay has passed (90% of wells passed) make a list of failed wells and proceed to the balancing process.
C. Balance each primer set (GRC1, GRC2 and SPEC).
   - Remove the negative wells from each primer set.
   - If a primer has failed then repeat from start of protocol.
   - If the assay has passed, then balance the primers and prepare the primer pool.



1.0 Raw data	
1.0.01	Acquire the results file following the upload post sequencing run. MalariaGEN will return the data to run the testing and balancing analysis.
1.0.02	Open a new Excel spreadsheet for the SPEC data.
1.0.03	Save file as “GbS_QC_DDMMYYYY”
1.0.04	Select the first tab and name it “**RAW_DATA_QC**”.
1.0.05	Create a new sheet and label “**TIDIED-UP**”.
1.0.06	Copy and paste one column at a time from the from **RAW_DATA_QC** tab into the “**TIDIED-UP**” sheet • “Rpt” = this is an identifier for each well • “Region” = The target • “Region_reads”
1.0.07	Rename the columns in the “**TIDIED-UP'** sheet as shown below: • “Rpt” = **well** • “Region” = **Target** • “Region_reads = **Reads**
1.0.08	**Well** column: Remove any extraneous characters from the well column leaving just the well numbers (e.g. 1 to 96). NOTE - characters up to and including the hash/hatch.
1.0.09	**Target** column: Remove any extraneous characters from the “Target” column. Leaving only the target name as per the primer list (Appendix 1, 2 and 3).
1.0.10	**Reads** column: The Reads are left as is.

1.1 Data sorting

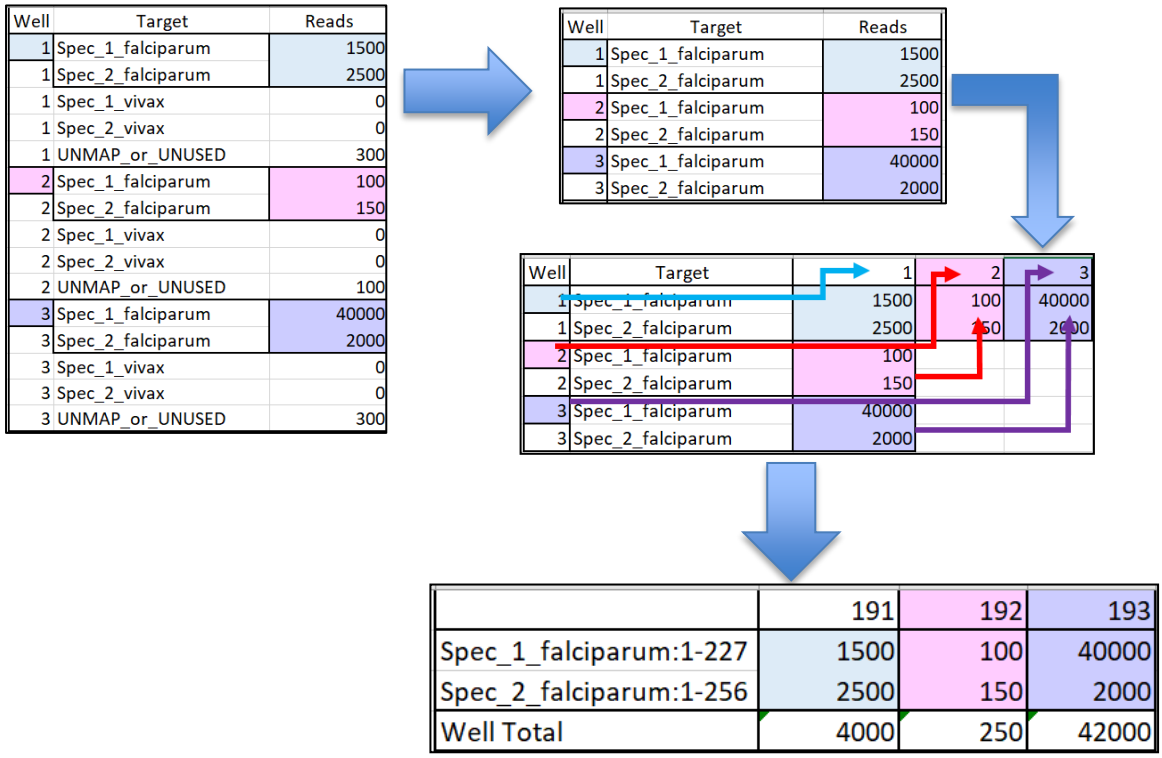
Well	Target	Reads
1	Spec_1_falciparum	1500
1	Spec_2_falciparum	2500
1	Spec_1_vivax	0
1	Spec_2_vivax	0
1	UNMAP_or_UNUSED	300
2	Spec_1_falciparum	100
2	Spec_2_falciparum	150
2	Spec_1_vivax	0
2	Spec_2_vivax	0
2	UNMAP_or_UNUSED	100
3	Spec_1_falciparum	40000
3	Spec_2_falciparum	2000
3	Spec_1_vivax	0
3	Spec_2_vivax	0
3	UNMAP_or_UNUSED	300

1.1.01	Select the "**TIDIED-UP**" sheet.
1.1.02	Select the well, Target and Reads columns.
1.1.03	Organise the Tags into numerical order: From the menu, select "Data" and then "Sort". From the Sort box select the following: Sort by = Well Sort On = Values Order = A to Z Then select OK.
1.1.04	Select each well group of data and add a border as above. This will help visual each data set.

1.2 Threshold selection.

The SPEC data are analysed to determine a suitable threshold to separate negative and positive wells. To do this the data is graphed.

1.2.01	Create a new sheet and label **"Threshold"**.
1.2.02	Copy and paste the data from the **'TIDIED-UP'** sheet into the **"Threshold"** sheet.
1.2.03	Delete the rows with vivax (Spec_1_vivax and Spec_2_vivax) and unmapped (UNMAP_or_UNUSED) target names and reads.
1.2.04	Rearrange the data to resemble to layout below, with the "**Well**" across the columns and the "**Target**" in the rows. N.B. delete the "Target" from column title.



Well	Target	Reads
1	Spec_1_falciparum	1500
1	Spec_2_falciparum	2500
1	Spec_1_vivax	0
1	Spec_2_vivax	0
1	UNMAP_or_UNUSED	300
2	Spec_1_falciparum	100
2	Spec_2_falciparum	150
2	Spec_1_vivax	0
2	Spec_2_vivax	0
2	UNMAP_or_UNUSED	100
3	Spec_1_falciparum	40000
3	Spec_2_falciparum	2000
3	Spec_1_vivax	0
3	Spec_2_vivax	0
3	UNMAP_or_UNUSED	300

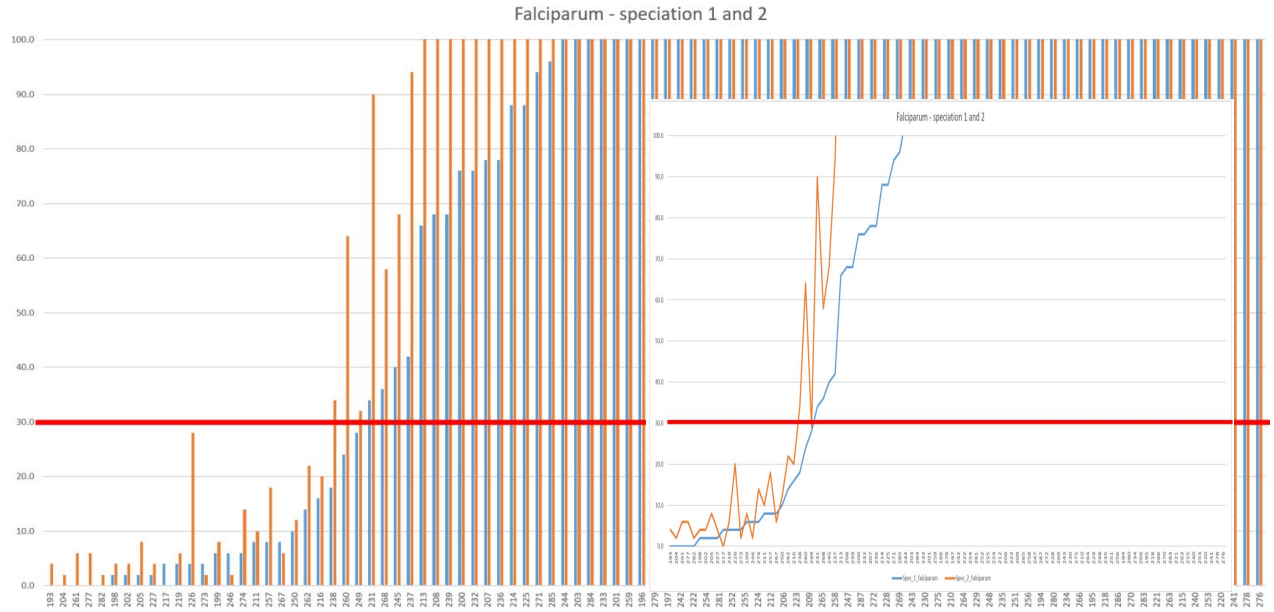
Well	Target	Reads
1	Spec_1_falciparum	1500
1	Spec_2_falciparum	2500
2	Spec_1_falciparum	100
2	Spec_2_falciparum	150
3	Spec_1_falciparum	40000
3	Spec_2_falciparum	2000

Well	Target	1	2	3
1	Spec_1_falciparum	1500	100	40000
1	Spec_2_falciparum	2500	150	2000
2	Spec_1_falciparum	100		
2	Spec_2_falciparum	150		
3	Spec_1_falciparum	40000		
3	Spec_2_falciparum	2000		

	191	192	193
Spec_1_falciparum:1-227	1500	100	40000
Spec_2_falciparum:1-256	2500	150	2000
Well Total	4000	250	42000

1.2.05	Calculate the falciparum well total reads: • **Well Total** (Columns) – At the bottom of the columns, calculate total reads.
1.2.06	Sort the columns by the "**Well Total**" to place the lowest performing well reads to the left and the highest performing well reads to the right. • Select columns B onwards. Then sort the data by selecting "data" from header, next select options and change the orientation to "left to right". Select the row corresponding to "**Well total**" and sort by selecting "values smallest to largest".

1.3	Threshold selection - Graphing the data.
1.3.01	Create a graph of the data table in the "**Threshold**" sheet
1.3.02	Select all the data and select "Insert" from the menu. From the "Insert" menu select "insert column or bar graph". Then select "2-D column" and "Clustered Column".
1.3.03	Adjust the size of the graph to fit the window. • Select the reads axis and adjust the scale to zero to one hundred.



Falciparum read data plotted as both a histogram and a line graph (inset) showing the reads on a scale between 0 and 100. Therefore, highlighting the most suitable threshold (line in red). In this example, a threshold of 30 has been selected but this will vary from assay to assay.

1.3.04	Review the data and select a suitable threshold.
1.3.05	Change the graph type (as per insert) if this helps visualisation of the trend.

1.4 QC negative data.

Well	Target	Reads	Well Calls
1	Spec_1_falciparum	1500	PASS
1	Spec_2_falciparum	2500	
1	Spec_1_vivax	0	
1	Spec_2_vivax	0	
1	UNMAP_or_UNUSED	300	
2	Spec_1_falciparum	100	PASS
2	Spec_2_falciparum	150	
2	Spec_1_vivax	0	
2	Spec_2_vivax	0	
2	UNMAP_or_UNUSED	100	
3	Spec_1_falciparum	40000	PASS
3	Spec_2_falciparum	2000	
3	Spec_1_vivax	0	
3	Spec_2_vivax	0	
3	UNMAP_or_UNUSED	300	
4	Spec_1_falciparum	1000	PASS
4	Spec_2_falciparum	1900	
4	Spec_1_vivax	0	
4	Spec_2_vivax	2	
4	UNMAP_or_UNUSED	320	
5	Spec_1_falciparum:1-227	15	FAIL
5	Spec_2_falciparum:1-256	30	
5	Spec_1_vivax:1-230	0	
5	Spec_2_vivax:1-256	0	
5	UNMAP_or_UNUSED	50	

1.4.01	Select the "**TIDIED-UP**" sheet.
1.4.02	Conditional format the reads to highlight the falciparum positive wells: From the menu: • Select "Conditional Formatting" and then "New Rule". From the "New Formatting Rule" window select the following: • Select a "Rule Type" = Format only cells that contain • From the "Format only cells with" select the following: ▪ Cell Values ▪ Greater than ▪ Value = 30* • Select "Format" and "Fill". Then select the background colour (e.g. purple). • Then select OK on the "format" window • Then OK on the "New Formatting Rule" window.
* The value will vary depending on the dataset.	
1.4.03	For each well determine if it has passed or failed: ○ PASS – Reads exceeding threshold for the falciparum speciation primers. ○ FAIL – Reads below threshold or no reads present for the falciparum speciation primers
1.4.04	Highlight the FAILED wells in red (as shown above).

1.5 NEGATIVE/FAILED WELLS.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

	1	2	3	4	5	6	7	8	9	10	11	12
A	97	105	113	121	129	137	145	153	161	169	177	185
B	98	106	114	122	130	138	146	154	162	170	178	186
C	99	107	115	123	131	139	147	155	163	171	179	187
D	100	108	116	124	132	140	148	156	164	172	180	188
E	101	109	117	125	133	141	149	157	165	173	181	189
F	102	110	118	126	134	142	150	158	166	174	182	190
G	103	111	119	127	135	143	151	159	167	175	183	191
H	104	112	120	128	136	144	152	160	168	176	184	192

	1	2	3	4	5	6	7	8	9	10	11	12
A	193	201	209	217	225	233	241	249	257	265	273	281
B	194	202	210	218	226	234	242	250	258	266	274	282
C	195	203	211	219	227	235	243	251	259	267	275	283
D	196	204	212	220	228	236	244	252	260	268	276	284
E	197	205	213	221	229	237	245	253	261	269	277	285
F	198	206	214	222	230	238	246	254	262	270	278	286
G	199	207	215	223	231	239	247	255	263	271	279	287
H	200	208	216	224	232	240	248	256	264	272	280	288

GREEN = PASS
RED = FAIL
GREY = Negative control

1.5.01	Create a plate template in the pattern of the wells used.
1.5.02	Using the QC data make a list of failed wells and mark them onto the plate templates in red.
1.5.03	Use the SPEC plate maps to determine the negative wells on the GRC1 and GRC2 plates including negative controls.
1.5.04	Make a list of failed wells and the negative control for each plate tested.
The highlighting of wells will emphasise if edge effect has occurred during library production. Make a note of column and/or row affected.	
1.5.05	Passed: If more than 90% pass then continue with the balancing process.
1.5.06	Failed: If more than 10% fail then the assay will need to be repeated.

2. DATA ANALYSIS & PRIMER REBALANCING.

N.B. – refer to 'GbS_rebalancing_example' Excel file for an example of what is described in the following instructions. This SOP assumes that the operator is proficient in the use of Microsoft excel.	
2.1 Raw data	
2.1.01	If not already done so acquire the results files for all targets following the upload post sequencing run.
2.1.02	Select one of the primer panels. Analysis should be completed in the following order SPEC then GRC1 and finally GRC2. (From this point on all three data outputs will be used for analysed)
2.1.03	Open a new Excel spreadsheet.
2.1.04	Save file as "GbS_rebalancing_TARGET_DDMMYYYY". (e.g. GbS_rebalancing_GRC1_12052019)
2.1.05	Select the first tab and name it "**RAW_DATA**".
We are only interested in three of the columns in the results file: 'Rpt' (well number/sample), 'Region' (Target) and 'Region_reads' (number of reads for that particular combination of well and target).	
2.1.06	Create a new sheet and label '**TIDIED-UP**'.
2.1.07	Copy and paste one column at a time from the from **RAW_DATA** tab into the '**TIDIED-UP**' sheet • "Rpt" • "Region" • "Region_reads".
2.1.08	Rename the columns in the '**TIDIED-UP**' sheet as shown below: • "Rpt" = **Well** • "Region" = **Target** • "Region_reads = **Reads**
2.1.09	**Well** column: Remove any extraneous characters from the well column leaving just the well numbers (e.g. 1 to 96). NOTE - characters up to and including the hash/hatch.
2.1.10	**Target** column: Remove any extraneous characters from the "Target" column. Leaving only the target name as per the primer list (Appendix 1, 2 and 3).
2.1.11	**Reads** column: The Reads are left as is.

2.2	Removal of negative wells
2.2.01	In the **'TIDIED-UP'** tab delete the data associated to the negative control well as determined in 8.5.04. Select all the rows associated with the specific well and delete.
2.2.02	Delete the data associated to the negative wells as determined in 8.5.04. • Select all the rows associated with the one of the FAILED well and delete. • Repeat for remaining failed well.

2.3	Creating the Pivot table
2.3.01	Once the three columns have been completed and the negative wells removed then Pivot the data into a table in a new tab.
2.3.02	Creating the Pivot table PART 1: • 1: Select cell A1 in **'TIDIED-UP'** tab**.** • 2: Select PivotTable (can be found on the INSERT menu). • 3: In the pop-up window, the 3 table columns will be displayed (i.e. Well, Target and Reads). • Select "New Worksheet" in the bottom-half of the window. • Then click OK.
2.3.03	Creating the Pivot table PART 2: • 1: In the "PivotTable Fields" window ▪ Click and drag "**Well"** into the COLUMNS box**.** ▪ Click and drag "**Target"** option in to the ROWS box. ▪ Click and drag "**Reads"** option into the VALUES box. • 2: Then click OK.
2.3.04	Rename the newly created tab "**Pivot**".

2.4	Calculating subtotals and sorting
2.4.01	Create a new worksheet called 'SUBTOTALS CALCULATED, SORTED'.
2.4.02	Copy the whole pivot table and paste special (Values) into a new sheet: 1. In the Pivot table worksheet select all cells (Ctrl A) and copy (Ctrl C). 2. Go to the new worksheet: 'SUBTOTALS CALCULATED, SORTED', select cell A1 and “paste” the data in by depressing the right click on the mouse, move to “Paste options”, then select “Paste Special”, select “Values” and then “OK”.
2.4.03	Delete rows “Grand Total” & “UNMAP_or_UNUSED” at bottom of table.
2.4.04	Delete column “Grand Total” at end of table.
2.4.05	Calculate the total reads: • Well Subtotal (Columns) – At the bottom of the columns, calculate total reads. • Target Subtotal (rows) – At the end of the rows, calculate total reads.
2.4.06	Conditionally format the table in order to highlight any particular wells or targets, which are relatively under or over performing. From the menu, select “Conditional Formatting” and then “New Rule”. From the “New Formatting Rule” window select the following: • Select a “Rule Type” = Format all cells based on their values From the “Edit the Rule Description” select the following: • Format Style = 3-Color Scale • Minimum: Lowest value – Red • Midpoint: Percentile 50 – Yellow • Maximum: Highest value – Green Then select OK on the “New Formatting Rule” window.
2.4.07	Sort the wells and targets by the totals to place the lowest performing well/target combination in the top-left corner and the highest performing well/target combination in the bottom-right (see GbS_rebalancing_example). • Select all rows (from row 1 down to and including “Well Subtotal”). Sort the data by selecting “data” from header, next sort by “Target Subtotal” using “values smallest to largest”. • Select columns B onwards up to target subtotal. Sort the data by selecting “data” from header, next select options and change the orientation to “left to right”. Select the row corresponding to “Well Subtotal) and sort by selecting “values smallest to largest”.

2.5 Failed Primers

It is important to determine if any of the primers from the panel have not produced reads. If this occurs, it would suggest that a set of primers have failed to yield any Libraries.

If this is the case, the whole process of testing and balancing will need to be repeated. This would be most likely that either one or both of the primer pair has been missed during pooling.

If this is the case then using the failed primer pool would not yield libraries and a full dataset will never be produced. Therefore, a new primer pool would be required. We recommend repeating the initial production of the “equivolume plate” and then repeating the testing and balancing process.

2.5.01	With the data sorted review “Target subtotal” for each Target.
2.5.02	Highlight in Blue any Targets where the “Target subtotal” is less than the threshold selected during the QC. These will be categorised as FAILED.
2.5.03	If one or more Targets have failed then stop the process at this point.
2.5.04	If all Targets have produced adequate reads then proceed to next section.

2.6 Target read factions + median

The purpose of calculating the read fractions per target is to provide the input for the primer-rebalancing algorithm. The output of this is a list of pool weightings whose level is dependent on the read fractions observed in the unbalanced primer pool. Calculating pool weightings is complicated by the fact that there is sample-to-sample variability in read fractions (see GbS_rebalancing_example).

2.6.01	Copy the whole 'SUBTOTALS CALCULATED, SORTED' sheet and paste into a new tab (by selecting paste and then paste values). Label the new tab “Target read fractions, median”.
2.6.02	Delete row “Well Subtotal”. (i.e. delete bottom row)
2.6.03	Delete column “Target Subtotal”. (i.e. delete last column)
2.6.04	Select all the remaining data points and delete data from table. Then format the vacant cells within the table as number - percentage.
2.6.05	Fill out this cross table with target read fractions: = Reads for a particular well-target combination ÷ total reads for that well = Read/Well Subtotal
2.6.06	In the column at the end of the read fractions table input the title “Median”, and then calculate the **median** read fraction for each individual target.

2.7 Rebalancing calculations

2.7.01	Create a new tab and label it “Rebalancing calculations”
2.7.02	Add the following headers (see GbS_rebalancing_example). Target | Median fraction | Scale to 100% | Pool weighting | Scale to min=1 | Clipped to max=10
2.7.03	In the **Target** column copy and paste the list of targets under the cell**.**
2.7.04	In the **Median fraction** column copy and paste, the corresponding calculated median values**.** Format cells to percentage and two decimal places.
2.7.05	In the **Scale to 100%** column scale the Median fraction to 100% by using the calculation below: • Calculate the sum of the median read fractions for each target • Scale the sum of the median read fractions to 1 by dividing each fraction by (sum of fractions). = Median fraction/(sum “median read fractions”)
2.7.06	In the **pool weighting** column calculate the pool weighting as shown below: = (read fraction)$^{-0.561}$ N.B. This figure has been determined empirically to be optimal.
The pool weightings can be interpreted directly as the volumes to add of each target’s primer pair to the pool. However, in order to reduce inaccuracies associated with pipetting small volumes it is advisable to scale all the weightings so that the minimum volume pipetted is 1µl.	
2.7.07	To **scale to min=1** divide all the pool weightings by the value of the minimum weighting: = Pool weighting divided by minimum of all Pool weightings = Pool weighting÷min(all Pool weightings) = D2/MIN(D$2:D$67)
Primer pairs which generate only very small reads can be overweighted by the primer rebalancing algorithm, which leads to them dominating the reads from the resultant rebalanced pool. It is therefore advisable to ‘clip’ the maximum pool weighting to 10x the minimum. Given that the minimum pool weight has been scaled to 1, the maximum volume of primer pair that can be added as 10µl.	
2.7.08	In the **Clipped to max=10** column reduce any calculation greater than 10 to 10 by using the following calculation: =IF(Scale to min=1>=10,10, Scale to min=1) =IF(E2>=10,10,E2)
2.7.09	At the end of the **Clipped to max=10** column calculate the sum of the pool weightings: =SUM(all Clipped to max=10**)** =SUM(F2:F68)

2.8 Rebalancing calculations - Volumes	
2.8.01	In the next cell down calculate the interquartile mean (IQM) pool weighting. This uses the interquartile mean rather than the arithmetic mean so as to allow us to ignore the effect of clipping any over-weighted targets to 10x the minimum, The TRIMMEAN function requires 2 arguments: TRIMMEAN(array, percent) The values for the array are in cells F2:F68 The trim percent is entered as 50% (or 0.5) = TRIMMEAN(F2:F68,0.5)
2.8.02	In the next cell down calculate the central primer ($[centre]_{pool}$) concentration for the pool in nanomolar (nM): = (IQM pool weighting/sum of weightings) and multiply by 250,000 = (F71/F70)*250000 250,000nM = the concentration of the primer pairs in the source plate
2.8.03	Calculate the dilution factor required to dilute this pool to the 40nM working concentration. = central primer concentration/40 = F72/40
2.8.04	In the next cell down calculate the volume of diluent required to dilute the primer pool to 40nM: = (sum of the pool weightings*dilution factor)-total volume = (F70*F73)-F70
2.8.05	Sort the table of primers and volumes to match the layout of the "equivolume plate" (see Appendix 1, 2, and 3).

2.9 PRIMER DILUENT

The primer diluent (T1E0.1 +0.01% v/v Triton X-100) is used in section 9 for the diluting of the pooled primers to 40nM before aliquoting.

To create the primer diluent do the following: Dilute 10mM Tris/1mM EDTA pH 8 (T10E1) 1in10 with 10mM Tris pH 8 (e.g. Qiagen EB) then spiking in the 0.01%v/v Triton X-100 detergent.

- Add 5mL of 10x TE (pH 8) to a 50mL falcon tube.
- Then add 45mL of EB (10mM Tris).
- Dispense 5µL of neat Triton X-100 onto the inner surface of the tube slowly, using a P10 pipette, i.e. not into the diluent.
- Vortex thoroughly until all the Triton X-100 is in solution.

3. PREPARATION OF PRIMER POOLS

Due to the potential for surface adsorption to significantly alter the concentration of the primers in the aliquots, it is advisable to use only low-binding plasticware for storage.	
3.0.01	Wipe down all surfaces and pipettes with tissue wipes (Azowipes).
3.0.02	Defrost the 96 well “equivolume plate” (250uM) containing the pooled forward and reverse primers for the panel and briefly centrifuge. **The “equivolume plate” was prepared in the testing and balancing Laboratory protocol: section 3.1 (containing 15µL of a 1:1 mixture of forward and reverse primers, @ 250µM).**
3.0.03	Build the rebalanced primer pool by ‘piling up’ volumes of primer pairs according to the list of weightings into an eppendorf, obtaining the primers from the relevant well of the storage plate. All pipetting can be carried out with a 20µL pipette and care should be taken to not cross contaminate the primers.
3.0.04	Add the volume of Primer Diluent (T1E0.1 +0.01% v/v Triton X-100) required (see section 2.9) to dilute the primer pool down to 40mM. The volume is calculated as per 2.8.04.
3.0.05	Mix thoroughly.
3.0.06	Aliquot the rebalanced primer pool into 10µL aliquots using low-bind tubes and clearly label.
3.0.07	Store the aliquots in clearly labelled container at -20°C.

4. STORAGE

4.0.01	Store the primer pools and stock plates at -20°C or colder.

APPENDIX 1 – GRC1 PRIMERS

Well	Name	Forward sequence	Reverse Sequence
A1	CRT_220	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATCTTTTGAAACACAAGAAGmAA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTATTTCCCTTGTCATGTTTGAmAA
B1	CRT_326	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGAGCATGGGTAAGAAGCTTAmUA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTCCTCTTGTATGTATCAACGTmUT
C1	CRT_371	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGGTACAACGTATCATATTTmUA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTACGAACAAGCCATTTGATATmUA
D1	CRT_72_74_75_76	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTAACAGATGGCTCACGTTmUA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTGAGTTTCGGATGTTACAAAAmCT
E1	DHFR_16_51_59	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTTTTCGATATTTATGCCATAmUG	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTACATTATCCACAGTTTCTTTmGT
F1	DHFR_306	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGATGATGATGAAGAAGAAGAmUG	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTCGATCACTTTGTTTATTTCmCA
G1	DHPS_436_437	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTTTGTTGAACCTAAACGTmGC	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTTATAATTGGTTTCGCATCAmCA
H1	K13_resistance_1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATGAATTTAGAACTTCGCCAmUT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTCCATATGCCTTATTAGAAGmCT
A2	K13_resistance_3	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCATAGCTGATGATCTAGGmGG	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTCTGAGGTGTATGATCGTTTAmAG
B2	K13_resistance_5	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTAGACATAGGTGTACACATAmCG	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTCTTAGATAGGGATAGTGAGmUT
C2	MDR1_86	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGTATGTGCTGTATTATCAGmGA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTCATGAAATTGTCCATCTTGAmUA
D2	Pf_PF3D7_1460900-1_Pf3D7_14	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCCCAAAAGACAATAAGAAAmGA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTGCAAGAGTACTGTTTTATTTmCG
E2	Pf3D7_01_v3_145515_294I_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCCTGAGTTTTAAGTGAATGmAA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTAGTTTGTTGATGAAAGAAAGmGA
F2	Pf3D7_01_v3_180554_D714N	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACACCCAGAAATATAAATGGAmAC	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTTCAAAACGACGTTATTACAmAT
G2	Pf3D7_01_v3_535211_2521F_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAAATAATAAGAACAACGATGmCT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTCACTCATCAATTAATTGTTCmAT
H2	Pf3D7_02_v3_470013_G75E_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAATATGTTGTTTGTGTGGTCmAT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTTGCCATGATAAACCTTTTCmAT
A3	Pf3D7_02_v3_714480_D258G	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGAGGTGATCACTATGTTTAmUT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTTCATCCAAGGTTTTGTCTTmAC
B3	Pf3D7_03_v3_155697_150P_B	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACGTCTCATTACCAAATTCAmAA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTTTATCAACTGAAGGTACAAmCG
C3	Pf3D7_03_v3_656861_129V_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATTATTATCATCACAATGGTmUG	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTAGTATGAAGGGTGATGATACmUT
D3	Pf3D7_04_v3_1037656_2776I	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTAAATGCTGAAGATGAACCAmAG	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTATGGTAAATAAAGTGAGTGTmGC
E3	Pf3D7_04_v3_139051_K438N_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAAATTCTAGTAGTAAATACAmCA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTGCATATTCTTTTAAATCGTTTmCT
F3	Pf3D7_04_v3_426436_D560A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGGAGTTGGCTGTAATAATTmUT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTCTTCCATGACAAGGAAGAATmUA
G3	Pf3D7_04_v3_531138_A992E_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTCATCCATATGTATCCACmUC	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTACAAAAGCAGCAATACACATmAT
H3	Pf3D7_04_v3_881571_1081R_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACAAGGTACATTATTATGGAAmAC	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTAATATTATATGCAACACCCAmCC
A4	Pf3D7_05_v3_1204155_1338I_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGATGAGTGGAATAAAATTTCAmGA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTGTCATATTCGAAGGATCAAAmCT
B4	Pf3D7_05_v3_172801_E218K_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATTATTGGACGTTCAGAAACmUG	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTGGTTCCAATACTAATATGGATmGG
C4	Pf3D7_05_v3_369740_907L_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCCTCCTATAATCATAGTGAAmUG	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTTGTCATTATTATTTGATCTTmUT
D4	Pf3D7_06_v3_1289212_125T_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGGGAACTATTTAGATTACAmAA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTTTCCAAATTTCCCTTTACTmGT
E4	Pf3D7_06_v3_900278_P696S_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGTTCATCCTTTATTTCAACAmGA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTGTCCATTGGATTTAATTCCmUC
F4	Pf3D7_07_v3_1066698_G483S_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCATTATGTTTAAAGGAAAGmGA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTGTGTTCACAAATATCTTCATmGT

Well	Name	Forward sequence	Reverse Sequence
G4	Pf3D7_07_v3_1256331_L321F_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGTAATATAAATATGTATGATGGAAmGG	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTTTCACTTTCTTGAACTACAmCC
H4	Pf3D7_07_v3_1358910	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGGAGGTTATTCAACTAAGmGC	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTAGCAAAAATTCTCCATGCmCA
A5	Pf3D7_07_v3_619957_675R_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTATGCTAAAACTGAACGAmAA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTGTGTTATATGGAAGAAAAGmAT
B5	Pf3D7_07_v3_704373_389E	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGAAGGATTAAGGAGAAACACmAG	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTATCCCTATCTCTTTCTCTTTmCC
C5	Pf3D7_08_v3_1056829_L474I_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGACAATATGGCTAGTAACAmGT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTATCATCATTGTCTAAAGCTTmCG
D5	Pf3D7_08_v3_1314831_1342K_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGAAGATTTTAAAGAAGAAGAATmGT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTTCCCTTATATTCCATTCTTmUT
E5	Pf3D7_08_v3_150033_1315I_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATCAACAAGACGTTTCTGATmUT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTGGTATAACACGTTCCAATACmUT
F5	Pf3D7_08_v3_399774_421K_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTTCATCATTGTGTTGAATGmUC	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTTGCTCTTGTGAATGAAAGTmUA
G5	Pf3D7_08_v3_417335_R244K_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGGATCATATCTTCGGACTTmUT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTACAGTAGAGCAAACAAAAGAmAA
H5	Pf3D7_08_v3_549993	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGTGCTTGTACAAATAATCmCA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTATCCTATAACGAGGTATTCmCA
A6	Pf3D7_09_v3_452690_1018I	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCAGGAACCATACTTTTGTTmUA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTATATTTCGACCTGCTTCAAmUG
B6	Pf3D7_09_v3_900277_1534E_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGATTTGGAAATAACTGATGmAA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTGTCCATGTTTAAATGCTTTmUG
C6	Pf3D7_10_v3_1383789_N114H	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTTAGGTTGGTTAGAATGGAmGT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTATGAACCCAACTTTCTTTACmAT
D6	Pf3D7_10_v3_1386850_927K_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGTTAAATATATTGAAGACGTmGG	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTGAGGACAAGGAAAATAATAmCA
E6	Pf3D7_10_v3_361684	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACTTCATCAGCATTTTCAAmCC	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTCTCATTCATTAGGTGGTGmGT
F6	Pf3D7_11_v3_1006911_D124E_B	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTAATTTTGCAAAATAGCGTmGG	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTCGTTTTGGTCATTCATGTCTmUA
G6	Pf3D7_11_v3_1020397_G700E_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTATAATGCATGTGTCACCTmUC	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTACAGAAACATTAAACACAACmCA
H6	Pf3D7_11_v3_1295068_E405K	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTAATTGGGGAAATTATAAmCA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTCCCTTCATCATCATTATCATmUT
A7	Pf3D7_11_v3_1815412_E765Q	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATGAGTTGTTATATTCATGTGmCT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTAACATATGCTGCAGATTTTGTmUA
B7	Pf3D7_11_v3_1935031_I139L	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGATGTTCTTTTATGAAATCAmCA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTACGTCAATACAAGAATTAACmCA
C7	Pf3D7_11_v3_477922_H147Y_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAACAACCTTAGCATTGATTGmAT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTATATTGACTTTCATCATTTmGA
D7	Pf3D7_12_v3_1667593_2381N_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGCTGCTGAATATACATATCmUG	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTTCTTTTCTAATTCCTTTTCmCA
E7	Pf3D7_12_v3_2171901_V140D_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGAAGATTCTAAGGAACAAAmAT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTCTGATTCCACAAAAGAAGAAmGA
F7	Pf3D7_12_v3_858501_Q469K	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAAATAGAAAATTTGCCACATmGC	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTAGACCCCTGTTTTAGTAAGAmAT
G7	Pf3D7_12_v3_974663	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGAGAAGGAAGACCTTGTTmGT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTAGAATCCTTAAGAGACTGTmUT
H7	Pf3D7_13_v3_1056452_1234D	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTTTCATCGCAGGAAAATACmUT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTCGTCATTAATGTGTTTATTmCA
A8	Pf3D7_13_v3_1419519	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTTCTTGACCAAACATAmAT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTAAGTACAAAAACCAGmCA
B8	Pf3D7_13_v3_1867630_M4911I	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTCCGATTGGTAGTTATACAmGA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTAAAGGAGATGTATCATTTGCmAC
C8	Pf3D7_13_v3_2377887_2002S_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATTAAAGAAGAATGTGGAGCmUG	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTGGAACGGCAAGGATATTTTAmAT
D8	Pf3D7_13_v3_2573828_I1153M	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTTCATAACATATAGCAGCmAT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTATGACACCAATCGTTAATCmAC
E8	Pf3D7_13_v3_388365_S1236R	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGTCTTTGTCTCTTTTCCTTmUC	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTATCCAACTCAAAACAGCTTAmUT

Well	Name	Forward sequence	Reverse Sequence
F8	Pf3D7_14_v3_137622_1179V_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACCATTCATATTTTCTAGGTTTmGT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTACGAAATTTATGAAGATGAGmCA
G8	Pf3D7_14_v3_1757603_D1365G	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTAAATGACGAAGAACTAGCmUA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTCTTCAATATGTAAATCATTCAmCA
H8	Pf3D7_14_v3_2164225_2830S_B	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCTACAAGGAGAAATGACAAmAT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTCATCTGTAGAATCGGTAAGmAA
A9	Pf3D7_14_v3_2733656_557C_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCAAAACCGTTTCATCTACAmAT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTAAAAGATTCAGCATCGCTAmUC
B9	Pf3D7_14_v3_3126219	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAACAAGAGAATCAGGGCAmAT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTCATCTCTAGGTAATAACGTACmCA
C9	PlasII_ref	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGAAAATGCTCTTTTCACCmUT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTCATCTAAATTCTGCAACATTTTmAT
D9	PlasIV_ref	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGACAATGCTTTATTTACAmUT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTCATTCATATCTCTAAAGAATTTmAT

GRC1 = 68 Primers in total.

APPENDIX 2 – GRC2 PRIMERS

Well	Name	Forward sequence	Reverse Sequence
A1	CRT_271	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTTCCAATTGTTCACTTCTTmGT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTATTTTACCTCTACGACTGTmGT
B1	CRT_356	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGTTAGTTGTATACAAGGTCmCA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTACGTTGTACCATCATAAACAmUT
C1	CRT_97	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTTTGCTAAAAGAACTTTAAAmCA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTTGGTAGGTGGAATAGATTCmUC
D1	DHFR_108_164	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTTGTAGTTATGGGAAGAACmAA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTATTCTTGATAAACAACGGAAmCC
E1	DHPS_613	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAATGGAATACCTCGTTATAGmGA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTTCATTTTGTTGTTCATCATmGT
F1	EXO_415	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAATTTACCTGAAGACGTTAAmAA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTATCCCATTCGATATCTATACmCT
G1	K13_resistance_2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCATTATCAATACCTCCAACAmAC	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTATCGTATGAAAGCATGGGTmAG
H1	K13_resistance_4	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAATTACTTGAAACATACCATmAC	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTATAGGTGGATTTGATGGTGmUA
A2	K13_resistance_6	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGGGTATAGTTAACGGATTTmCT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTAAAATTGTTGATGCAAATATmUG
B2	MDR1_1034_1042	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTGCATTTAGTTCAGATGATmGA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTCCAAACCAATAGGCAAAACTmAT
C2	MDR1_1226_1246	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTGCAGAAGATTATACTGTmAT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTACTAACACGTTTAACATCTTmCC
D2	MDR1_184	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTTACATATGCCAGTTCCTTmUT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTGCAACAGTTCTTATTCCCATmUA
E2	Pf_PF3D7_1318100_Pf3D7_13	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGATTGAGGACAAATTACATmGT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTCATATAGTTGTAGGGGAGGTmAG
F2	Pf_PF3D7_1447900_Pf3D7_14_v	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTATCCATCTCATTTGCTTTTmGT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTGAATAGTTGATAGAGGTACCmGA
G2	Pf3D7_01_v3_179347_311G_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCACATATCCAGCCCTCAmAA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTGCTTGTGTTATTAATCCTGTmAA
H2	Pf3D7_01_v3_283144_H664D_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATGGATTAAACGCTGATATGmUC	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTATCATCATTTTCCTCATCATmCG
A3	Pf3D7_02_v3_376222_K1929E_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGATGTGATTTCTCTACGAACmAA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTAGGTATAACTTCACGAAATTmUT
B3	Pf3D7_02_v3_529709_F487L	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAAAAGACAAGAGTACAAAAGmGA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTACCAGCTATTTCTTGATATGmUG
C3	Pf3D7_02_v3_839620_260L	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGAATGATTTGAAAATTGCAmCT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTAAATCGTGTGCATCCATTATmUT
D3	Pf3D7_03_v3_548178_R2L_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGGGTAAGTTACATTCTTCTmCA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTGTTCATTATGATTAATTATATGmCT
E3	Pf3D7_04_v3_1102392_E808D_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTAGAGGTGTTGATGTTAATATmGG	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTATCATCAAAATATACATCCCCmAC
F3	Pf3D7_04_v3_110442_G285E_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATTGGAAAATTCATGTAGTGmCA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTAGGAAAGACAGCAACAATAAmAA
G3	Pf3D7_04_v3_286542_H586N	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTATATAATGCGTCTCTGTCCmAT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTGAAAAGGAAACAAATAAGGmAT
H3	Pf3D7_04_v3_529500_1477Y_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAATAGGAGACAGAATTTGATmUC	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTAAGAATTCCTTAGGACACGmUA
A4	Pf3D7_04_v3_648101_51V	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAATTTCACATTATACCTTCCmAA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTGATACAAATTCATATAAATAmCA
B4	Pf3D7_04_v3_891732_R4468S	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCATTCCTCCTAGTTTAAACCmAA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTGAGCAAATTCTTGAGCATTAmAC
C4	Pf3D7_05_v3_350933	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGCATCATTTTGCmUC	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTATATATAATTCAACAACAmGC
D4	Pf3D7_05_v3_796714_396K_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGTTAATGAAAAGAACCCAAmAA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTATGGTGTTTTACGTTTTAGmGG
E4	Pf3D7_06_v3_1282691_803K_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAAATGTACAAGTGAGGAGTmUA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTGCTTCATTTGTAAATAAAATGmCT
F4	Pf3D7_06_v3_574938_I2934L_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGACGATGATGAAAACATGAmAT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTACATTTATACATGCCCAACAmAT

Well	Name	Forward sequence	Reverse Sequence
G4	Pf3D7_07_v3_1044052_686K_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGAACACGCTTATCATTTATmCT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTATGACAACCAAAATGAAGGAmAT
H4	Pf3D7_07_v3_1213486_S543N_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATTTCTCCTAACTTACGTCAmGT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTAACAAACACATACATAAAACmCC
A5	Pf3D7_07_v3_1308383_G1945R	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCATTACCTTTACCTTTTCCmUC	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTTGATTATGATGATGATGACmGA
B5	Pf3D7_07_v3_1359218_K388N_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGTAAATTTGAATGGCATACmGT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTTCACGCTTTTCCTTAATTGmUA
C5	Pf3D7_07_v3_461139	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTATATATCACAACAATATTTAmGA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTCTGAGATTATTTAATTCTTCTTTmAC
D5	Pf3D7_07_v3_635985_T598A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTACTGCGCCTATCATTATTmAT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTAAAAGGGATGGAGATTACAAmUG
E5	Pf3D7_08_v3_1313202_799F_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGAATAAATGGATTGAGAGAmCA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTATCTTGCAATCCATCTTTTmCC
F5	Pf3D7_08_v3_339406_1283C_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTATTTCAAATATCCAACCGCmUT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTATAGATGGAAAGCAACCTAmCA
G5	Pf3D7_08_v3_413067_1044V_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAATGTGTACGAGAAACATCmAC	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTCATAAGTTTCTTCTAAATAGATAmGT
H5	Pf3D7_09_v3_1379145_R398Q_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCCATTTCAATCTTTTCGTTTmAT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTCAGGATGTGTTATGGTATCmAA
A6	Pf3D7_09_v3_163977_403D_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTCATTCATCAAGTGATCCAmAA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTGTAACTAATGGTTTTGCAACmAC
B6	Pf3D7_09_v3_599655_E654D_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATCATGACAAAGAAACATGTmGG	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTGTAGATTGTTCATATCTTCmAT
C6	Pf3D7_10_v3_1385894_815P_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCATTCCACATGTACGTAAAGmAA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTGAGTGATAAACCTGCTTATmCT
D6	Pf3D7_10_v3_317581_311I_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTATCTTGTCTCATGTCATGmUG	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTCAGCTTCTATACCTAATGCTmUG
E6	Pf3D7_10_v3_336274_I1677V_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTGCTATAGTCATGATCATCmGT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTACCTGGAGATTCATGTAATTmCA
F6	Pf3D7_11_v3_1018899_1199L_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAAAAGGTTCACAGAATATTmUT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTGTGGTATAAGTAACATAAGTAmGT
G6	Pf3D7_11_v3_1294107_84A_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTATCCAATAGACCACGAmAG	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTAAGTCTATATTGAGTTCCAGmCT
H6	Pf3D7_11_v3_1802201_450S	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTGTATTTTCCTTTTCCCTGmAT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTAAGGAGATAGTTTACCTGGTmUT
A7	Pf3D7_11_v3_1935227_R73S_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGGATCTAAGGATGTAATTTmGT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTAGGAATTCAAGAACTTTAGCmAT
B7	Pf3D7_11_v3_408668	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCACAGGATGATGAATATAATAAmCT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTGACGGACATTCTTTCAATmGC
C7	Pf3D7_11_v3_828596_K240E_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGTCCTTTTATATCTGTTTGmCT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTGCATACATTAATAAAAGTGAmCA
D7	Pf3D7_12_v3_1663492_1014E_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGTATGCACCGATTTTCTATmAT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTATATTTTCGAATCAGCGCTAmUT
E7	Pf3D7_12_v3_1934745_241L_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACCTGGAATATTATCACCTTmCT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTGACAAATCATGAAAATCAAmGA
F7	Pf3D7_12_v3_857245_E50G_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTAAGATGTCCTATCAAGACGmUT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTACGACATACTGCTATTTATGmUT
G7	Pf3D7_13_v3_1233218_N277S_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGGTTCTACATTTTCCAGATmUT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTACCAACTTTGTAAAGCTGTAmAA
H7	Pf3D7_13_v3_1466422	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTTACTTGTAACAGTGCATmCA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTGTATACGTCGTCATAATTmGG
A8	Pf3D7_13_v3_159086_21R	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTAACAACACACTTTTATTCmCT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTCTACTTGGTAGGTATACGTCmAT
B8	Pf3D7_13_v3_2161975_D252V	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCCAATTCCATGTATATTCATmCA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTATATGAACGAACTGGACAAmGA
C8	Pf3D7_14_v3_107014_215K	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTATTCCATACAACATCAAmCA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTAAAGGTATACATGGTATATCmAT
D8	Pf3D7_14_v3_2355751_H1589Q	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTATCCAAACCCATTTAACGmAG	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTAAATGTTTCGTTATATATGAmCA
E8	Pf3D7_14_v3_2625887_M238I_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTTATTCGATCTGTACCTTmCA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTAAAGCTTGGTTAGAAGATTTmCT

Well	Name	Forward sequence	Reverse Sequence
F8	Pf3D7_14_v3_3046108_417V_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCAGGAAATGAGATAATTGACmGT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTTTTCTCATTGGGACGGAmUT
G8	Pf3D7_14_v3_438592_N348T_A	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGAGAAAATGAATCTATGCCTmCA	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTATGTGGTCAAGTGATGTATCmAT
H8	PlasI_ref	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGAACAAGCCGTTTTTACCmUT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTCATCTAAACCTTCAAAGAATTTmGT
A9	PlasIII_ref	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGAACAAGCCGTTTATTCCmAT	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTCACTTGCAGATTCTACGAATTGmAT
B9	W1421_Pf3D7_14_v3_289610_T4	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTAGGTGACCCATTTATGmAG	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTTAGCTTTAGCATCATTCAmCG

GRC2 = 66 Primers in total.

APPENDIX 3 – SPECIATION PRIMERS

Well	Name	Forward sequence	Reverse Sequence
A1	Spec1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACCATCCAATTTGATTmGG	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTCTTGAGGCAGTTTGTTmCC
A2	Spec2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTAGCGTGTATTGTTGCCTmUG	TCGGCATTCCTGCTGAACCGCTCTTCCGATCTCCTTTAATGTAGTTTCCTCACAmGC

SPEC = 2 Primers in total.

Appendix 4 – Primer pooling: