Standard Operating Procedure (SOP)	
SOP Title	Preparation of PCR_2 tag plates.
SOP number	GbS01
SOP Version	2

1. SCOPE

This SOP describes the laboratory procedure to produce 'tag' plates for use in the PCR_2 step of the GbS process (Library preparation - GbS03).

A central feature of the GbS process is normalisation of library yields. These are achieved during the PCR itself; rather than requiring upfront or downstream processing.

Whilst PCR_1 involves a limited cycle PCR during which a low concentration of seeding primers specifically anneal to the genomic template, PCR_2 involves the addition of indexed tag primers annealing to and extending from the common 5'-tails incorporated during PCR_1. The normalisation effect relates to the number of PCR cycles and tag concentrations used in PCR_2 being sufficient to drive the reaction into plateau for a wide range of input DNA concentrations.

Due to the degree of miniaturisation of the PCR, it is impractical to add a solution of tag primer to PCR_1 prior to PCR_2. This is due to both the very small volume of liquid that would need to be added accurately (if adding concentrated tag primers), and the dilution effect on the PCR reagents (if adding more dilute primers). The only practical way of enabling PCR_2 involves the transfer of a volume of PCR_1 to another plate containing lyophilised tag primers. Thorough mixing of the reaction volume with these dried primers re-suspends them in solution.

Given that the samples destined for GbS typically are stored in 96 well plates, it is therefore sensible to use up to 96 i7 barcodes to indicate which well was the source for a given cluster. Each plate of samples destined to be pooled together is simultaneously barcoded with a single i5 tag, which therefore allows for up to 16x 96 well plates to be pooled together (for a maximum of 1536 samples simultaneously).

As we are drying the tag primers down into plates, the tags will necessarily have a fixed layout. We are running PCRs in 96 well plates, therefore the 96 i7 tags will be present in the same layout in each tag plate, and one different i5 tag will be present per plate.

2. MATERIALS REQUIRED:

2.1 REAGENTS AND MATERIALS		
Reagents	***Supplier***	***Cat. No.***
Buffer Elution Buffer (Buffer EB, 10 mM Tris-Cl, pH 8.5)	Qiagen	19086
i7 tag plates (at least 100µL @ 300µM, See appendix 1)		
i5 tag oligos in screwcap tubes (x16) (at least 130µL @ 100µM, See appendix 2)		
Triton X-100		
Tris EDTA (1x)		
Materials	***Supplier***	***Cat. No.***
96 well qPCR plate, skirted (AB2800)	Fisher Scientific	10032013
Qiagen seal	Qiagen	19570
Lo-bind Eppendorf tubes (1.5 mL)		
50mL Falcon centrifuge tubes (x10)		
15mL Falcon centrifuge tubes (x16)		
Centrifuge (requires a 96 well plate adaptor)		
Tube/plate vortexer		
Ice tray/cooling block/		
Tissue wipes (Azowipes)		
Repeater & tips (40µL)		
8 Multi-channel (200µL)		
12 Multi-channel (10µL)		
Pipettes (1000, 200, 100 and 20µL)		
Filtered tips of various volumes		
Stripette gun & Stripettes (25 & 50mL)		

3. METHODOLOGY

3.1 i5 Oligo plate production	
Elution Buffer + Triton X-100 (EB-TX100)	
3.1.01	Add Triton X-100 to Buffer EB at a final concentration of 0.01%v/v. • Add 40mL of Buffer EB to a 50mL Falcon tubes • Dispense 4µL of neat Triton X-100 - Onto the inner surface of the tube slowly, using a P10 pipette, i.e. not into the buffer • Vortex thoroughly until all the Triton X-100 is in solution • Each assay will require seven 50mL Falcon tubes
i5 Oligo	
3.1.02	Defrost the sixteen i5 oligo tubes and thoroughly vortex
3.1.03	From the information sheet add the suggested volume to produce a concentration of 100µM. N.B. The i5 oligos should be ordered in at a concentration of 100µM.
3.1.04	Add 12,375µL of prepared buffer EB-TX100 to sixteen 15mL Falcon tubes.
3.1.05	Label each tube with the unique i5 oligo identifier.
3.1.06	Add 125µl of the corresponding i5 tag oligo (100µM) to the correct Falcon tube and vortex thoroughly. (N.B. 1 oligo per tube).

3.2 i7 Oligo plate production	
3.2.01	Defrost the stock i7 oligo 96 well plate and thoroughly vortex.
3.2.02	Pulse centrifuge the stock i7 oligo 96 well plate.
3.2.03	Dilute the 300µM to 50µM with TE (x1) into a new 96 well plate. (N.B. 1 in 6 dilution): • Add 7.5µL of each i7 tag primer to a new 96 well plate. • Add the calculated volume of TE (x1) for each i7 primer.
3.2.04	Label 16 plates with the i7 oligos.
3.2.05	Transfer 2.5µL of each specific diluted i7 tag oligo (50µM) to its corresponding well of the new 96 well plates (i.e. create a stamp). Plates can be centrifuged (Store @ 4°C until required). Plates can be frozen for medium and the long term.
3.2.06	The remaining volume (~5µL) in the i7 oligo dilution plate can be disposed of.

3.3	COMBINED PLATES (I7 + I5)
3.3.01	Retrieve sixteen pre-aliquoted i7 oligo plates and thoroughly vortex (from section 3.2).
3.3.02	Label each one of the i7 oligo plates with a unique i5 oligo identifier.
3.3.03	Add 122.5µL of the corresponding diluted i5 Tag Oligo (from section 3.1) to each well.
3.3.04	Seal the i7 + i5 oligo stock plate with a sticky lid and briefly vortex them using the plate vortexer to fully mix the primers in each well into homogenous solutions.
3.3.05	Repeat 3.3.02 to 3.3.04 for other plates making sure that each i7 oligo plate receives the allocated diluted i5 oligo.
The processing of the combined plates (1µM) can be paused at this point indefinitely. The combined 96 well plates are to be stored at -20°C.	

3.4	OLIGO STOCK PLATES (I7 + I5)
3.4.01	Label sixteen 96 well plates with the i5 oligo identifier, which will be dispensed in each one (N.B. 1 oligo per plate).
3.4.02	Transfer 40µL of each specific diluted well from the "combined plates" (section 3.3, I7 + I5, 1µM) to its corresponding well of the new 96 well plates (i.e. create a stamp).
3.4.03	Add 40µL of EB-TX100 into each well of the sixteen 96 well plates.
The oligo stock plates (500nM) processing can be paused at this point indefinitely. The oligo stock plates (500nM) are to be stored at -20°C.	

3.5 PCR_2 PLATE PRODUCTION – 96 WELL PLATE (1 TAG).

Each oligo stock plate contains sufficient volume to allow the stamping of at least 20 PCR_2 plates

(80µL stock and 4µL per 96 well PCR plate = 20 plates)

Given likely GbS pipeline throughput and the unknown longevity of dried-down primers, it may be optimal to stamp out from three or four of each set of tag stock plates at any one time, retaining the others in the freezer until required.

3.5.01	Transfer 4µL of each well from the oligo stock plate into each of the twenty 96 well PCR plates and label accordingly.
3.5.02	Centrifuge all stamped plates in the plate centrifuge (1000g for 30sec is sufficient).
3.5.03	Lyophilise the primers by placing plates in the fan oven (set to 50°C) and leave for at least one hour or until all wells are all dry.

Drying time is entirely dependent on the heater output, fan speed, oven size and number/layout of plates present in the oven, so there is really no substitute for visually inspecting each plate to confirm that the liquid has evaporated. The dried down tag primer will be invisible to the eye, so a fully dried plate will look identical to an empty plate to the naked eye. Over-drying is not a concern in this process.

3.6 PCR_2 PLATE PRODUCTION –384 WELL PLATE (3 TAGS).

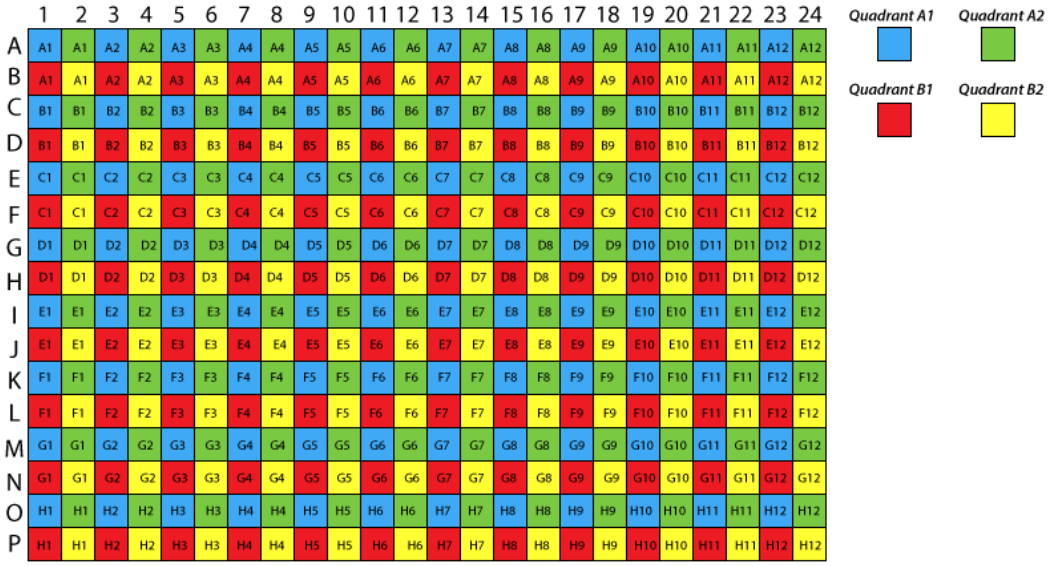
Each oligo stock plate contains sufficient volume to allow the stamping of at least 20 x 384-well PCR_2 plates

(80µL stock and 4µL per 384 well PCR plate = 20 plates)

Given likely GbS pipeline throughput and the unknown longevity of dried-down primers, it may be optimal to stamp out from three or four of each set of tag stock plates at any one time, retaining the others in the freezer until required.

3.6.01	Transfer 4µL of each well/quadrant in interweaved format (see below) from the oligo stock plate into each of the twenty 384 well PCR plates and label accordingly. Leave quadrant 4 empty. 384 well plate – i5 = 1, 2, & 3 384 well plate – i5 = 4, 5, & 6 384 well plate – i5 = 7, 8, & 9 384 well plate – i5 = 10, 11, & 12 384 well plate – i5 = 13, 14, 15 & 16
3.6.02	Centrifuge all stamped plates in the plate centrifuge (1000g for 30sec is sufficient).
3.6.03	Lyophilise the primers by placing plates in the fan oven (set to 50°C) and leave for at least one hour or until all wells are dry.

The diagram below shows the 96-well plate locations in each of the corresponding quadrants



3.7 PCR_2 PLATE PRODUCTION –384 WELL PLATE (4 TAGS).

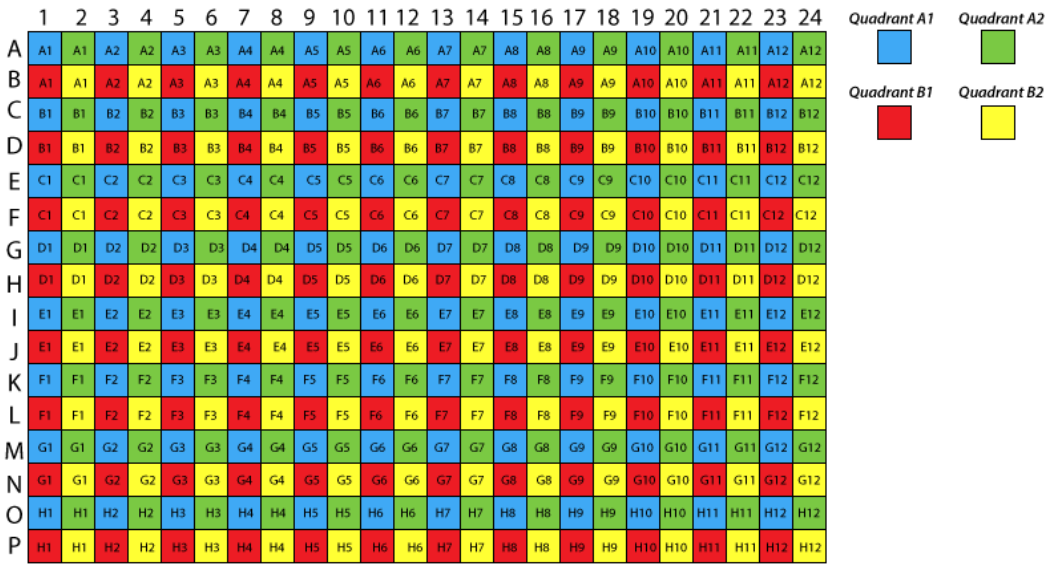
Each oligo stock plate contains sufficient volume to allow the stamping of at least 20 x 384-well PCR_2 plates

(80µL stock and 4µL per 384 well PCR plate = 20 plates)

Given likely GbS pipeline throughput and the unknown longevity of dried-down primers, it may be optimal to stamp out from three or four of each set of tag stock plates at any one time, retaining the others in the freezer until required.

3.7.01	Transfer 4µL of each well/quadrant in interweaved format (see below) from the oligo stock plate into each of the twenty 384 well PCR plates and label accordingly. 384 well plate – i5 = 1, 2, 3 & 4 384 well plate – i5 = 5, 6, 7 & 8 384 well plate – i5 = 9, 10, 11 & 12 384 well plate – i5 = 13, 14, 15 & 16
3.7.02	Centrifuge all stamped plates in the plate centrifuge (1000g for 30sec is sufficient).
3.7.03	Lyophilise the primers by placing plates in the fan oven (set to 50°C) and leave for at least one hour or until all wells are dry.

The diagram below shows the 96-well plate locations in each of the corresponding quadrants



4. STORAGE OF PREPARED OLIGO PLATES

STORAGE
Seal each plate using a tape lid, and collect together in batches of 10 of each tag group and place into a Ziploc bag and seal.
Store the completed oligo PCR plates in a cool, dark, dry place, e.g. a cupboard in an air-conditioned room. Under these conditions, the oligo plates should last at least 6-12 months.

5. QUALITY CONTROL OF PREPARED OLIGO PLATES

QC
Given the use of the tag plates as part of the input to the process the i7 tags can be regarded as having been QC'd already. Furthermore, we will only be using i5 tags which have also historically passed a separate QC. Therefore, both tag primers in any well can be regarded as having been tested already. However, it is advisable on each occasion that a new batch of tag plates is prepared from a new synthesis batch of i5s or i7s to perform a comparison GbS run (ideally using every well of the tag plate) against a previous batch to serve as confirmation that the tags are performing correctly.

PCR_2 REACTION CONCENTRATION
Tag primers are at 200nM in the final ~10ul PCR_2 reactions. The stock plates contain the tag primers (i5 & i7 combined) at 500nM. The 4µl stamp is created before drying down the tag plates. There is therefore 2pmol of each tag primer present, which we rehydrate with the 10µl PCR_1 volume when transferred. 2 pmol in 10µl = 200nM

APPENDIX 1 – I7 PRIMERS (300µM)

Index sequencing is performed as a separate read using the iPCRtagseq sequencing oligo (see below for sequence).
LARGE YIELD – AT LEAST 100µL @ 300µM

Oligo name	Index	Sequence
iPCRtagT1	AACGTGAT	CAAGCAGAAGACGGCATACGAGAT**AACGTGAT**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT2	AAACATCG	CAAGCAGAAGACGGCATACGAGAT**AAACATCG**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT3	ATGCCTAA	CAAGCAGAAGACGGCATACGAGAT**ATGCCTAA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT4	AGTGGTCA	CAAGCAGAAGACGGCATACGAGAT**AGTGGTCA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT5	ACCACTGT	CAAGCAGAAGACGGCATACGAGAT**ACCACTGT**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT6	ACATTGGC	CAAGCAGAAGACGGCATACGAGAT**ACATTGGC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT7	CAGATCTG	CAAGCAGAAGACGGCATACGAGAT**CAGATCTG**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT8	CATCAAGT	CAAGCAGAAGACGGCATACGAGAT**CATCAAGT**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT9	CGCTGATC	CAAGCAGAAGACGGCATACGAGAT**CGCTGATC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT10	ACAAGCTA	CAAGCAGAAGACGGCATACGAGAT**ACAAGCTA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT11	CTGTAGCC	CAAGCAGAAGACGGCATACGAGAT**CTGTAGCC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT12	AGTACAAG	CAAGCAGAAGACGGCATACGAGAT**AGTACAAG**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT13	AACAACCA	CAAGCAGAAGACGGCATACGAGAT**AACAACCA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT14	AACCGAGA	CAAGCAGAAGACGGCATACGAGAT**AACCGAGA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT15	AACGCTTA	CAAGCAGAAGACGGCATACGAGAT**AACGCTTA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT16	AAGACGGA	CAAGCAGAAGACGGCATACGAGAT**AAGACGGA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT17	AAGGTACA	CAAGCAGAAGACGGCATACGAGAT**AAGGTACA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT18	ACACAGAA	CAAGCAGAAGACGGCATACGAGAT**ACACAGAA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT19	ACAGCAGA	CAAGCAGAAGACGGCATACGAGAT**ACAGCAGA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT20	ACCTCCAA	CAAGCAGAAGACGGCATACGAGAT**ACCTCCAA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT21	ACGCTCGA	CAAGCAGAAGACGGCATACGAGAT**ACGCTCGA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT22	ACGTATCA	CAAGCAGAAGACGGCATACGAGAT**ACGTATCA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT23	ACTATGCA	CAAGCAGAAGACGGCATACGAGAT**ACTATGCA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT24	AGAGTCAA	CAAGCAGAAGACGGCATACGAGAT**AGAGTCAA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT25	AGATCGCA	CAAGCAGAAGACGGCATACGAGAT**AGATCGCA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT26	AGCAGGAA	CAAGCAGAAGACGGCATACGAGAT**AGCAGGAA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T

Oligo name	Index	Sequence
iPCRtagT27	AGTCACTA	CAAGCAGAAGACGGCATACGAGAT**AGTCACTA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT28	ATCCTGTA	CAAGCAGAAGACGGCATACGAGAT**ATCCTGTA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT29	ATTGAGGA	CAAGCAGAAGACGGCATACGAGAT**ATTGAGGA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT30	CAACCACA	CAAGCAGAAGACGGCATACGAGAT**CAACCACA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT31	CAAGACTA	CAAGCAGAAGACGGCATACGAGAT**CAAGACTA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT32	CAATGGAA	CAAGCAGAAGACGGCATACGAGAT**CAATGGAA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT33	CACTTCGA	CAAGCAGAAGACGGCATACGAGAT**CACTTCGA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT34	CAGCGTTA	CAAGCAGAAGACGGCATACGAGAT**CAGCGTTA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT35	CATACCAA	CAAGCAGAAGACGGCATACGAGAT**CATACCAA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT36	CCAGTTCA	CAAGCAGAAGACGGCATACGAGAT**CCAGTTCA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT37	CCGAAGTA	CAAGCAGAAGACGGCATACGAGAT**CCGAAGTA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT38	CCGTGAGA	CAAGCAGAAGACGGCATACGAGAT**CCGTGAGA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT39	CCTCCTGA	CAAGCAGAAGACGGCATACGAGAT**CCTCCTGA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT40	CGAACTTA	CAAGCAGAAGACGGCATACGAGAT**CGAACTTA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT41	CGACTGGA	CAAGCAGAAGACGGCATACGAGAT**CGACTGGA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT42	CGCATACA	CAAGCAGAAGACGGCATACGAGAT**CGCATACA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT43	CTCAATGA	CAAGCAGAAGACGGCATACGAGAT**CTCAATGA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT44	CTGAGCCA	CAAGCAGAAGACGGCATACGAGAT**CTGAGCCA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT45	CTGGCATA	CAAGCAGAAGACGGCATACGAGAT**CTGGCATA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT46	GAATCTGA	CAAGCAGAAGACGGCATACGAGAT**GAATCTGA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT47	GACTAGTA	CAAGCAGAAGACGGCATACGAGAT**GACTAGTA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT48	GAGCTGAA	CAAGCAGAAGACGGCATACGAGAT**GAGCTGAA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT49	GATAGACA	CAAGCAGAAGACGGCATACGAGAT**GATAGACA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT50	GCCACATA	CAAGCAGAAGACGGCATACGAGAT**GCCACATA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT51	GCGAGTAA	CAAGCAGAAGACGGCATACGAGAT**GCGAGTAA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT52	GCTAACGA	CAAGCAGAAGACGGCATACGAGAT**GCTAACGA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT53	GCTCGGTA	CAAGCAGAAGACGGCATACGAGAT**GCTCGGTA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT54	GGAGAACA	CAAGCAGAAGACGGCATACGAGAT**GGAGAACA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT55	GGTGCGAA	CAAGCAGAAGACGGCATACGAGAT**GGTGCGAA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT56	GTACGCAA	CAAGCAGAAGACGGCATACGAGAT**GTACGCAA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T

Oligo name	Index	Sequence
iPCRtagT57	GTCGTAGA	CAAGCAGAAGACGGCATACGAGAT**GTCGTAGA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT58	GTCTGTCA	CAAGCAGAAGACGGCATACGAGAT**GTCTGTCA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT59	GTGTTCTA	CAAGCAGAAGACGGCATACGAGAT**GTGTTCTA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT60	TAGGATGA	CAAGCAGAAGACGGCATACGAGAT**TAGGATGA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT61	TATCAGCA	CAAGCAGAAGACGGCATACGAGAT**TATCAGCA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT62	TCCGTCTA	CAAGCAGAAGACGGCATACGAGAT**TCCGTCTA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT63	TCTTCACA	CAAGCAGAAGACGGCATACGAGAT**TCTTCACA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT64	TGAAGAGA	CAAGCAGAAGACGGCATACGAGAT**TGAAGAGA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT65	TGGAACAA	CAAGCAGAAGACGGCATACGAGAT**TGGAACAA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT66	TGGCTTCA	CAAGCAGAAGACGGCATACGAGAT**TGGCTTCA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT67	TGGTGGTA	CAAGCAGAAGACGGCATACGAGAT**TGGTGGTA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT68	TTCACGCA	CAAGCAGAAGACGGCATACGAGAT**TTCACGCA**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT69	AACTCACC	CAAGCAGAAGACGGCATACGAGAT**AACTCACC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT70	AAGAGATC	CAAGCAGAAGACGGCATACGAGAT**AAGAGATC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT71	AAGGACAC	CAAGCAGAAGACGGCATACGAGAT**AAGGACAC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT72	AATCCGTC	CAAGCAGAAGACGGCATACGAGAT**AATCCGTC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT73	AATGTTGC	CAAGCAGAAGACGGCATACGAGAT**AATGTTGC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT74	ACACGACC	CAAGCAGAAGACGGCATACGAGAT**ACACGACC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT75	ACAGATTC	CAAGCAGAAGACGGCATACGAGAT**ACAGATTC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT76	AGATGTAC	CAAGCAGAAGACGGCATACGAGAT**AGATGTAC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT77	AGCACCTC	CAAGCAGAAGACGGCATACGAGAT**AGCACCTC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT78	AGCCATGC	CAAGCAGAAGACGGCATACGAGAT**AGCCATGC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT79	AGGCTAAC	CAAGCAGAAGACGGCATACGAGAT**AGGCTAAC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT80	ATAGCGAC	CAAGCAGAAGACGGCATACGAGAT**ATAGCGAC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT81	ATCATTCC	CAAGCAGAAGACGGCATACGAGAT**ATCATTCC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT82	ATTGGCTC	CAAGCAGAAGACGGCATACGAGAT**ATTGGCTC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT83	CAAGGAGC	CAAGCAGAAGACGGCATACGAGAT**CAAGGAGC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT84	CACCTTAC	CAAGCAGAAGACGGCATACGAGAT**CACCTTAC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT85	CCATCCTC	CAAGCAGAAGACGGCATACGAGAT**CCATCCTC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT86	CCGACAAC	CAAGCAGAAGACGGCATACGAGAT**CCGACAAC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T

Oligo name	Index	Sequence
iPCRtagT87	CCTAATCC	CAAGCAGAAGACGGCATACGAGAT**CCTAATCC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT88	CCTCTATC	CAAGCAGAAGACGGCATACGAGAT**CCTCTATC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT89	CGACACAC	CAAGCAGAAGACGGCATACGAGAT**CGACACAC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT90	CGGATTGC	CAAGCAGAAGACGGCATACGAGAT**CGGATTGC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT91	CTAAGGTC	CAAGCAGAAGACGGCATACGAGAT**CTAAGGTC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT92	GAACAGGC	CAAGCAGAAGACGGCATACGAGAT**GAACAGGC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT93	GACAGTGC	CAAGCAGAAGACGGCATACGAGAT**GACAGTGC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT94	GAGTTAGC	CAAGCAGAAGACGGCATACGAGAT**GAGTTAGC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT95	GATGAATC	CAAGCAGAAGACGGCATACGAGAT**GATGAATC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T
iPCRtagT96	GCCAAGAC	CAAGCAGAAGACGGCATACGAGAT**GCCAAGAC**GAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T

APPENDIX 2 – I5 PRIMERS - LYOPHILISED

Oligo name	Index	Sequence
GbS_i5_1	TATAGCCT	AATGATACGGCGACCACCGAGATCTACAC**TATAGCCT**ACACTCTTTCCCTACACGACGCTCTTCCGATC*T
GbS_i5_2	ATAGAGGC	AATGATACGGCGACCACCGAGATCTACAC**ATAGAGGC**ACACTCTTTCCCTACACGACGCTCTTCCGATC*T
GbS_i5_3	CCTATCCT	AATGATACGGCGACCACCGAGATCTACAC**CCTATCCT**ACACTCTTTCCCTACACGACGCTCTTCCGATC*T
GbS_i5_4	GGCTCTGA	AATGATACGGCGACCACCGAGATCTACAC**GGCTCTGA**ACACTCTTTCCCTACACGACGCTCTTCCGATC*T
GbS_i5_5	AGGCGAAG	AATGATACGGCGACCACCGAGATCTACAC**AGGCGAAG**ACACTCTTTCCCTACACGACGCTCTTCCGATC*T
GbS_i5_6	TAATCTTA	AATGATACGGCGACCACCGAGATCTACAC**TAATCTTA**ACACTCTTTCCCTACACGACGCTCTTCCGATC*T
GbS_i5_7	CAGGACGT	AATGATACGGCGACCACCGAGATCTACAC**CAGGACGT**ACACTCTTTCCCTACACGACGCTCTTCCGATC*T
GbS_i5_8	GTACTGAC	AATGATACGGCGACCACCGAGATCTACAC**GTACTGAC**ACACTCTTTCCCTACACGACGCTCTTCCGATC*T
GbS_i5_9	CTCTCTAT	AATGATACGGCGACCACCGAGATCTACAC**CTCTCTAT**ACACTCTTTCCCTACACGACGCTCTTCCGATC*T
GbS_i5_10	TATCCTCT	AATGATACGGCGACCACCGAGATCTACAC**TATCCTCT**ACACTCTTTCCCTACACGACGCTCTTCCGATC*T
GbS_i5_11	AGAGTAGA	AATGATACGGCGACCACCGAGATCTACAC**AGAGTAGA**ACACTCTTTCCCTACACGACGCTCTTCCGATC*T
GbS_i5_12	GTAAGGAG	AATGATACGGCGACCACCGAGATCTACAC**GTAAGGAG**ACACTCTTTCCCTACACGACGCTCTTCCGATC*T
GbS_i5_13	CTATTAAG	AATGATACGGCGACCACCGAGATCTACAC**CTATTAAG**ACACTCTTTCCCTACACGACGCTCTTCCGATC*T
GbS_i5_14	AAGGCTAT	AATGATACGGCGACCACCGAGATCTACAC**AAGGCTAT**ACACTCTTTCCCTACACGACGCTCTTCCGATC*T
GbS_i5_15	GAGCCTTA	AATGATACGGCGACCACCGAGATCTACAC**GAGCCTTA**ACACTCTTTCCCTACACGACGCTCTTCCGATC*T
GbS_i5_16	TTATGCGA	AATGATACGGCGACCACCGAGATCTACAC**TTATGCGA**ACACTCTTTCCCTACACGACGCTCTTCCGATC*T

APPENDIX 3 – PLATE PRODUCTION: