2 Materials and Methods

2.1 Common buffer formulae

Phosphate Buffered Saline (PBS)

36.65 g	Sodium chloride
11.80 g	Disodium hydrogen phosphate (Na_2HPO_4)
6.60 g	Sodium dihydrogen phosphate (NaH_2PO_4)
up to 5 l	Double-distilled water

10x Tris-Buffered EDTA (TBE)

109.0 g	Tris
55.6 g	Boric Acid
40 ml	0.5 M EDTA
up to 1 l	Double-distilled water
	… pH to 8.3

LB Broth

10 g	Tryptone
5 g	Yeast extract
10 g	Sodium chloride
up to 1 l	Double-distilled water
	… pH to 7.0

2x LB Broth

20 g	Tryptone
10 g	Yeast extract
10 g	Sodium chloride
up to 1 l	Double-distilled water
	… pH to 7.0

LB Agar

10 g	Tryptone
5 g	Yeast extract
5 g	Sodium chloride
up to 1 l	Double-distilled water
	… pH to 7.5
20 g	Agar

ExoSAP Buffer

20 ml	1 M Tris pH 8.0
10 ml	1 M Magnesium Chloride
70 ml	Double-distilled water

2.2 Cell Culture Protocols & Media

2.2.1 Media for HeLa and HT1080 cell lines

500 ml Modified Eagle's Medium (Sigma, #M2279)

10% FBS (Gibco #10270-106)

2 mM L-Glutamine

100 units ml^{-1} Penicillin

100 μg ml^{-1} Streptomycin

5% Non-essential amino acids (Gibco, #11140-035)

2.2.2 Media for TE671 and HEK293FT cell lines

500 ml Dulbecco's Modified Eagle's Medium (Sigma, #D5796)

10% FBS (Gibco #10270-106)

2 mM L-Glutamine

100 units ml^{-1} Penicillin

100 μg ml^{-1} Streptomycin

2.2.3 Passaging Cells

All cells were grown in a Galaxy R incubator (Scientific Laboratory Supplies) at 37°C, 5% CO_2 in 75 cm^2 culture flasks with 0.2 μm vent caps (Corning, #430641). They were passaged when between 80% and 95% confluent. HeLa, HT1080 and HEK293FT cells were split at 1:10, and TE671 cells at 1:6.

1. The media from the culture flask was decanted into 1% Virkon (Antec International, #330003).
2. The cells were washed twice with 10 ml of 1x PBS, and the wash decanted into 1% Virkon.
3. The cells were washed twice with 3 ml of 1x Trypsin-EDTA (Gibco, #25300-054), and the wash decanted into 1% Virkon.
4. The flask was incubated at 37°C, 5% CO_2 for 5 mins.
5. During the incubation, 15 ml of the appropriate cell culture medium was added to a new 75 cm^2 flask.
6. The cells are dislodged by sharply tapping the flask 2-5 times, and the cells suspended in 10 ml of the appropriate cell culture medium.

7. The appropriate volume of cell suspension was added to the new flask according to the recommended split ratios.

2.3 Chapter 3 Protocols

DNA samples of the 48 CEPH grandparents were a gift from Andrew Dunham, Wellcome Trust Sanger Institute, and were originally purchased from Coriell Cell Repositories. Samples used were:

Repository #	Cell line #	Age	Gender	Family #	Relation to proband
NA06985	GM06985	69	F	1341	Mat
NA06993	GM06993	74	M	1341	Mat
NA06994	GM06994	68	M	1340	Pat
NA07000	GM07000	66	F	1340	Pat
NA07002	GM07002	63	F	1333	Pat
NA07007	GM07007	95	M	1331	Pat
NA07016	GM07016	71	M	1331	Mat
NA07017	GM07017	61	M	1333	Mat
NA07022	GM07022	63	M	1340	Mat
NA07034	GM07034	71	M	1341	Pat
NA07049	GM07049	68	M	1333	Pat
NA07050	GM07050	62	F	1331	Mat
NA07055	GM07055	70	F	1341	Pat
NA07056	GM07056	65	F	1340	Mat
NA07340	GM07340	83	F	1331	Pat
NA07341	GM07341	61	F	1333	Mat
NA07345	GM07345	69	F	1345	Mat
NA11879	GM11879	66	M	1347	Pat
NA11880	GM11880	65	F	1347	Pat
NA11881	GM11881	62	M	1347	Mat
NA11882	GM11882	61	F	1347	Mat
NA11917	GM11917	66	M	1423	Pat
NA11918	GM11918	64	F	1423	Pat
NA11919	GM11919	67	M	1423	Mat
NA11920	GM11920	66	F	1423	Mat
NA11992	GM11992	86	M	1362	Pat
NA11993	GM11993	80	F	1362	Pat
NA11994	GM11994	80	M	1362	Mat
NA11995	GM11995	84	F	1362	Mat
NA12003	GM12003	97	M	1420	Pat
NA12004	GM12004	92	F	1420	Pat
NA12005	GM12005	77	M	1420	Mat
NA12006	GM12006	75	F	1420	Mat
NA12043	GM12043	74	M	1346	Pat

NA12044	GM12044	70	F	1346	Pat
NA12045	GM12045	74	M	1346	Mat
NA12144	GM12144	71	M	1334	Pat
NA12145	GM12145	70	F	1334	Pat
NA12146	GM12146	61	M	1334	Mat
NA12154	GM12154	92	M	1408	Pat
NA12155	GM12155	86	M	1408	Mat
NA12156	GM12156	81	F	1408	Mat
NA12236*	GM12236	86	F	1408	Pat
NA12239	GM12239	61	F	1334	Mat
NA12248	GM12248	89	M	1416	Pat
NA12249	GM12249	77	F	1416	Pat
NA12250	GM12250	66	M	1416	Mat
NA12251	GM12251	63	F	1416	Mat

* DNA sample no longer available from Coriell

2.3.1 Selection of promoters for re-sequencing

An in-house script (written by Dr. David Beare) was used to extract the genomic sequence from -2000 bases to +50 bases relative to the TSSs of all chromosome 22 genes with a confirmed 5' end, according to the latest published annotation (Collins et al. 2003).

NCBI BLAST was then used to map these sequences back against the human genome. The results were analysed manually, and promoter sequences that matched more than one location in the genome were eliminated.

2.3.2 Primer design

Primers were designed using Primer3 (Rozen and Skaletsky 2000). All parameters were used at default settings except for the ones in the table below:

Parameter	**Value used**
Primer optimum size	20
Primer minimum size	16
Primer maximum size	24
PRIMER_MAX_POLY_X	4
PRIMER_SELF_ANY	6.0
PRIMER_SELF_END	2.0
PRIMER_MIN_GC	18
PRIMER_MAX_GC	82.5
PRIMER_MIN_TM	50
PRIMER_MAX_TM	70

2.3.3 Optimisation of genomic PCR

All PCRs were carried out using the Hot Start Taq (Abgene #SP-0034) and associated reagents at their stock concentrations.

Standard protocol

A PCR reaction premix sufficient for the number of reactions to be carried out was prepared according to the following formula:

Reagent	1X / µl
10x Buffer	1.5
1 mM dNTPs	1.5
DMSO	0.75
ddH_2O	9.06
Taq	0.09
10 ng ml^{-1} DNA	0.5
Total premix	13.4
Primer (15 µM)	1.6

The following cycling protocol was used:

- 95°C for 15:00
- 95°C for 0:30, 60°C for 0:30, 72°C for 0:30 – 38 cycles
- 72°C for 10:00

Stepped activation

The same premix formula as the standard protocol was used, but the following cycling protocol was used:

- 95°C for 2:00, 60°C for 0:30, 72°C for 0:30 – 7 cycles
- 95°C for 0:30, 60°C for 0:30, 72°C for 0:30 – 31 cycles
- 72°C for 10:00

65°C Annealing

The same premix formula as the standard protocol was used, but the following cycling protocol was used:

- 95°C for 15:00
- 95°C for 0:30, 65°C for 0:30, 72°C for 0:30 – 38 cycles
- 72°C for 10:00

55°C Annealing

The same premix formula as the standard protocol was used, but the following cycling protocol was used:

- 95°C for 15:00
- 95°C for 0:30, 55°C for 0:30, 72°C for 0:30 – 38 cycles
- 72°C for 10:00

1.1 M Betaine/7% DMSO

A PCR reaction pre-mix sufficient for the number of reactions to be carried out was prepared according to the following formula:

Reagent	1X / µl
10x Buffer	1.5
1 mM dNTPs	1.5
DMSO	1.05
5M Betaine	3.3
ddH_2O	5.46
Taq	0.09
10 ng ml^{-1} DNA	0.5
Total premix	13.4
Primer (15 µM)	1.6

The following cycling protocol was used:

- 95°C for 15:00
- 95°C for 0:30, 60°C for 0:30, 72°C for 0:30 – 38 cycles
- 72°C for 10:00

2.3.4 High-throughput PCR of promoter fragments

1. Oligonucleotide primers were ordered from Illumina, and were supplied at a concentration of 30 µM. Polymerase and associated buffer was Hot Start Taq (Abgene #SP-0034).
2. A TECAN Genesis RSP150 robot was used to aliquot 8 µl of 3 µM primer into a batch of twelve 384-well PCR plates (Eppendorf, # 951020516), such that each plate was divided into 4 identical quadrants, each containing the same 96 primer pairs.
3. A PCR reaction pre-mix sufficient for the number of reactions to be carried out was prepared according to the following formula:

Reagent	**1X / µl**	**6000X / µl**
10x Taq Buffer	1.5	9000
1 mM dNTPs	1.5	9000
DMSO	0.75	4500
ddH_2O	5.86	35160
Hot Start Taq	0.09	540

4. For each 96-well plate of STSs to be sequenced, four 96-well microtitre plates (Greiner, # 650161) were filled with 145.5 µl of premix per well.
5. For each of the 48 DNA samples to be amplified 7.5 µl of 10 ng μl^{-1} DNA was added to each of 8 wells of premix in a column.
6. A TECAN Genesis RSP150 robot was used to aliquot 7 ul of premix/DNA solution into the 384-well PCR plates containing the pre-aliquoted primers, such that all the wells in each quadrant contained the same DNA sample.
7. The PCR plates were centrifuged at 1000 rpm for 1 min on an Eppendorf 5403 centrifuge.

8. PCR was carried out using the following reaction cycle on a thermocycler (MJ, #PTC-225):
    a. 95°C for 15 mins
    b. 95°C for 30 secs, 60/65°C for 30 secs, 72°C for 30 secs –> 38 cycles
    c. 72°C for 10 mins

2.3.5 Cleanup of PCR products

1. A premix of Shrimp Alkaline Phosphatase (USB, #70092X) and Exonuclease I (USB, #70073X) sufficient for the number of reactions to be cleaned was prepared, according to the following formula:
   1 ml ExoSAP buffer
   1 ml ddH_2O
   1 ml Shrimp Alkaline Phosphatase
   0.1 ml Exonuclease I
2. 2 µl was added to each PCR reaction and the plates centrifuged at 1000 rpm for 1 min on an Eppendorf 5403 centrifuge.
3. The PCR plates were incubated for 1 hour at 37°C and for 15 mins at 90°C on a thermocycler (MJ, #PTC-225), and stored at -20°C until sequencing.

2.3.6 Sequencing of PCR products

Cleaned PCR fragments were submitted to the Sanger Institute Sequencing Centre. They were sequenced from both ends using the di-deoxy chain terminator method (Sanger et al. 1977), with V3.1 Bigdye terminator chemistry (West et al. 2005). The resulting sequencing reactions were analyzed on 3730 ABI sequencing machines (Applied Biosystems, USA).

2.4 Chapter 4 Protocols

2.4.1 Creation of pools and design of oligos

The results of the haplotype predictions were analysed by eye, and a set of individuals chosen for each promoter such that the proportions of the different haplotypes present

was as close to equal as possible. 10 ng μl^{-1} solutions of the individual DNA samples were mixed in order to keep the concentration at that level.

Oligos for cloning the promoters into the Gateway vectors were designed by simply taking the sequence of the primers used for SNP-mining and adding the att-sites to the 5' ends.

2.4.2 PCR of promoters from pool templates

1. A PCR reaction premix sufficient for the number of reactions to be carried out was prepared according to the following formula:

Reagent	**1X / µl**
10x KOD Buffer	2
2 mM dNTPs	2
25 mM $MgSO_4$	0.8
DMSO	1
ddH_2O	8
KOD (2.5 U μl^{-1})	0.4

2. 14.2 µl of premix was aliquoted into 96-well PCR plates (ABgene, #AB-0800), and 0.8 µl of each primer mix was added to the reactions.
3. 5 µl of each template was added to the well containing the corresponding primers.
4. PCR was carried out using the following reaction cycle on a thermocycler (MJ, #PTC-225).
    a. 95°C for 4 mins
    b. 95°C for 1 min, 65°C for 30 secs, 72°C for 1 min –> 30 cycles (-0.3°C annealing temperature per cycle)
    c. 72°C for 5 mins
5. First round PCR products were diluted 1:200 in ddH_2O and used as templates for the second round of PCR.
6. A PCR reaction premix sufficient for the number of reactions to be carried out was prepared according to the following formula:

Reagent	1X / µl
10x KOD Buffer	2.5
2 mM dNTPs	2.5
25 mM $MgSO_4$	1
DMSO	1.25
ddH_2O	11.25
15 µM Primer	1
KOD (2.5 U μl^{-1})	0.5

7. 20 µl of premix was aliquoted into 96-well PCR plates.
8. 5 µl of template was added to each well.
9. PCR was carried out using the following reaction cycle on a thermocycler (MJ, #PTC-225).
    a. 95°C for 2 mins
    b. 95°C for 15 secs, 45°C for 30 secs, 68°C for 1 min – 5 cycles
    c. 95°C for 15 secs, 55°C for 30 secs, 68°C for 1 min – 20 cycles
    d. 68°C for 5 mins

2.4.3 Gateway cloning into pDONR223

The protocol included with the BP Clonase II kit (Invitrogen, #11789-020), was followed, except that reactions were all scaled down by half in order to conserve reagent.

1. A custom reaction buffer, called BP3 buffer, was made up and used instead of the included BP buffer. The formula for 5x BP3 buffer is:
   100 mM Tris-Cl, pH 7.5
   20 mM EDTA
   30 mM spermidine
   25% glycerol
   225 mM NaCl
2. A reaction premix sufficient for the number of reactions to be carried out was prepared according to the following formula:

Reagent	Volume (1X)
5X BP3 Buffer	2 µl
pDONR223	100 ng (minimum 1 µl)
ddH_2O	Up to 2 µl

3. 5 µl of premix per reaction was aliquoted into the wells of a 96-well PCR plate (ABgene, #AB-0800).
4. 4 µl of cleaned-up PCR insert was added to the premix, and mixed by pipetting.
5. 1 µl of BP clonase II was added to each reaction and mixed by pipetting
6. The reactions were incubated at 16^{o}C overnight on a thermocycler (MJ, #PTC-225).
7. 1 µl of proteinase K (included in the kit) was added to each reaction.
8. The reactions were incubated at 37^{o}C for 10 mins.

2.4.4 Transformation and preparation of pDONR223 haplotype libraries

1. 0.5 µl of each BP reaction was aliquoted into a 96-well PCR plate (Costar, #6511).
2. The plate was pre-chilled to -20^{o}C, and then placed in a metal heating block inside a benchtop cooler (StrataCooler) to equilibrate to 4^{o}C for 5 mins.
3. 10 µl of library-efficient DH5α cells (Invitrogen, #18263-012) were added to the plasmid and the plate incubated at 4^{o}C for 30 mins.
4. The cells were heat-shocked at 42^{o}C for 45 secs using a thermocycler (MJ, PTC-225).
5. The plate was placed back in the 4^{o}C heating block for 2 mins.
6. 90 µl of SOC media (Invitrogen, #15544-034) was added to each transformation.
7. The plates were incubated at 37^{o}C for 1 hour.
8. The transformations were plated on to Hybond-N+ nylon membranes (Amersham, #AMNK9655) laid on LB agar plates containing 100 ng ml^{-1} of spectinomycin (Sigma, #S-4014), and the plates incubated at 37^{o}C overnight.
9. Colonies were scraped into 10 ml of LB broth with a plastic spreader, and the cells pelleted using a Beckman centrifuge (J6-M6) centrifuge at 3000 rpm for 15 mins.
10. Plasmids were prepared from the cell pellets using the Qiaquick Spin Miniprep kit (Qiagen, #27104) as per manufacturer's instructions.

2.4.5 Gateway cloning into pGL3 Basic GW

The protocol included with the LR Clonase II kit (Invitrogen, #11791-020), was followed, except that reactions were all scaled down by half in order to conserve reagent.

1. A custom reaction buffer, called LR4 buffer, was made up and used instead of the included LR buffer. The formula for 5X LR4 buffer is:
   200 mM Tris-Cl, pH 7.5
   10 mM EDTA
   35 mM spermidine-HCl
   320 mM NaCl
   25% glycerol
2. A reaction premix sufficient for the number of reactions to be carried out was prepared according to the following formula:

Reagent	**Volume (1X)**
5X LR Buffer	2 µl
pGL3 Basic GW+	100 ng (minimum 1 µl)
TE	Up to 4 µl

3. 7 µl of premix per reaction was aliquoted into a 96-well PCR plate ABgene, #AB-0800.
4. 2 µl of prepared pDONR223 containing the inserts to be cloned was added to each reaction and mixed by pipetting.
5. 1 µl of LR clonase II was added to each reaction and mixed by pipetting.
6. The reactions were incubated at 16^{o}C overnight on a thermocycler (MJ, #PTC-225).
7. 1 µl of proteinase K (included in the kit) was added to each reaction.
8. The reactions were incubated at 37^{o}C for 10 mins.

2.4.6 Colony PCR of clones from pGL3 Basic GW haplotype libraries

PCR was carried out using the KOD Hot-start DNA polymerase kit (Novagen, #71086) and associated reagents

1. Colonies were picked from agar plates into 1 ml of LB broth containing 100 ng ml^{-1} ampicillin in a deep 96-well plate.

2. Cultures were incubated overnight in an Innova 4000 shaker incubator (New Brunswick Scientific) at 37°C, 275 rpm.
3. PCR templates were prepared by pipetting 50 µl of the overnight cultures into a pipette tip, expelling it back into the culture, and pipetting 50 µl ddH_2O several times using the same tip.
4. 100 µl of the cultures were mixed with 20 µl 50% glycerol and stored at -70°C to produce long term stocks.
5. A PCR reaction premix sufficient for the number of reactions to be carried out was prepared according to the following formula:

Reagent	**1X / µl**
10x KOD Buffer	1.5
2 mM dNTPs	1.5
25 mM $MgSO_4$	0.6
DMSO	0.75
ddH_2O	4.75
15 µM Primers	0.6
KOD (2.5 U μl^{-1})	0.3

   The primers used were RVPrimer 3 (CTAGCAAAATAGGCTGTCCC) and GLPrimer2 (CTTTATGTTTTTGGCGTCTTCCA), and were pre-designed by Promega to amplify across the multi-cloning site
6. 15 µl was aliquoted into the wells of a 96-well PCR plate (ABgene, #AB-0800).
7. 5 µl of each template was added to the reactions and mixed thoroughly by pipetting.
8. PCR was carried out using the following reaction cycle on a thermocycler (MJ, #PTC-225):
   a. 94°C for 2 mins
   b. 94°C for 30 secs, 60°C for 30 secs, 68°C for 1 min –> 25 cycles
   c. 68°C for 5 mins

2.4.7 Sequencing of colony PCR products

Cleaned fragments were sequenced from both ends using the di-deoxy chain terminator method (Sanger et al. 1977), with V3.1 Bigdye terminator chemistry (West

et al. 2005). The resulting sequencing reactions were analyzed on 3700 ABI sequencing machines (Applied Biosystems, USA).

2.4.8 Preparation of plasmids for high-throughput transfection

The Millipore Montage96 Plasmid prep kit (Millipore, #LSKP096) was used to prepare reporter plasmid for transfection. A modified version of the protocol was used as follows:

1. Ice scrapings from glycerol stocks of each plasmid were inoculated into 1 ml starter cultures of 2x LB broth in deep 96-well plates (Costar, #3961). Cultures were incubated for 6-8 hours at 37^{o}C, 275 rpm in a shaker incubator.
2. 20 µl of starter culture were transferred to fresh 1 ml cultures in a new plate, and incubated overnight at 37^{o}C, 275 rpm in a shaker incubator.
3. Cultures were centrifuged at 2600 rpm in a Sorvall RT7 centrifuge (RTH-250 rotor), and the supernatant decanted away.
4. Pellets were re-suspended in 130 µl of solution 1 (Millipore kit) using a pipette to ensure re-suspension.
5. 130 µl of lysis buffer (Millipore kit) was added to each well, and the plates shaken gently on a Stovall belly dancer for 1 min, and incubated at room temperature until 5 mins after addition of the lysis buffer.
6. 130 µl of neutralisation buffer (Millipore kit) was added to each well, and the plates shaken gently on a Stovall belly dancer for 2 mins.
7. Cell lysates were centrifuged at 2600 rpm for 15 mins.
8. The supernatants were transferred to a new plate and re-centrifuged at 2600 rpm for 15 mins.
9. The supernatants were transferred to a Multiscreen$_{96}$ lysis clearance plate (Millipore kit, #MANANLY), and the lysates filtered into a Multiscreen$_{96}$ plasmid plate (Millipore kit, #MANUPSD) using an eppendorf plate vacuum manifold at 0.27 bar (8 in Hg).
10. Lysates were filtered through the plasmid plates at 0.81 bar (24 inHg), with the filtrate directed to waste.
11. The wells were washed 5 times by adding 100 µl of HPLC-grade water and filtering at 0.81 bar, with the filtrate discarded each time.

12. 35 µl of Tris-HCl pH 8.0 was added to each well, and the purified plasmid re-suspended by shaking vigorously on a Sorvall belly dancer for 30 mins.

2.4.9 Co-transfection of cell lines with reporter plasmids

Just prior to setting up the transfection reactions, the cells to be transfected were split according to the protocol detailed above. All transfection experiments were done using cells at passages 3-6. The Effectene transfection reagent (Qiagen, #301427) was used to transfect the cells, and all reagents came from the kit unless otherwise stated.

1. The cells in the cell suspension were counted using a Neubauer 2.5 μm^2 haemocytometer.
2. The concentration of the suspension was adjusted with appropriate growth medium to 6.67 x 10^4 cells ml^{-1}, and 1 x 10^4 cells (150 µl) were seeded into the wells of 96-well cell culture plates (Falcon, #3072). Sufficient wells were seeded to carry out each transfection in quadruplicate, with 4 wells per plate to be used for negative control transfections.
3. 354 ng of each plasmid to be transfected was aliquoted into 96-well PCR plates, with the top wells of every 3rd column containing the same mass of pGL3 Basic (Promega, #E1741).
4. 71 ng of pRL-CMV (Promega, #E2261) was added to each well, and the volumes made up to a total of 47.5 µl with EC Buffer.
5. A dilution of 3.4 µl in 80 µl of Enhancer reagent was made in EC buffer, and 80 µl of this solution was added to each transfection and mixed by pipetting 6 times. The reactions were incubated at room temperature for 5 mins.
6. During the 5 min incubation, a 1:40 dilution of Effectene reagent in EC buffer was prepared. 85 µl of this solution was added to each transfection and mixed by pipetting 6 times, and the reactions incubated at room temperature for 10 mins.
7. 50 µl of each transfection reagent was pipetted into 4 wells pre-seeded with cells, and mixed by pipetting once.
8. The plates were incubated at 37^{o}C, 5% CO_2 for 48 hours.

2.4.10 Assay of firefly and renilla luciferase levels

All assays were carried out using the Dual-Luciferase Reporter Assay Kit (Promega, #E1960), and all reagents are from this kit unless otherwise stated.

1. The media from the transfections was aspirated into 1% Virkon.
2. The cells were washed once in 100 µl 1x PBS, with the wash solution aspirated into 1% Virkon.
3. 23 µl of 1x passive lysis buffer was added to each well, and the plates shaken vigorously for 30 mins on a Sorvall belly dancer.
4. 20 µl of the cell lysates were transferred to 96-well Optiplate luminometer plates (PerkinElmer, #P12-106-001).
5. The levels of firefly and renilla luciferase were assayed using a Berthold LB96V luminometer equipped with dual injectors, one for each of the two luciferase substrates. The injectors were programmed to dispense 30 µl of luciferase assay reagent II (LAR II) and 30 µl Stop & Glo reagent, with each injection followed by a 1.6 sec delay and a 10 sec measurement time.

2.5 Chapter 5 Protocols

2.5.1 Preparation of total RNA from cell lines

RNA samples were prepared from HT1080, TE671, HEK293FT and HeLa cells using the RNeasy mini kit (Qiagen #74104) according to manufacturer instructions.

2.5.2 Sample preparation and hybridisation on whole genome expression arrays

RNA samples for hybridisation were prepared according to the Affymetrix GeneChip Expression Analysis Manual (Affymetrix, California). 5 μg of total RNA per replicate per cell line was prepared and hybridised on Affymetrix U133 Plus 2.0 arrays as per manufacturers instructions. Hybridisations were carried out in an Affymetrix GeneChip oven overnight at 42^{o}C, 60 rpm. Array washes were done on an Affymetrix GeneChip Fluidics 450 Workstation, and the arrays scanned with an Affymetrix GeneChip Scanner. All protocols are outlined in detail in the Affymetrix GeneChip manual, and were followed without deviation. Data analysis was carried out using the Bioconductor package (Gentleman et al. 2004) in collaboration with Juanma Vaquerizas and the European Bioinformatics Institute (see section 5.2). Present, marginal and absent calls were made using the PANP algorithm (Warren et al. 2006).