

Extraction of total white blood cells (WBC) from an EDTA-tube

1. Put 5ml of total blood to fresh 50ml Falcon tube for WBC isolation.
2. Determine volume of 1xBoyle's solution required (1:10, 5ml blood + 45ml 1xBoyles's Solution).
3. Incubate at RT for 10min with frequent inversion.
4. Centrifuge 300xg 10min
5. Pour off red blood cell lysate and remove residual supernatant.
6. Pour 1xBoyle's Solution to each Falcon tube to 30ml.
7. Incubate at RT for 10min with frequent inversion.
8. Centrifuge 300xg 10min.
9. Pour off red blood cell lysate and remove residual supernatant.
10. Resuspend pellets in 30ml of 2mM EDTA – 1xPBS solution. Take all replicate pellets to a single 50ml Falcon tube.
11. Centrifuge 300xg 10min.
12. Remove supernatant and resuspend pellet in 10ml of 2mM EDTA – 1xPBS solution.
13. Count the cells.
14. Centrifuge 300xg 10min.
15. Whilst centrifugation is in progress, prepare the cryo solution by adding 10% DMSO to appropriate volumes of FCS.
16. Remove supernatant and resuspend pellet in 1ml of cryo solution.

Additional file 3: Table S2.

A

EpiTYPER primers				
	Forward Primer Sequence 5'- 3'	Reverse Primer Sequence 5'- 3'	Size (bp)	From publication
<i>H19</i> DMR	AGGAAGAGAGTTTTTATTAAAGGTTAAGGTGGTGAT	CAGTAATACGACTCACTATAGGGAGAAGGCTCAAACAAAATCCCCACAACC	255	Ollikainen <i>et al</i> 2010
<i>H19</i> CTCF6	AGGAAGAGAGTATGGGTATTTTGGAGGTTTTTTT	CAGTAATACGACTCACTATAGGGAGAAGGCTAACTTAAATCCCAAACCATAACACT	316	Ollikainen <i>et al</i> 2010
<i>LINE-1</i>	AGGAAGAGAGGTGTGAGGTGTAGTGTGTTTTGTT	CAGTAATACGACTCACTATAGGGAGAAGGCTATATCCACACCTAACTCAAAAAAT	433	Wang <i>et al</i> 2010

Bisulphite sequencing primers				
	Forward Primer Sequence 5'- 3'	Reverse Primer Sequence 5'- 3'	Size (bp)	From publication
<i>H19</i> CTCF6	AGGAAGAGAGGGAAAATGTAAGATTTTGGTGAATAT	CAGTAATACGACTCACTATAGGGAGAAGGCTTCCAATTCATAAATAATAAAAAATCTC	465	Coolen <i>et al</i> 2007

Genotyping primers				
	Forward Primer Sequence 5'- 3'	Reverse Primer Sequence 5'- 3'	Size (bp)	
<i>H19</i> CTCF6	GGCTTCTCTCGGTCTCAC	TGTGGTAATGCCCGACCTG	241	

B

EpiTYPER PCR protocol	Bisulphite sequencing PCR protocol	Genotyping PCR protocol
95° 15min	95° 15min	95° 15min
5x 94° 1min 56° 30s 72° 30s	40x 94° 1min 56° 30s 72° 30s	40x 95° 1min 62° 30s 72° 30s
40x 94° 1min 30s 60° for <i>H19</i> DMR; 58° for <i>H19</i> CTCF6; 56° for <i>LINE-1</i> 72° 30s	72° 10min 4° ∞	72° 10min 4° ∞
72° 10min 4° ∞		