

Whole Genome Amplification Information (Appendix E) for Human Custom SNP Genotyping

	MDA (Multiple Displacement Amplification) Method	Omniplex Method
Details	phi29 DNA polymerase plus exonuclease-resistant random hexamer primers - isothermal amplification. Method based on strand-displacement synthesis originally developed for amplifying large circular templates	Converts randomly fragmented genomic DNA into a library - common primers ligated to ends and library is amplified via PCR reaction
Originating Companies	Molecular Staging	Rubicon
Available Kits	Amersham - GenomiPhi kit. Qiagen - Repli-G kit	Sigma - GenomePlex kit
WGA Service Labs	Qiagen	Rubicon - OmniPlex WGA Amplification Service
Minimum Amount Input gDNA Required	GenomiPhi kit - minimum 1ng - recommends at least 10 ng Repli-G kit - minimum 10 ng - recommends 100 ng	GenomePlex kit - minimum 10 ng Rubicon service - at least 50 ng at > 5 ng/ul
Purification of Amplification Products	Not required for Illumina GoldenGate assay. See below regarding impact on quantitation method	Purification of final product recommended. Purification method must retain both single and double-stranded DNAs!
Quantitation of Amplification Products	Pico green quantitation required if amplification products are not purified by spin-column or ethanol precipitation (to eliminate random hexamers). If amplification products have been purified then concentration can be measured by absorbance	Once purified, the DNA can be quantified by absorbance. Pico green quantitation will often underestimate the actual WGA DNA yield, since ssDNA might be generated during the amplification
Storage of Amplification Products	At 4C or -20C until ready for analysis or purification. The stability of the WGA DNA is equivalent to gDNA under the same conditions	At 4C or -20C until ready for analysis or purification. The stability of the WGA DNA is equivalent to gDNA under the same conditions
Amplification without gDNA Template	Will get amplification, hexamers will amplify at low efficiency	Will not get amplification if procedure done correctly
Quality Assessment of Amplification Products	Load 1 ug of WGA reaction on 1% agarose gel in TBE buffer. The majority of the product should be above 10Kb in length and generate a trail of smear by electrophoresis down to about 2 Kb	Load 5-10% of the final reaction onto a 1.5% agarose gel. The DNA size should range from ~75-1500 bp with the mean size ~400bp
Further Quality Assessment of WGA Products	Real-time PCR using primer sets for human gDNA. Yan, J., Fen, J., Hosono, S. and Sommer, S. Assessment of multiple displacement amplification in molecular epidemiology. <i>Biotechniques</i> , 37(1), July 2004 has details on two loci used by Molecular Staging service lab to assess quality of amplification products	Real-time PCR using primer sets for human gDNA. Yan, J., Fen, J., Hosono, S. and Sommer, S. Assessment of multiple displacement amplification in molecular epidemiology. <i>Biotechniques</i> , 37(1), July 2004 has details on two loci used by Molecular Staging service lab to assess quality of amplification products