ULS™

Technical Notes

Genome-pULSe: Whole Genome Amplification & Labeling for arrayCGH analysis

The Genome-pULSe, arrayCGH Genomic DNA Amplification and Labeling Kit offers a novel procedure that allows the uniform amplification and subsequent direct (non-enzymatic) labeling of whole genome DNA from small samples (Figure 1). This method has been designed to provide a quick and highly reproducible amplification and labeling procedure for arrayCGH analysis. The Genome-pULSe Kit consists of a REPLI-g® Mini Kit module and a ULS™ Labeling Kit module containing Cy™3- ULS and Cy™5-ULS. The Genome-pULSe Kit has been evaluated on BAC slides obtained from Dr. Karoly Szuhai, Department of Molecular Cell Biology, LUMC, Leiden, The Netherlands (12800 BACs, CodeLink™ slides)

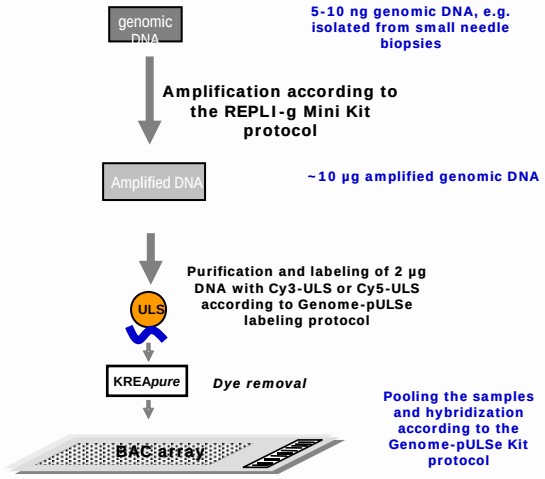
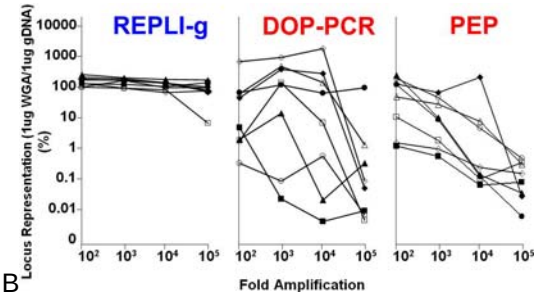


Figure 1. Overview of the Genome-pULSe Kit workflow

Method

Reference DNA was purchased from Promega and tumor DNA from ATCC. The DNA was amplified according to the standard REPLI-g Mini Kit protocol of the Genome-pULSe Kit. Where indicated, (amplified) genomic DNA was fragmented by sonication. Amplified and un-amplified genomic DNA samples where labeled with Cy3-ULS and Cy5-ULS according to the standard protocol of the Genome-pULSe Kit. Labeled DNA samples where pooled and hybridized on BAC slides according to the Genome-pULSe protocol.

A



B

Figure 2: A) Shows the mechanism of Whole Genome Amplification be the Phi29 Polymerase of the REPLI-g Mini Kit. Samples are denatured, random primed with hexamers. The high fidelity Phi29 Polymerase produces a high molecular weight product by Multiple Displacement Amplification (error rate 10^{-7}). B) Bias study. TaqMan validation of the locus representation of 8 different loci. REPLI-g was compared to DOP-PCR and PEP. These results clearly show that the Phi29 enzyme is introducing much less bias compared to DOP-PCR and PEP. DATA kindly provided by Dr. Gerald Schock, QIAGEN GmbH, Hilden, Germany

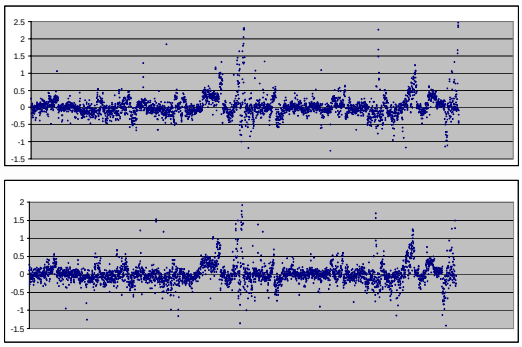


Figure 3: ArrayCGH ratio plots of ULS Labeled Genomic DNA (upper panel) and ULS labeled REPLI-g amplified genomic DNA (lower panel). 2 µg Tumor DNA (SK-BR3) and reference genomic DNA (amplified by REPLI-g or un-amplified) was fragmented by sonication and labeled with Cy3-ULS and Cy5-ULS. Labeled samples where pooled and precipitated in the presence of a 12.5 x excess of C0t-1 DNA. The precipitated labeled sample was hybridized on BAC slides and ratio plots where generated.

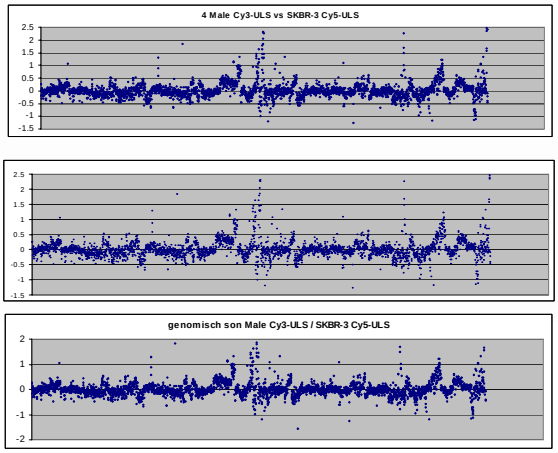


Figure 4: arrayCGH ratio plots of ULS labeled genomic DNA (Tumor and reference). Upper panel, REPLI-g amplified genomic DNA that has not been fragmented after amplification. Middle panel, REPLI-g amplified genomic DNA that has been fragmented by sonication after amplification. Lower panel, Genomic DNA (un-amplified) that has been fragmented by sonication before labeling and subsequent hybridization on BAC arrays.

Discussion and conclusions

- REPLI-g Whole Genome Amplification does not introduce bias when compared to DOP-PCR and PEP. The combination of REPLI-g and the direct non-enzymatic ULS labeling of amplified Genomic DNA in the Genome-pULSe Kit provides a method to reduce the introduction of bias in arrayCGH analysis.
- No fragmentation step is required, which makes the target preparation and labeling procedure very short and easy (Figure 1)
- When genomic material is limited, the Genome-pULSe procedure is an easy and reliable method for Whole Genome Amplification with REPLI-g and labeling with ULS for arrayCGH applications.