A Novel Approach to Whole Genome Amplification and Labeling of DNA Samples for Copy Number Variation Detection on BAC Microarrays

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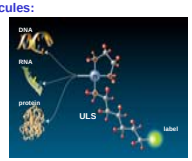
ULS™ principle

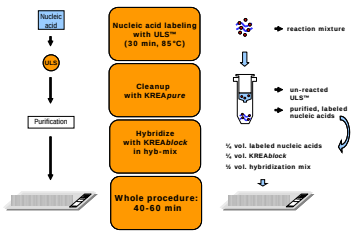
- The Universal Linkage System (ULS™) is a platinum-based labeling technology that allows for the direct coupling of a large variety of fluorescent labels and haptens to biomolecules like DNA, RNA and proteins
- The labeling reaction produces a stable coordinative bond with the biomolecule of interest
- ULS is a suitable technology in microarray applications for any of the following nucleotide target molecules:

* genomic DNA
* amplified unmodified aRNA, cRNA
* miRNA siRNA or other small RNA fragments
* total or mRNA for unbiased representation of the transcriptome (ideal for splice variant analysis and prokaryotic gene expression profiling)
* complex PCR products



ULS™ labeling of genomic DNA

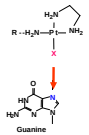
A 40-60 minute protocol for microarray applications



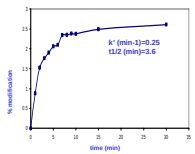
ULS™ : direct chemical labeling

Direct labeling using ULS™ allows precise control over the degree of modification, in contrast to enzymatic incorporation of modified nucleotides.

ULS labels DNA, RNA by forming a coordinative bond on the N7 position of guanine

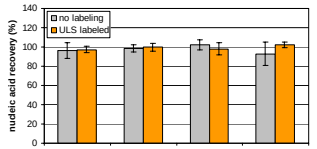


Half-life of the reaction is a tool to control the labeling reaction



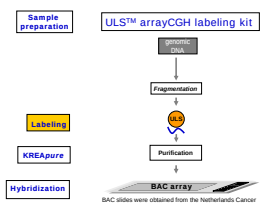
Kinetics of total RNA labeling at 85°C (aimed at 3 % labeling at t=infinity)

Use of KREA*pure* clean-up column for optimal recovery of labeled DNA



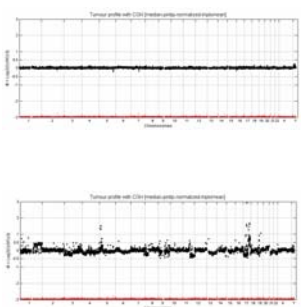
Recovery of nucleic acids of different lengths, both ULS labeled and not labeled using the KREA*pure* column purification procedure.

ULS™ labeling of DNA from FFPE material optimized for CGH analysis on BAC-arrays



BAC slides were obtained from the Netherlands Cancer Institute (3.6k BACs spotted on CodeLink slides)

ULS™ labeling of archival DNA (FFPE)

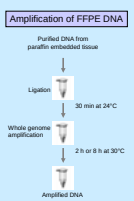


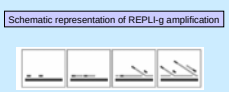
Self-self normal reference hybridization

Comparative genomic hybridization is performed on 3.5k, 1Mb resolution human BAC arrays. This is a genome-wide profile of an grade 3 infiltrating ductal breast carcinoma DNA isolated from formalin-fixed-paraffin-embedded (FFPE) tissue. DNA was labeled using ULS-Cy5 and hybridized together with normal reference DNA labeled with ULS-Cy3. Data provided by Erik van Beers, NKI, The Netherlands.

Tumor vs. normal reference hybridization

Principle of REPLI-g FFPE WGA



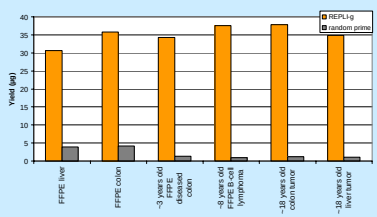


REPLI-g FFPE amplification of DNA isolated(*) from FFPE material is a two step process: an initial processing reaction preparing the damaged DNA for whole genome amplification and a second reaction involving the actual amplification.

REPLI-g WGA is performed using isothermal MDA (Multiple Displacement Amplification): the REPLI-g DNA polymerase moves along the DNA template strand displacing the complementary strand. The displaced strand becomes a template for replication allowing high yields of high-molecular-weight DNA to be generated.

(*) Direct amplification of DNA without prior isolation is also possible.

Yield of REPLI-g FFPE amplification compared to random priming



Yields of amplification with REPLI-G FFPE versus random priming (Exo-Klenow) of DNA isolated from FFPE material from different organs and storage periods.

Manufacturer's instructions were followed starting with **50 ng DNA**. Yields to be expected are < 40 µg for REPLI-g and ≥7 µg for random priming, respectively.

Low input FFPE material: REPLI-g® FFPE whole genome amplification combined with ULS™ labeling

ULS Labeling of REPLI-g FFPE amplified DNA compared to random prime labeling

sample	ULS	REPLI-G + ULS	random prime
FFPE liver	1.2	1.3	0.5
FFPE colon	1.2	1.3	0.4
~3 yrs old FFPE diseased colon	1.0	0.9	0.1
~8 yrs old FFPE B-cell lymphoma	1.0	0.9	0.0
~18 yrs old FFPE colon tumor	1.6	1.6	0.1
~18 yrs old FFPE liver tumor	2.0	1.9	0.2

Degrees of labeling of non-amplified and REPLI-g FFPE amplified and ULS labeled versus random prime of DNA isolated from freshly prepared to "very old" FFPE material from different organs.
Shown are the %DoL obtained using ULS on non-amplified DNA, ULS on REPLI-g FFPE amplified DNA, and using incorporation of labeled dCTP using random prime amplification (Exo-Klenow). % DoL to be expected are 1.0-2.5 for ULS and ≥ 0.7 for random priming, respectively.

SUMMARY

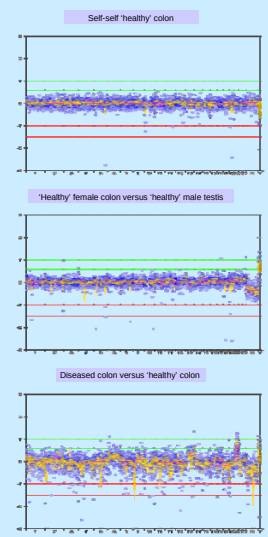
CGH on BAC arrays using DNA from FFPE material

High amounts of DNA available => no amplification needed
ULS provides a non-enzymatic and reproducible approach for high quality data using direct chemical labeling

Low amounts of DNA available => WGA needed
REPLI-g FFPE generates substantial amounts of DNA from all FFPE samples tested (regardless of tissue type and storage period)

REPLI-g FFPE in combination with ULS labeling is the method of choice when starting from as little as 10-50ng DNA

CGH analysis of REPLI-g FFPE amplified, ULS labeled DNA from FFPE material on BAC arrays



Comparative genomic hybridization is performed using 3.5k, 1Mb resolution human BAC arrays. Shown comparisons were performed using REPLI-g FFPE amplification of 50 ng of indicated DNA from FFPE material followed by labeling with Cy3-ULS and Cy5-ULS, respectively. Please note that chromosome plots shown are based on Lowess normalized data; no smoothening was applied.

Relevant literature:

Joosse et al. Automated array-CGH optimized for archival formalin-fixed, paraffin-embedded tumor material. BMC Cancer 7: 43 (2007)
Knijnenburg et al. Optimized amplification and fluorescent labeling of small cell samples for genomic array-CGH. Cytometry Part A 71A: 585-591
Van Beers et al. A multiplex PCR predictor for aCGH success of FFPE samples. Br J Cancer 94: 333-337

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