Materials List for:

Microarray-based Identification of Individual HERV Loci Expression: Application to Biomarker Discovery in Prostate Cancer

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URL: https://www.jove.com/video/50713
DOI: doi:10.3791/50713

Materials

Name	Company	Catalog Number	Comments
Trizol	Invitrogen	15596-026	
RNA poly-A control stock	Affymetrix	900433	
DNAse 1	Promega	M6101	1,000 U (1 U/µl)
Terminal transferase	Roche	3333574001	400 U. Including enzyme and coenzyme ($CoCl_2$).
DLR-1a	Affymetrix	900542	
Hybridization internal controls B2 and 20x Eukaryotic Hybridization Control	Affymetrix	900454	
GeneChip Hybridization, Wash and staining	Affymetrix	900720	Including PreHybridization Mix and 2x Hybridization Mix for 30 reactions
10x One-Phor-All Buffer PLUS			Composition in DEPC-treated water: 100 mM Tris-acetate pH 7.5; 100 mM magnesium acetate; 500 mM potassium acetate.
RNeasy Mini kit	Qiagen	74104	RNA cleanup protocol
WT-Ovation RNA amplification system	Nugen	2210-24	
QIAquik PCR purification kit	Qiagen	28104	
EQUIPMENT			
Material Name	Company	Catalogue Number	Comments
Nanodrop 1000	Thermo Scientific		
GeneChip Scanner 3000 7G	Affymetrix	GS30007G	Optional: autoloader
GeneChip Fluidics Station 450	Affymetrix	FS450	
GeneChip Hybridization 640 Oven	Affymetrix	640	Includes 4 GeneChip Probe array carriers
Workstation loaded with GeneChip Operating Software (GCOS) including the GeneChip Scanner 3000 High-Resolution Scanning Patch			
HERV-V2 chip	Affymetrix		Custom array.

			For microarray availability (for research use only), please contact: François Mallet Laboratoire Commun de Recherche Hospices Civils de Lyon-bioMérieux Medical Diagnostic Discovery Department Centre Hospitalier Lyon Sud, Bâtiment 3F 69495, Pierre Bénite cedex France Phone: 33 (0)4 72 67 87 85 Email: francois.mallet@biomerieux.com

HERV-V2 conception

Dedicated database and annotations

The construction of a dedicated database, grouping genomic HERV sequences belonging to 6 HERV families, has been achieved by the following procedure: (i) the most complete and representative sequence of each HERV family was selected from the literature and defined as a prototype sequence (**Figure 2**). (ii) The 6 prototypes were functionally annotated with reference to their LTR (U3/R/U5) and internal parts (gag/pol/env). (iii) RepeatMasker [44] was then applied using these functional sequences as input libraries. A genome-wide search of all related sequences was performed over the human genome on the basis of a minimum 80% homology (NCBI 36/hg18). (iv) Finally, the functional sequences retrieved by this process were assembled into distinct loci on the basis of their genomic location and eventually implemented in a dedicated HERV database. This database, called HERV-gDB3, contains 10,035 individual HERV loci[35].

Locus-specific probes design

Starting from HERV-gDB3, overlapping tracks of 25-mer candidate probes were firstly generated. Each candidate probe was then aligned against the human genome using KASH 45 in order to assess the cross-hybridization potentialities. This latter estimation was performed by a model developed specifically for this purpose and referred to as EDA+. Briefly, the principle of EDA+ is to take into account the instability brought by mismatches and gaps in a 25-mer target/probe hybridization complex. Candidate probes exhibiting low cross-hybridization risks (*i.e.* a low number of non-specific genomic targets) are selected and lastly assembled into probesets.

Custom HERV GeneChip microarray

The custom HERV GeneChip integrates 23,583 HERV probesets and can discriminate 5,573 distinct HERV elements, composed of solo LTRs, complete and partial proviruses (**Figure 2**). The standard Affymetrix control probes for unbiased amplification and hybridization were also included in the microarray.