Microarrays Unit: Sample delivery requirements

DNA samples

For genomic microarrays

For **CytoScan 750K or HD assay**, it is required a minimum amount of 1 µg of DNA, diluted in low EDTA TE buffer (0.1 mM EDTA, 10 mM Tris HCL, pH 8.0). If you do not have available low EDTA TE buffer, we can provide you.

For **CytoScan Xon assay**, it is required a minimum amount of 500ng of DNA, diluted in low EDTA TE buffer or nuclease free water.

For **OncoScan assay**, it is required a minimum amount of 500ng of DNA, diluted in low EDTA TE buffer or ATE buffer (from *QiAamp® DNA FFPE Tissue Kit*). DNA should be kept refrigerated for no more than 2 weeks, otherwise, kept at -20ºC or lower temperature. In case of low concentration, DO NOT concentrate.

For DNA eluted in other reagents, contact us.

For genotyping with the Biomark HD

For **Genotyping application**, it is required a minimum volume of 3 µL of DNA at a concentration of ≥ 20 ng/µL. The DNA should have a 260:280 ratio between 1.5-1.8 and should be preferably diluted in EDTA TE buffer (0.1 mM EDTA, 10 mM Tris HCL, pH 8.0). If you do not have available buffer, we can provide you.

RNA samples

For expression microarrays

It is required a minimum amount of 1 µg of RNA, diluted in nuclease free water. In case of having smaller amount ask us, there are available protocols for low input RNA (a minimum of 500 pg of total RNA).

For **miRNA samples (miRNA microarrays)**, it is required a minimum of 2 µg of RNA enriched with miRNA and in nuclease free water.

For gene expression with the Biomark HD

RNA (when Fluidigm retrotranscription service is requested): it is required a minimum volume 2.5 µL of RNA at a recommended concentration of 100-150ng/ µL. However, the RNA concentration range that can be used for the reverse transcription reaction goes from 50-250ng/ul. The success with a given sample in qPCR will depend on the level of gene expression for the genes of interest, the percentage of mRNA in the total RNA, and the number of cycles of preamplification performed prior to qPCR. RNA should have a 260:280 ratio between 1.5-1.8 and should be preferably diluted in nuclease free water. Prior to use, it is recommended to monitor the integrity of the RNA on a system such as the Agilent® 2100 bioanalyzer.

cDNA: it is required a minimum volume of 3 µL of cDNA, preferably diluted in nuclease free water. Preamplification will always be performed, unless >1000 copies/µL for all specific targets are present/expected in the cDNA.

Nucleic acid extraction methods

DNA and RNA sample extractions should be performed by the researcher. For nucleic acid extraction for microarrays applications, the following chemistries are recommended:

DNA*	DNA from FFPE samples
QIAGEN® - Gentra Puregene Kit 5 PRIME - PerfectPure DNA Blood Kit SDS/ProK digestion, phenol-chloroform extraction, Microcon® or Centricon®(Millipore) ultrapurification and concentration.	QIAGEN® - QIAmp DNA Blood and Tissue Kit (For CytoScan Assay) QIAGEN® - QIAamp DNA FFPE Tissue Kit (For OncoScan Assay)[1]

*Methods that include boiling or strong denaturants are not acceptable, because the DNA would be rendered single-stranded

RNA from FFPE samples	miRNA
PROMEGA - ReliaPrep™ FFPE Total RNA Miniprep System QIAGEN - RNeasy® FFPE Kit Life Technologies - RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE Beckman Coulter Genomics - Agencourt FormaPure® Kit NuGEN - Prelude™ FFPE Isolation Module	Applied Biosystems - mirVana™ miRNA Isolation Kit Applied Biosystems - RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE QIAGEN - miRNeasy Mini Kit Invitrogen - PureLink™ miRNA Isolation Kit Invitrogen - TRIzol® reagent (total RNA only) with additional overnight –20°C precipitation step during isopropanol precipitation.

Sample delivery

DNA samples will be preferably sent at 4 ºC or -20 ºC.

RNA samples will be sent at -80 ºC in dry ice.

All the samples must be sent, with the *Sample delivery form* attached, to:

Mar Mallo / Laura Palomo / Francesc Solé

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[1] For improved DNA yields, Thermo Fisher Scientific® recommends a modification: add a heating step at 98 °C for 15 minutes to improve the tissue digestion process to release DNA from tissue sections.

Samples return will be chargeable to the applicant.

Sample quality analyses

The sample quality analyses will be performed at sample arrival time, in order to assess the purity and integrity of DNA and RNA samples. This procedure will be done by the *Microarrays Unit*. If the samples meet the requirements for inclusion in the study, we will proceed to it (the sample quality analysis is included in the price). If it fails, we will charge it.