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Sample Requirements

Service	Input	Quantification	Concentration	Size	Quantity
Infinium® DNA Methylation	gDNA for bisulfite conversion	PicoGreen or Qubit or Nanodrop(1)	50 – 100 ng/µl	10-20kb (fragments size, if present ≥ 2kb)	500-1000 ng
hMeDIP-Seq	Fragmented gDNA	PicoGreen or Qubit or Nanodrop(1)	50 - 100 ng/µl		Tissues 0.5-1 µg Cells: 2.5-5 µg DNA 50ng
ChIP-Seq	dsDNA	Picogreen or Qubit	0.1 – 0.2 ng/µl	200-600 pb	5 - 10 ng
Expression Array (Human HT12)(2)	Total RNA	Nanodrop or Ribogreen	50 ng/µl (500ng/10µL)		500 ng
RNA-Seq	Total RNA				1-2µg

(1) Please note, the use of NanoDrop for gDNA quantification requires sample treatment with RNase, as NanoDrop may lead to overestimation of actual DNA concentration due to ssDNA, free nucleotides, oligonucleotides, RNA and/or proteins contamination.

(2) Please note, that the Mouse Expression array is now obsolete

If you have any queries and/or do not meet the **sample requirements**, please contact us by email: epics@ulb.ac.be or phone +32(0)2-55562 43.

DNA Methylation (Infinium® HumanMethylation BeadChip450 and MethylationEPIC BeadChip)

DNA Requirements

Bisulfite-converted genomic DNA (gDNA) is required.
Bisulfite conversion is included in our prices.
The DNA Quality Control assessment is not part of our Methylation Assay service. We highly recommend that you determine the size and quality of your gDNA on 1% agarose gel (see below).

DNA Format

Genomic DNA from sources such as cells, frozen tissues, plasma, or other biofluids are suitable.
Please note that Formalin-Fixed Paraffin-Embedded (FFPE) samples can be processed with a modified protocol. Please contact us for details.

Input Buffer

Input gDNA (to be converted by bisulfate) must be diluted in Ultra Pure Water or in Low TE buffer (10mM Tris-HCl/0.1mM EDTA pH=8).

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Sample Requirements

DNA Concentration
Submit samples in a concentration range 50 – 100 ng/µl. DO NOT precipitate DNA samples to increase sample concentration: this may cause loss of DNA, both in quantity and quality. If necessary, use a vacuum concentrator (e.g. SpeedVac). Determine gDNA concentration with Qubit or PicoGreen method. DNA quantification methods that rely on intercalating fluorescent dyes measure better dsDNA. NanoDrop is not suitable as it may lead to overestimation of actual DNA concentration due to ssDNA, free nucleotides, oligonucleotides, RNA and/or proteins measurement. Please, pre-treat samples with RNase prior to NanoDrop measurement and let us know.

DNA Purity
Absorbance measurements at 260, 280 nm are commonly used to assess DNA quality. Suitable DNA should have a 260/280 nm ratio of 1.8 – 2.0.
It is recommended to check for proteins, chaotropic salts (e.g. guanidinium isotiocynate) and phenol carry over, with absorbance at 230 nm. The DNA solution should also have a 260/230 nm ratio of 1.8 – 2.0.

DNA Size (Quality Control)
Check gDNA on 1% agarose gel. High quality gDNA should give a major band at 10-20 kb. Fragments, if present, should be ≥ 2kb. Please send an image of the gel with correctly annotated molecular weight marker/ladder, together with your samples.

hMeDIP-Seq (5-hmC DNA Immuno Precipitation Sequencing)

Our hMeDIP-Seq Service includes:

1. DNA fragmentation.
2. Library preparation
3. Immunoprecipitation using monoclonal 5-hmC antibody
4. Amplification of the enriched DNA sample.
5. Cluster generation and Sequencing.

The DNA Quality Control assessment is part of our hMeDIP-Seq service depending on the type of sample submitted (see §*DNA Size (Quality Control)* below).

DNA Format
Customers can submit cell pellets (2.5 – 5µg) or frozen tissues (0.5 – 1 µg). Fragmented double-stranded DNA (dsDNA) from gDNA or cDNA, chromatin immunoprecipitates (ChIP), degraded DNA from sources such as plasma, or other biofluids are suitable. Please note that Formalin-Fixed Paraffin-Embedded (FFPE) samples can be processed with a modified protocol. Please contact us for details.

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Sample Requirements

Input Buffer
Input DNA must be diluted in Ultra Pure Water or in Low TE buffer (10mM Tris-HCl/0.1mM EDTA pH=8).

DNA Concentration
For h-MeDIP-Seq samples need to be delivered in a concentration range 50 - 100ng/µl. DO NOT precipitate DNA samples to increase the sample concentration: this may cause loss of DNA, both in quality and quality. If necessary, use a vacuum concentrator (e.g. SpeedVac). Determine gDNA concentration with Qubit or PicoGreen method. DNA quantification methods that rely on intercalating fluorescent dyes measure better dsDNA. NanoDrop is not suitable as it may lead to overestimation of actual DNA concentration due to ssDNA, free nucleotides, oligonucleotides, RNA and/or proteins measurement, unless sample are pre-treated with RNase A.

DNA Purity
Absorbance measurements at 260, 280 nm are commonly used to assess DNA quality. Pure DNA should have a 260/280 nm ratio of 1.8 – 2.0.
It is recommended to check for proteins, chaotropic salts (e.g. guanidinium isotiocynate) and phenol carry over, with absorbance at 230 nm. The DNA solution should also have a 260/230 nm ratio of 1.8 – 2.0.

DNA Size (Quality Control)
If you submit fragmented DNA, check fragments size on 1% agarose gel or on a chip-based capillary electrophoresis system (e.g. Agilent 2100 Bioanalyzer). Fragments should range in size 100 – 500 bp. Please send an image of the gel with correctly annotated molecular weight marker or the chip-based capillary profile, together with your samples.

ChIP-Seq (Chromatin Immuno Precipitation Sequencing)

The DNA Quality Control assessment is not part of our ChIP-Seq service. We highly recommend that you determine the size and quality of the DNA fragments on 1% agarose gel or on a chip-based capillary electrophoresis system (e.g. Agilent Bioanalyzer).
DO NOT denaturate DNA
DO NOT use salmon sperm, prefer BSA instead.

DNA Format
Customers should submit sonicated or enzyme digested DNA (see below).
Fragmented double-stranded DNA (dsDNA) from gDNA or cDNA, chromatin immunoprecipitates (ChIP), degraded DNA from sources such as FFPE (Formalin Fixed Paraffin Embedded) tissue, plasma, or other biofluids are suitable.

Sample Requirements

Input Buffer
Input DNA must be diluted in Ultra Pure Water or in Low TE buffer (10mM Tris-HCl/0.1mM EDTA pH=8). DO NOT exceed recommended concentrations and avoid phosphate containing buffers.

DNA Concentration
DO NOT precipitate DNA samples in order to increase the sample concentration: this will cause loss of DNA, both in quality as well as quality. If necessary, use a vacuum concentrator (e.g. SpeedVac).
Determine dsDNA concentration with Qubit or PicoGreen method. DNA quantification methods that rely on intercalating fluorescent dyes measure better dsDNA. NanoDrop is not suitable as it may lead to overestimation of actual DNA concentration due to ssDNA, free nucleotides, oligonucleotides, RNA and/or proteins measurement, unless sample are pre-treated with RNase A

DNA Purity
Absorbance measurements at 260, 280 nm are commonly used to assess DNA quality. Pure DNA should have a 260/280 nm ratio of 1.8 – 2.0.
Check for contaminants (proteins and chaotropic sals such as guanidinium isotiocynate and phenol) with absorbance at 230 nm. The DNA solution should also have a 260/230 nm ratio of 1.8 – 2.0.

DNA Size
For ChIP-Seq, restriction enzyme digestion or sonication for preparing fragmented genomic DNA should be used. Check dsDNA fragments size on 1% agarose gel or on or on a chip-based capillary electrophoresis system (e.g. Agilent 2100 Bioanalyzer). Fragments should range in size from 200 – 600 bp with major bands at 300 – 400 bp. Please send an image of the gel with correctly annotated molecular weight marker or the Agilent 2100 Bioanalyzer profile, together with your samples.

Expression Array (Human HT12 Expression BeadChip)

The RNA Quality Control assessment is not part of our Human Expression Array service, but can be offered as an additional service. We highly recommend that you determine the quality of your RNA on a denaturating 1% agarose gel or (preferred) on a chip-based capillary electrophoresis system (e.g. Agilent 2100 Bioanalyzer).

Pricing for gene expression is based on standard labeling (from >50 ng/µL total RNA). For smaller amounts of starting material (25-50 ng/µL range of RNA), amplification will be required and additional charges will be applicable. Please contact us for details.

RNA Format
Total full-length RNA is suitable for Expression Array. We advise you to use Trizol or Qiagen RNeasy for extraction.
The quality of the RNA is the most important factor affecting the results.

Sample Requirements

RNA Buffer
RNA must be diluted in Ultra Pure Water or in TE buffer (10mM Tris-HCl/1mM EDTA pH=8).

RNA Purity
Absorbance measurements at 260, 280 nm are commonly used to assess RNA quality. Pure RNA should have a 260/280 nm ratio of 1.7 – 2.1.
Check for contaminants (proteins, DNA, chaotropic sals such as phenol and ethanol) with absorbance at 230 nm. The RNA solution should also have a 260/230 nm ratio of 1.7 – 2.1.

RNA Integrity
The integrity of RNA, or the proportion that is full-length, is important as reverse transcription of partially degraded mRNAs will typically generate relatively short cDNAs that can potentially lack portions of the coding region. Check total RNA on 1% denaturating agarose gel or on Agilent 2100 Bioanalyzer type. For full-length RNA (eukaryotic) the ratio of 28S to 28S rRNA should approach 1:2. The Agilent Bioanalyzer will also generates the RNA Integrity Number (RIN) that will analyze information contained inside and outside the 18S and 28S bands. We recommend a RIN higher than 7 (RIN >7).
Please send an image of the gel with correctly annotated molecular weight marker or the Agilent 2100 Bioanalyzer profile, together with your samples.

RNA-Seq
Under construction