

E-PREST-11	MGX – Pré-requis échantillons	Date de création : 21/1/22 Date de modification : 02/12/2025 Version 8
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Instructions for sample preparation, QC, storage & shipment

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The prerequisites described in this document generally correspond to twice the quantity necessary to carry out the experiment.

Half of the requested material will be used to build the library.

The remaining half will be kept as a backup in case of a problem during the first construction attempt.

If you are unable to provide the platform with the quantities and qualities of material requested, the platform staff will contact you and will ask you to decide to continue to work with these samples or to provide new ones.

In the event that you decide to continue working with non-compliant samples, the steps carried out by the platform will be invoiced, even if they fail.

If your experiment is composed of more than 16 samples, please dispatch them in column in a skirted 96 wells plate such as the Eppendorf Twin Tec 96-well PCR plate skirted).

Single Cell 3'RNAseq (10X Genomics) is not concerned by the above statement : all the material (cells) will be used during the first construction attempt.

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Application	Library	Sample preparation	Minimal amount , concentration & method of quantification	QC	Comment
DNA sequencing (Illumina)	Sonication & PCR amplification	Method 1 Proteinase K, ϕ ol/ CHCl_3 extraction, EtOH/NaOAc precipitation Method 2 PureLink Genomic DNA kit (Thermo Fisher Sci.) or similar	400 ng 20 ng/ μL T ₁₀ E ₁ or H ₂ O microfluorimetry (<i>e.g.</i> Qubit).	260/230 ≥ 2 ; 260/280 ≥ 1.8 Check DNA integrity, <i>i.e.</i> agarose gel or capillary electrophoresis (<i>e.g.</i> BioAnalyzer).	Highly recommended RNase treatment, then inactivation. Store @ -20°C. Ship @ 4°C.
DNA sequencing (Illumina)	Tagmentation & PCR amplification	Method 1 Proteinase K, ϕ ol/ CHCl_3 extraction, EtOH/NaOAc precipitation Method 2 PureLink Genomic DNA kit (Thermo Fisher Sci.) or similar	Bacteria: 2 ng 0.1 ng/ μL H ₂ O microfluorimetry (<i>e.g.</i> Qubit). Large Genomes: 200 ng 10 ng/ μL H ₂ O microfluorimetry (<i>e.g.</i> Qubit).	Check DNA integrity, <i>i.e.</i> agarose gel or capillary electrophoresis (<i>e.g.</i> BioAnalyzer).	Highly recommended RNase treatment, then inactivation. Store @ -20°C. Ship @ 4°C.

Application	Library	Sample preparation	Minimal amount , concentration & method of quantification	QC	Comment
DNA sequencing (Illumina)	Sonication, PCR-free	Method 1 Proteinase K, ϕ ol/ CHCl_3 extraction, EtOH/NaOAc precipitation Method 2 PureLink Genomic DNA kit (Thermo Fisher Sci.) or similar	2 μg 40 ng/ μL T ₁₀ E1 or H ₂ O microfluorimetry (<i>e.g.</i> Qubit).	260/230 ≥ 2 ; 260/280 ≥ 1.8 Check DNA integrity, <i>i.e.</i> agarose gel or capillary electrophoresis (<i>e.g.</i> BioAnalyzer).	Highly recommended RNase treatment, then inactivation. Store @ -20°C. Ship @ 4°C.
DNA sequencing (Illumina)	Tagmentation, PCR-free	Method 1 Proteinase K, ϕ ol/ CHCl_3 extraction, EtOH/NaOAc precipitation. Method 2 PureLink Genomic DNA kit (Thermo Fisher Sci.) or similar.	50 ng 1 ng/ μL H ₂ O microfluorimetry (<i>e.g.</i> Qubit).	Check DNA integrity, <i>i.e.</i> agarose gel or capillary electrophoresis (<i>e.g.</i> BioAnalyzer).	Highly recommended RNase treatment, then inactivation. Store @ -20°C. Ship @ 4°C.
DNA sequencing (MinION)	Long reads	Use a method that is appropriate to prepare (very) long DNA fragments. Might be species specific.	5 μg 100 ng/ μL H ₂ O microfluorimetry (<i>e.g.</i> Qubit).	260/230 ≥ 2 ; 260/280 ≥ 1.8 Check DNA integrity, <i>i.e.</i> agarose gel or capillary electrophoresis (<i>e.g.</i> BioAnalyzer).	Highly recommended RNase treatment, then inactivation Store @ 4°C. Ship @ 4°C.

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Application	Library	Sample preparation	Minimal amount , concentration & method of quantification	QC	Comment
TF binding sites, histones modif.	ChIP-seq	Immunoprecipitated chromatin is heated to reverse the DNA-protein crosslinks, incubated with RNase, then proteinase K. Glycogen may be added, if necessary. ϕ ol/CHCl ₃ extraction, EtOH/NaOAc precipitation.	15 ng 0.5 ng/ μ L T ₁₀ E ₁ or H ₂ O microfluorimetry (<i>e.g.</i> Qubit).	Using purified chromatin input, check DNA integrity, <i>i.e.</i> agarose gel or capillary electrophoresis (<i>e.g.</i> BioAnalyzer).	Store @ -20°C. Ship @ 4°C.
Genotyping	RAD-seq	PureLink Genomic DNA kit (Thermo Fisher Sci.), DNeasy Blood & Tissue Kit (Qiagen) or similar product.	2 μ g 25ng/ μ L H ₂ O microfluorimetry (<i>e.g.</i> Qubit).	260/230 $\geq$ 2 ; 260/280 $\geq$ 1.8 Check DNA integrity, <i>i.e.</i> agarose gel or capillary electrophoresis (<i>e.g.</i> BioAnalyzer).	Mandatory RNase treatment, then inactivation. Store @ -20°C. Ship @ 4°C.
DNA methylation	WGBS	Method 1 Proteinase K, ϕ ol/CHCl ₃ extraction, EtOH/NaOAc precipitation. Do NOT use Trizol ! Method 2 PureLink Genomic DNA kit (Thermo Fisher Sci.) or similar.	200 ng 5 ng/ μ L H ₂ O microfluorimetry (<i>e.g.</i> Qubit).	260/230 $\geq$ 2 ; 260/280 $\geq$ 1.8 Check DNA integrity, <i>i.e.</i> agarose gel or capillary electrophoresis (<i>e.g.</i> BioAnalyzer).	Highly recommended RNase treatment, then inactivation. Store @ -20°C. Ship @ 4°C.

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Application	Library	Sample preparation	Minimal amount , concentration & method of quantification	QC	Comment
DNA methylation	RRBS	Method 1 Proteinase K, ϕol/CHCl_3 extraction, EtOH/NaOAc precipitation. Do NOT use Trizol ! Method 2 PureLink Genomic DNA kit (Thermo Fisher Sci.) or similar. Do NOT vortex samples !!	200 ng 5 ng/ μL H_2O microfluorimetry (<i>e.g.</i> Qubit).	260/230 ≥ 2 ; 260/280 ≥ 1.8 Check DNA integrity, <i>i.e.</i> agarose gel or capillary electrophoresis (<i>e.g.</i> BioAnalyzer).	Highly recommended RNase treatment, then inactivation. Store @ -20°C . Ship @ 4°C .
RNA sequencing (Illumina)	RNAseq, polyA+	Method 1 Trizol-like. After addition of CHCl_3 to the Trizol lysate and centrifugation, extract the aqueous upper phase with CHCl_3 again. After EtOH/NaOAc precipitation, wash the pellet TWICE with 70 % EtOH. Method 2 Quick RNA (Zymo) or similar.	1 μg Min. 100 ng/ μL H_2O Max. 500ng/ μl H_2O spectrophotometry	260/230 ≥ 2 ; 260/280 ≥ 2 . Check RNA integrity, <i>i.e.</i> denaturing agarose gel or capillary electrophoresis (<i>e.g.</i> BioAnalyzer).	Highly recommended DNase treatment, then inactivation. Store @ -80°C . Ship on dry ice.

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Application	Library	Sample preparation	Minimal amount , concentration & method of quantification	QC	Comment
RNA sequencing (Illumina)	RNAseq, polyA+, small amounts	Method 1 Trizol-like. After addition of CHCl₃ to the Trizol lysate and centrifugation, extract the aqueous upper phase with CHCl₃ again. After EtOH/NaOAc precipitation, wash the pellet TWICE with 70 % EtOH. Method 2 Quick RNA (Zymo) or similar.	25 ng 1 ng/μL H ₂ O capillary electrophoresis	Check RNA integrity, <i>i.e.</i> capillary electrophoresis (<i>e.g.</i> BioAnalyzer).	Mandatory DNase treatment, then inactivation. Store @ -80°C. Ship on dry ice.
RNA sequencing (Illumina)	RNAseq, rRNA- depleted	Method 1 Trizol-like. After addition of CHCl₃ to the Trizol lysate and centrifugation, extract the aqueous upper phase with CHCl₃ again. After EtOH/NaOAc precipitation, wash the pellet TWICE with 70 % EtOH. Method 2 Quick RNA (Zymo) or similar.	<i>Bacteria, NEBNext® rRNA Depletion Kit</i> → 2 μg <i>Mammals, Illumina Ribo-Zero Plus rRNA Depletion Kit</i> → 200 ng <i>For both kits,</i> 200 ng/μL H₂O spectrophotometry	260/230 ≥ 2 ; 260/280 ≥ 2. Check RNA integrity, <i>i.e.</i> denaturing agarose gel or capillary electrophoresis (<i>e.g.</i> BioAnalyzer).	Mandatory DNase treatment, then inactivation. Store @ -80°C. Ship on dry ice.

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RNA sequencing (Illumina)	BRB-seq	Method 1 Trizol-like. After addition of CHCl₃ to the Trizol lysate and centrifugation, extract the aqueous upper phase with CHCl₃ again. After EtOH/NaOAc precipitation, wash the pellet TWICE with 70 % EtOH. Method 2 Quick RNA (Zymo) or similar.	500 ng 15 µl H₂O 33 ng/µl	260/230 ≥ 1,6 ; 260/280 ≥ 2. Check RNA integrity, <i>i.e.</i> denaturing agarose gel or capillary electrophoresis (<i>e.g.</i> BioAnalyzer → RIN >6).	Highly recommended DNase treatment, then inactivation. Store @ -80°C. Ship on dry ice.
RNA sequencing (MinION)	Direct RNAseq	Method 1 Trizol-like. After addition of CHCl₃ to the Trizol lysate and centrifugation, extract the aqueous upper phase with CHCl₃ again. After EtOH/NaOAc precipitation, wash the pellet TWICE with 70 % EtOH. Method 2 Quick RNA (Zymo) or similar.	3 µg 150 ng/µL H₂O spectrophotometry	260/230 ≥ 2 ; 260/280 ≥ 2. Check RNA integrity, <i>i.e.</i> denaturing agarose gel or capillary electrophoresis (<i>e.g.</i> BioAnalyzer).	Highly recommended DNase treatment, then inactivation. Store @ -80°C. Ship on dry ice.

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Small RNA sequencing (Illumina)	small RNAseq	Method 1 Trizol-like. After addition of CHCl₃ to the Trizol lysate and centrifugation, extract the aqueous upper phase with CHCl₃ again. After EtOH/NaOAc precipitation, wash the pellet TWICE with 70 % EtOH. Method 2 Quick RNA (Zymo) or similar.	4 ng 0.3 ng/μL H₂O Max 15 μl capillary electrophoresis	260/230 ≥ 2 ; 260/280 ≥ 2. Check RNA integrity, <i>i.e.</i> denaturing agarose gel or capillary electrophoresis (<i>e.g.</i> BioAnalyzer).	Highly recommended DNase treatment, then inactivation. Store @ -80°C . Ship on dry ice.

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Single Cell 3'RNAseq (10X Genomics)	Single Cell 3'RNAseq	Any methods that produces suspensions of viable single cells	20 000 cells or nuclei 1000 cells or nuclei/ μ l Cell counter	-Cell viability >90 % -No cell aggregates -Low debris	Highly recommended Optimization of tissue dissociation protocols. • Prepare cell suspensions with > 90% viable cells. • Additional wash and straining steps may be necessary to remove excess amount of ambient RNA and debris, respectively. • Accurately determine cell viability (e.g. microscopy and LIVE/DEAD™ Cell Viability Assay from ThermoFisher Scientific as an alternative to trypan blue). • Determine cell viability after cell handling steps (e.g. flow-sort) and just prior to loading if the samples will not be processed immediately.

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Amplicon sequencing (MinION)	Amplicon full length sequencing	Purified PCR product	Min 400 fmole (eg 130ng for a 1 Kb PCR product) Max volume 20µl	260/230 ≥ 1,8 ; 260/280 ≥ 2. agarose gel or capillary electrophoresis (<i>e.g.</i> BioAnalyzer).	Ship@4°C
Ready-to- load library	Illumina compatible library	Any methods that produces Illumina compatible library.	10 nM in 20µl H ₂ O/EB capillary electrophoresis microfluorimetry (<i>e.g.</i> Qubit).	Less than 1 % adapter doublets and PCR primer Homogenous insert sizes for libraries to be mutliplexed in the same lane/flow cell	Highly recommended Construction method that includes Unique Dual Adapters (UDI)