




E-EXP-43	MGX – Pré-requis échantillons	Date : 21/1/22 Version 2
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## **Instructions for sample preparation, QC, storage & shipment**

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Application	Library	Sample preparation	Minimal amount , concentration & method of quantification	QC	Comment
DNA sequencing (Illumina)	Sonication & PCR amplification	<p><b>Method 1</b> Proteinase K, <math>\phi</math>ol/CHCl<sub>3</sub> extraction, EtOH/NaOAc precipitation</p> <p><b>Method 2</b> PureLink Genomic DNA kit (Thermo Fisher Sci.) or similar</p>	400 ng 20 ng/ $\mu$ L T <sub>10</sub> E <sub>1</sub> or H <sub>2</sub> O microfluorimetry (e.g. Qubit).	260/230 $\geq$ 2 ; 260/280 $\geq$ 1.8 Check DNA integrity, i.e. agarose gel or capillary electrophoresis (e.g. BioAnalyzer).	<b>Highly recommended</b> RNase treatment, then inactivation. Store @ -20°C. Ship @ 4°C.
DNA sequencing (Illumina)	Tagmentation & PCR amplification	<p><b>Method 1</b> Proteinase K, <math>\phi</math>ol/CHCl<sub>3</sub> extraction, EtOH/NaOAc precipitation</p> <p><b>Method 2</b> PureLink Genomic DNA kit (Thermo Fisher Sci.) or similar</p>	2 ng 0.1 ng/ $\mu$ L H <sub>2</sub> O microfluorimetry (e.g. Qubit).	Check DNA integrity, i.e. agarose gel or capillary electrophoresis (e.g. BioAnalyzer).	<b>Highly recommended</b> RNase treatment, then inactivation. Store @ -20°C. Ship @ 4°C.
DNA sequencing (Illumina)	Sonication, PCR-free	<p><b>Method 1</b> Proteinase K, <math>\phi</math>ol/CHCl<sub>3</sub> extraction, EtOH/NaOAc precipitation</p> <p><b>Method 2</b> PureLink Genomic DNA kit (Thermo Fisher Sci.) or similar</p>	2 $\mu$ g 40 ng/ $\mu$ L T <sub>10</sub> E <sub>1</sub> or H <sub>2</sub> O microfluorimetry (e.g. Qubit).	260/230 $\geq$ 2 ; 260/280 $\geq$ 1.8 Check DNA integrity, i.e. agarose gel or capillary electrophoresis (e.g. BioAnalyzer).	<b>Highly recommended</b> RNase treatment, then inactivation. Store @ -20°C. Ship @ 4°C.

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DNA sequencing (Illumina)	Tagmentation, PCR-free	<b>Method 1</b> Proteinase K, $\phi$ ol/CHCl <sub>3</sub> extraction, EtOH/NaOAc precipitation. <b>Method 2</b> PureLink Genomic DNA kit (Thermo Fisher Sci.) or similar.	50 ng 1 ng/ $\mu$ L H <sub>2</sub> O microfluorimetry ( <i>e.g.</i> Qubit).	Check DNA integrity, <i>i.e.</i> agarose gel or capillary electrophoresis ( <i>e.g.</i> BioAnalyzer).	<b>Highly recommended</b> 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 $\mu$ g 100 ng/ $\mu$ L H <sub>2</sub> 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).	<b>Highly recommended</b> RNase treatment, then inactivation Store @ 4°C. Ship @ 4°C.
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. $\phi$ ol/CHCl <sub>3</sub> extraction, EtOH/NaOAc precipitation.	20 ng 0.66 ng/ $\mu$ L T <sub>10</sub> E <sub>1</sub> or H <sub>2</sub> 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.


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Genotyping	RAD-seq	PureLink Genomic DNA kit (Thermo Fisher Sci.), DNeasy Blood & Tissue Kit (Qiagen) or similar product.	2µg 25ng/µL H <sub>2</sub> 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).	<b>Mandatory</b> RNase treatment, then inactivation. Store @ -20°C. Ship @ 4°C.
DNA methylation	WGBS	<b>Method 1</b> Proteinase K, φI/CHCl <sub>3</sub> extraction, EtOH/NaOAc precipitation. Do NOT use Trizol ! <b>Method 2</b> PureLink Genomic DNA kit (Thermo Fisher Sci.) or similar.	200 ng 5 ng/µL H <sub>2</sub> 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).	<b>Highly recommended</b> RNase treatment, then inactivation. Store @ -20°C. Ship @ 4°C.
DNA methylation	RRBS	<b>Method 1</b> Proteinase K, φI/CHCl <sub>3</sub> extraction, EtOH/NaOAc precipitation. Do NOT use Trizol ! <b>Method 2</b> PureLink Genomic DNA kit (Thermo Fisher Sci.) or similar. Do NOT vortex samples !!	200 ng 5 ng/µL H <sub>2</sub> 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).	<b>Highly recommended</b> RNase treatment, then inactivation. Store @ -20°C. Ship @ 4°C.

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RNA sequencing (Illumina)	RNAseq, polyA+	<p><b>Method 1</b> Trizol-like. After addition of CHCl<sub>3</sub> to the Trizol lysate and centrifugation, extract the aqueous upper phase with CHCl<sub>3</sub> again. After EtOH/NaOAc precipitation, wash the pellet TWICE with 70 % EtOH.</p> <p><b>Method 2</b> Quick RNA (Zymo) or similar.</p>	2 µg 200 ng/µL H <sub>2</sub> O 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).	<b>Highly recommended</b> DNase treatment, then inactivation. Store @ -80°C. Ship on dry ice.
RNA sequencing (Illumina)	RNAseq, polyA+, small amounts	<p><b>Method 1</b> Trizol-like. After addition of CHCl<sub>3</sub> to the Trizol lysate and centrifugation, extract the aqueous upper phase with CHCl<sub>3</sub> again. After EtOH/NaOAc precipitation, wash the pellet TWICE with 70 % EtOH.</p> <p><b>Method 2</b> Quick RNA (Zymo) or similar.</p>	20 ng 0.15 ng/µL H <sub>2</sub> O capillary electrophoresis	Check RNA integrity, <i>i.e.</i> capillary electrophoresis ( <i>e.g.</i> BioAnalyzer).	<b>Mandatory</b> DNase treatment, then inactivation. Store @ -80°C. Ship on dry ice.

Application	Library	Sample preparation	Minimal amount , concentration & method of quantification	QC	Comment
RNA sequencing (Illumina)	RNAseq, rRNA-depleted	<p><b>Method 1</b> Trizol-like. After addition of CHCl<sub>3</sub> to the Trizol lysate and centrifugation, extract the aqueous upper phase with CHCl<sub>3</sub> again. After EtOH/NaOAc precipitation, wash the pellet TWICE with 70 % EtOH.</p> <p><b>Method 2</b> Quick RNA (Zymo) or similar.</p>	<p><i>Bacteria, NEBNext® rRNA Depletion Kit</i> → 2 µg</p> <p><i>Mammals, Illumina Ribo-Zero Plus rRNA Depletion Kit</i> → 200 ng</p> <p><i>For both kits,</i> 200 ng/µL H<sub>2</sub>O spectrophotometry</p>	260/230 ≥ 2 ; 260/280 ≥ 2. Check RNA integrity, <i>i.e.</i> denaturing agarose gel or capillary electrophoresis ( <i>e.g.</i> BioAnalyzer).	<b>Mandatory</b> DNase treatment, then inactivation. Store @ -80°C. Ship on dry ice.
RNA sequencing (MinION)	Direct RNAseq	<p><b>Method 1</b> Trizol-like. After addition of CHCl<sub>3</sub> to the Trizol lysate and centrifugation, extract the aqueous upper phase with CHCl<sub>3</sub> again. After EtOH/NaOAc precipitation, wash the pellet TWICE with 70 % EtOH.</p> <p><b>Method 2</b> Quick RNA (Zymo) or similar.</p>	80 µg 100 ng/µL H <sub>2</sub> O 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).	<b>Highly recommended</b> DNase treatment, then inactivation. Store @ -80°C. Ship on dry ice.

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Small RNA sequencing (Illumina)	small RNAseq	<p><b>Method 1</b> Trizol-like. After addition of CHCl<sub>3</sub> to the Trizol lysate and centrifugation, extract the aqueous upper phase with CHCl<sub>3</sub> again. After EtOH/NaOAc precipitation, wash the pellet TWICE with 70 % EtOH.</p> <p><b>Method 2</b> Quick RNA (Zymo) or similar.</p>	2 ng 0.1 ng/μL H <sub>2</sub> O 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).	<b>Highly recommended</b> DNase treatment, then inactivation. Store @ -80°C . Ship on dry ice.
Ready-to-load library	Illumina compatible library	Any methods that produces Illumina compatible library.	10 nM in 20μl H <sub>2</sub> O/EB capillary electrophoresis qPCR	Less than 1 % adapter doublets and PCR primer Homogenous insert sizes for libraries to be mutliplexed in the same lane/flow cell	<b>Highly recommended</b> Construction method that includes Unique Dual Adapters (UDI)