# Instructions for sample preparation, QC, storage & shipment

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 mateiral (cells) will be used during the first construction attempt.

| Application | Library | Sample preparation | Minimal amount , concentration & method of quantification | QC | Comment |
|---|---|---|---|---|---|
| DNA sequencing (Illumina) | Sonication & PCR amplification | ***Method 1***<br>Proteinase K, φol/$CHCl_3$ extraction, EtOH/NaOAc precipitation<br>***Method 2***<br>PureLink Genomic DNA kit (Thermo Fisher Sci.) or similar | 400 ng<br>20 ng/µL $T_{10}E_1$ or $H_2O$<br>microfluorimetry (*e.g.* Qubit). | 260/230 ≥ 2 ; 260/280 ≥ 1.8<br>Check DNA integrity, *i.e.* agarose gel or capillary electrophoresis (*e.g.* BioAnalyzer). | **Highly recommended**<br>RNase treatment, then inactivation.<br>Store @ -20°C.<br>Ship @ 4°C. |
| DNA sequencing (Illumina) | Tagmentation & PCR amplification | ***Method 1***<br>Proteinase K, φol/$CHCl_3$ extraction, EtOH/NaOAc precipitation<br>***Method 2***<br>PureLink Genomic DNA kit (Thermo Fisher Sci.) or similar | ***Bacteria:***<br>2 ng<br>0.1 ng/µL $H_2O$<br>microfluorimetry (*e.g.* Qubit).<br>***Large Genomes:***<br>200 ng<br>10 ng/µL $H_2O$<br>microfluorimetry (*e.g.* Qubit). | Check DNA integrity, *i.e.* agarose gel or capillary electrophoresis (*e.g.* BioAnalyzer). | **Highly recommended**<br>RNase treatment, then inactivation.<br>Store @ -20°C.<br>Ship @ 4°C. |

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| DNA sequencing (Illumina) | Sonication, PCR-free | ***Method 1***<br>Proteinase K, φol/$CHCl_3$ extraction, EtOH/NaOAc precipitation<br>***Method 2***<br>PureLink Genomic DNA kit (Thermo Fisher Sci.) or similar | 2 µg<br>40 ng/µL $T_{10}E_1$ or $H_2O$<br>microfluorimetry (*e.g.* Qubit). | 260/230 ≥ 2 ; 260/280 ≥ 1.8<br>Check DNA integrity, *i.e.* agarose gel or capillary electrophoresis (*e.g.* BioAnalyzer). | **Highly recommended**<br>RNase treatment, then inactivation.<br>Store @ -20°C.<br>Ship @ 4°C. |
| DNA sequencing (Illumina) | Tagmentation, PCR-free | ***Method 1***<br>Proteinase K, φol/$CHCl_3$ extraction, EtOH/NaOAc precipitation.<br>***Method 2***<br>PureLink Genomic DNA kit (Thermo Fisher Sci.) or similar. | 50 ng<br>1 ng/µL $H_2O$<br>microfluorimetry (*e.g.* Qubit). | Check DNA integrity, *i.e.* agarose gel or capillary electrophoresis (*e.g.* BioAnalyzer). | **Highly recommended**<br>RNase treatment, then inactivation.<br>Store @ -20°C.<br>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<br>100 ng/µL $H_2O$<br>microfluorimetry (*e.g.* Qubit). | 260/230 ≥ 2 ; 260/280 ≥ 1.8<br>Check DNA integrity, *i.e.* agarose gel or capillary electrophoresis (*e.g.* BioAnalyzer). | **Highly recommended**<br>RNase treatment, then inactivation<br>Store @ 4°C.<br>Ship @ 4°C. |

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| 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. φol/$CHCl_3$ extraction, EtOH/NaOAc precipitation. | 15 ng<br>0.5 ng/µL $T_{10}E_1$ or $H_2O$<br>microfluorimetry (*e.g.* Qubit). | Using purified chromatin input, check DNA integrity, *i.e.* agarose gel or capillary electrophoresis (*e.g.* BioAnalyzer). | Store @ -20°C.<br>Ship @ 4°C. |
| Genotyping | RAD-seq | PureLink Genomic DNA kit (Thermo Fisher Sci.), DNeasy Blood & Tissue Kit (Qiagen) or similar product. | 2µg<br>25ng/µL $H_2O$<br>microfluorimetry (*e.g.* Qubit). | 260/230 ≥ 2 ; 260/280 ≥ 1.8<br>Check DNA integrity, *i.e.* agarose gel or capillary electrophoresis (*e.g.* BioAnalyzer). | **Mandatory**<br>RNase treatment, then inactivation.<br>Store @ -20°C.<br>Ship @ 4°C. |
| DNA methylation | WGBS | ***Method 1***<br>Proteinase K, φol/$CHCl_3$ extraction, EtOH/NaOAc precipitation. Do NOT use Trizol !<br>***Method 2***<br>PureLink Genomic DNA kit (Thermo Fisher Sci.) or similar. | 200 ng<br>5 ng/µL $H_2O$<br>microfluorimetry (*e.g.* Qubit). | 260/230 ≥ 2 ; 260/280 ≥ 1.8<br>Check DNA integrity, *i.e.* agarose gel or capillary electrophoresis (*e.g.* BioAnalyzer). | **Highly recommended**<br>RNase treatment, then inactivation.<br>Store @ -20°C.<br>Ship @ 4°C. |

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| DNA methylation | RRBS | ***Method 1***<br>Proteinase K, φol/$CHCl_3$ extraction, EtOH/NaOAc precipitation. Do NOT use Trizol !<br>***Method 2***<br>PureLink Genomic DNA kit (Thermo Fisher Sci.) or similar.<br>Do NOT vortex samples !! | 200 ng<br>5 ng/µL $H_2O$<br>microfluorimetry (*e.g.* Qubit). | 260/230 ≥ 2 ; 260/280 ≥ 1.8<br>Check DNA integrity, *i.e.* agarose gel or capillary electrophoresis (*e.g.* BioAnalyzer). | **Highly recommended**<br>RNase treatment, then inactivation.<br>Store @ -20°C.<br>Ship @ 4°C. |
| RNA sequencing (Illumina) | RNAseq, polyA+ | ***Method 1***<br>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.<br>***Method 2***<br>Quick RNA (Zymo) or similar. | 1 µg<br>100 ng/µL $H_2O$<br>spectrophotometry | 260/230 ≥ 2 ; 260/280 ≥ 2.<br>Check RNA integrity, *i.e.* denaturing agarose gel or capillary electrophoresis (*e.g.* BioAnalyzer). | **Highly recommended**<br>DNase treatment, then inactivation.<br>Store @ -80°C.<br>Ship on dry ice. |

| Application | Library | Sample preparation | Minimal amount , concentration & method of quantification | QC | Comment |
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| RNA sequencing (Illumina) | RNAseq, polyA+, small amounts | ***Method 1***<br>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.<br>***Method 2***<br>Quick RNA (Zymo) or similar. | 25 ng<br>1 ng/µL $H_2O$<br>capillary electrophoresis | Check RNA integrity, *i.e.* capillary electrophoresis (*e.g.* BioAnalyzer). | **Mandatory**<br>DNase treatment, then inactivation.<br>Store @ -80°C.<br>Ship on dry ice. |
| RNA sequencing (Illumina) | RNAseq, rRNA-depleted | ***Method 1***<br>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.<br>***Method 2***<br>Quick RNA (Zymo) or similar. | *Bacteria*, *NEBNext® rRNA Depletion Kit*<br>→ 2 µg<br><br>*Mammals*, *Illumina Ribo-Zero Plus rRNA Depletion Kit*<br>→ 200 ng<br><br>*For both kits*,<br>200 ng/µL $H_2O$<br>spectrophotometry | 260/230 ≥ 2 ; 260/280 ≥ 2.<br>Check RNA integrity, *i.e.* denaturing agarose gel or capillary electrophoresis (*e.g.* BioAnalyzer). | **Mandatory**<br>DNase treatment, then inactivation.<br>Store @ -80°C.<br>Ship on dry ice. |

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| RNA sequencing (Illumina) | BRB-seq | ***Method 1***<br>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.<br>***Method 2***<br>Quick RNA (Zymo) or similar. | 500 ng<br>15 µl $H_2O$<br>33 ng/µl | 260/230 ≥ 1,6 ;<br>260/280 ≥ 2.<br>Check RNA integrity, *i.e.* denaturing agarose gel or capillary electrophoresis (*e.g.* BioAnalyzer → RIN >6). | **Highly recommended**<br>DNase treatment, then inactivation.<br>Store @ -80°C.<br>Ship on dry ice. |
| RNA sequencing (MinION) | Direct RNAseq | ***Method 1***<br>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.<br>***Method 2***<br>Quick RNA (Zymo) or similar. | 3 µg<br>150 ng/µL $H_2O$<br>spectrophotometry | 260/230 ≥ 2 ; 260/280 ≥ 2.<br>Check RNA integrity, *i.e.* denaturing agarose gel or capillary electrophoresis (*e.g.* BioAnalyzer). | **Highly recommended**<br>DNase treatment, then inactivation.<br>Store @ -80°C.<br>Ship on dry ice. |

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| Small RNA sequencing (Illumina) | small RNAseq | ***Method 1***<br>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.<br>***Method 2***<br>Quick RNA (Zymo) or similar. | 4 ng<br>0.3 ng/µL $H_2O$<br>Max 15 µl<br>capillary electrophoresis | 260/230 ≥ 2 ; 260/280 ≥ 2.<br>Check RNA integrity, *i.e.* denaturing agarose gel or capillary electrophoresis (*e.g.* BioAnalyzer). | **Highly recommended**<br>DNase treatment, then inactivation.<br>Store @ -80°C .<br>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<br>1000 cells or nuclei/µl<br>Cell counter | -Cell viability >90 %<br>-No cell aggregates<br>-Low debris | **Highly recommended**<br>Optimization of tissue dissociation protocols.<br>• Prepare cell suspensions with > 90% viable cells.<br>• Additional wash and straining steps may be necessary<br>to remove excess amount of ambient RNA and debris,<br>respectively.<br>• Accurately determine cell viability (e.g. microscopy and<br>LIVE/DEAD™ Cell Viability Assay from ThermoFisher<br>Scientific as an alternative to trypan blue).<br>• Determine cell viability after cell handling steps (e.g.<br>flow-sort) and just prior to loading if the samples will<br>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)<br>Max volume 20µl | 260/230 ≥ 1,8 ;<br>260/280 ≥ 2.<br>agarose gel or capillary electrophoresis (*e.g.* BioAnalyzer). | Ship@4°C |
| Ready-to-load library | Illumina compatible library | Any methods that produces Illumina compatible library. | 10 nM in 20µl $H_2O$/EB<br>capillary electrophoresis microfluorimetry (*e.g.* Qubit). | Less than 1 % adapter doublets and PCR primer<br>Homogenous insert sizes for libraries to be mutliplexed in the same lane/flow cell | **Highly recommended**<br>Construction method that includes Unique Dual Adapters (UDI) |