Illumina Short-Read Sequencing

Application	Amount	Volume (µL)	Conc. (ng/µL)	Quality	Size (bp)	Comment
DNA Sequencing	**General remarks** and **requirements** for DNA samples: - Buffer: TRIS-HCL 10 mM or lowTE - 260/280 ratio 1.8-2.0 (or according to quality column) - In case of genome sequencing: high molecular for best results **All samples of an order must be adjusted to a uniform concentration within the specifications.** **Samples that do not meet the requirements listed here cannot be processed and will be rejected!**					
Whole Genome - Low input (incl. PCR)	150 – 300ng	>=15	>10	see general remarks above	-	FFPE possible with constraints in output quality
Whole Genome - PCR Free	>300 ng	>=15	>20	see general remarks above	-	
Bacterial genome sequencing	15 ng	>=15	1	see general remarks above		
Whole Exome Sequencing	>75 ng	>=15	5	see general remarks above	-	FFPE possible if amount based on qPCR measurement is sufficient
Gene Panel	600 ng	>=15	40	260/280 ratio 1.7-2.2 260/230 ratio 1.2	-	-
Amplicons	>75 ng	>=15	5	260/280 ratio >1.5	-	less material possible on request
Microbiome 16S	150 ng	>=15	10	260/280 ratio >1.5	-	less material possible on request
Microbiome Shotgun Metagenomics	75 ng	>=15	5	260/280 ratio >1.5	-	less material possible on request

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RNA Sequencing	**General remarks** and **requirements** for totalRNA samples: - Buffer: nuclease free water - DNAse treated and cleaned up - 260/280 ratio >2.0 - RQN>=8; RQN Δ between samples <1 - ultra low input (< 1 ng total amount) possible, but must be planned beforehand **All samples of an order must be adjusted to a uniform concentration within the specifications.** **Samples that do not meet the requirements listed here cannot be processed and will be rejected!**					
Whole Transcriptome – standard input	>375 ng	>=15	25 - 80	see general remarks above	-	-
Whole Transcriptome - low input	>15 ng	>=15	1 - 5	see general remarks above	-	very low input amount may lead to constraints in output quality
Expression Profiling (mRNA) – standard input	>375 ng	>=15	25 - 80	see general remarks above	-	-
Expression Profiling (mRNA) – low input	>15 ng	>=15	1 - 5	see general remarks above	-	very low input amount may lead to constraints in output quality
3´Prime RNA Seq -standard input	>375 ng	>=15	25 - 80	see general remarks above		-
3´Prime RNA Seq -low input	>15 ng	>=15	1 - 5	see general remarks above		very low input amount may lead to constraints in output quality
Small RNA Profiling (miRNA, lnc-RNA etc.)	50 ng	>=15	50	see general remarks above	-	Low input possible on request

Application	Amount	Volume (µL)	Conc. (ng/µL)	Quality	Size (bp)	Comment
Sequencing of prepared NGS libraries	50 ng	>=15	5	No primer dimer residuals	-	Buffer: TRIS-HCL 10 mM

Single-Cell RNA-Sequencing

10X Genomics Chromium	1.000 – 20.000 cells /sample	50 µl	500-1.000 cells/µl	Vitality >80 %	-	-
BD Rhaspody	1.000 – 30.000 cells /sample	620 µl	50-500 cells/µl	Vitality >80 %		

Spatial Transcriptomics

10X Genomics Visium	Tissue sections placed on Visium slides, RNA extracted from tissue section RIN >7

Bionano Optical Mapping

Bionano Saphyr Chip	1 µg	-	>36	260/280 = 1.8 260/230 = 2.0-2.2	Megabase range	-

Pacific Biosciences Long-Read Sequencing

Revio System

Application	Amount	Volume (µL)	Conc. (ng/µL)	Quality	Size (bp)	Comment
Transcriptome						
Iso-Seq	>2-3 µg	>15	>200	RIN 8-10 260/280 = 2.0 260/230 = 2.2	-	DNase digested, buffer: RNase-free water
Whole Genome Sequencing						
HiFi Reads – Standard Protocol	≥8 µg	>120	~70	260/280 = 1.8 260/230 = 2.0-2.2	>50kb	homogenous HMW DNA, RNase digested, buffer: EB, DNA size distribution: 80% ≥ 50Kb
HiFi Reads – Low Input	>3 µg	>50	60	260/280 = 1.8 260/230 = 2.0-2.2	>50kb	homogenous HMW DNA, RNase digested, buffer: EB, DNA size distribution: 80% ≥ 50Kb
HiFi Reads – Ultra-Low Input	>50 ng	>15	>3	260/280 = 1.8 260/230 = 2.0-2.2	>50kb	homogenous HMW DNA, RNase digested, buffer: EB DNA size distribution: 80% ≥ 50Kb
Trageted Sequencing						
Amplicon Sequencing	2-4 µg (depends on size)	50	-	-	-	clean, target-specific, buffer: EB
Metagenomics						
Multiplexed Microbial	>2-3 µg per sample	20-100	-	260/280 = 1.8 260/230 = 2.0-2.2	>30kb	Homogenous HMW DNA, RNase digested, buffer: EB DNA size distribution: 80% ≥ 30Kb

Application	Amount	Volume (µL)	Conc. (ng/µL)	Quality	Size (bp)	Comment
Kinnex workflows						
Full-lenght RNA	2-3 µg (depends on # samples to multiplex)	50	> 200	RIN 8-10 260/280 = 2.0 260/230 = 2.2	-	DNase digested, buffer: RNase-free water Up to 12-plex barcoded cDNA
16S rRNA Amplicons	>500 ng - 3 µg (depends on # samples to multiplex)	50	-	-	~ 1500 bp	Purified amplicons Applicants are required to order Kinnex 16S Forward and Reverse primers. These can be sourced from any oligo vendor. For optimal results, HPLC purification is recommended. Up to 384-plex samples
Single-cell RNA	50 ng of 10x Chromium 3` or 5` single cell DNA.	25	-		500-1500 bp	Applicants must provide purified Single-cell cDNA generated using the 10x Chromium Next GEM Single Cell 3' kit v3.1/ 5' kit v2 standard throughput. cDNA in the respective buffer.

Sequel II/IIe Systems

Application	Amount	Volume (µL)	Conc. (ng/µL)	Quality	Size (bp)	Comment
Transcriptome (Iso-Seq)	>2 µg	>15	>130	RIN 8-10 260/280 = 2.0 260/230 = 2.2	-	DNase digested, buffer: RNase free water
Whole Genome Sequencing						
HiFi Reads – Standard Protocol	>6 µg	>120	50	260/280 = 1.8 260/230 = 2.0-2.2	>50kb	homogenous HMW DNA, RNase digested, buffer: EB DNA size distribution: 80% ≥ 50Kb
HiFi Reads – Low Input	>1 µg	>50	20	260/280 = 1.8 260/230 = 2.0-2.2	>50kb	homogenous HMW DNA, RNase digested, buffer: EB DNA size distribution: 80% ≥ 50Kb
HiFi Reads – Ultra-Low Input	>30 ng	>15	2	-	>50kb	homogenous HMW DNA, RNase digested, buffer: EB DNA size distribution: 80% ≥ 50Kb
Continous Long Reads	>6 µg	>120	50	260/280 = 1.8 260/230 = 2.0-2.2	>50kb	homogenous HMW DNA, RNase digested, buffer: EB
Amplicon Sequencing	500 ng – 3 µg (depends on size)	50	-	-	-	clean, target-specific, buffer: EB
Multiplexed Microbial	>1 µg per sample	20-100	-	260/280 = 1.8 260/230 = 2.0-2.2	>30kb	-

Oxford Nanopore Long-Read Sequencing

Application	Amount	Volume (µL)	Conc. (ng/µL)	Quality	Size (bp)	Comment
Transcriptome						
Direct mRNA Sequencing	>500 ng polyA+ or 1.5 µg Total-RNA	>12 >12	>40 >125	260/280 = 2.0 260/230 = 2.0-2.2	-	DNase digested, buffer: RNase free water
cDNA Sequencing	>200 ng polyA+ or >1 µg Total-RNA	>10 >10	>20 >100	260/280 = 2.0 260/230 = 2.0-2.2	-	200 ng cDNA can be used as input DNase digested, buffer: RNase free water
cDNA PCR Sequencing	>20 ng polyA+ or >600 ng Total-RNA	>15 >15	>1.3 >40	260/280 = 2.0 260/230 = 2.0-2.2	-	DNase digested, buffer: RNase free water
Whole Genome Sequencing						
Ligation Sequencing	>2 µg	>50	>40	260/280 = 1.8 260/230 = 2.0-2.2	>30 kb	homogenous HMW DNA, RNase digested, buffer: EB
Ligation Sequencing with Size-Selection	>10 µg	>50	>200	260/280 = 1.8 260/230 = 2.0-2.2	>30 kb	homogenous HMW DNA, RNase digested, buffer: EB
Ligation Sequencing Whole-Genome Amplification	>1 ng	>10	>0.1	260/280 = 1.8 260/230 = 2.0-2.2	>30 kb	homogenous HMW DNA, RNase digested, buffer: EB
Native Barcoding	>1 µg per sample	>50	>20	260/280 = 1.8 260/230 = 2.0-2.2	>30 kb	homogenous HMW DNA, RNase digested, buffer: EB
Ultra Long Reads	>50 µg	>750	>70	260/280 = 1.8 260/230 = 2.0-2.2	>100 kb	homogenous UHMW DNA, RNase digested, buffer: EEB (please inquire about EEB buffer)
Rapid - gDNA	>300 ng	>10	>30	260/280 = 1.8 260/230 = 2.0-2.2	>30 kb	homogenous HMW DNA, RNase digested, buffer: EB

Application	Amount	Volume (µL)	Conc. (ng/µL)	Quality	Size (bp)	Comment
Oxford Nanopore Long-Read Sequencing						
Amplicon Sequencing						
Amplicons by Ligation	>300 ng per sample	>20	>15	260/280 = 1.8 260/230 = 2.0-2.2	-	clean, target-specific, buffer: EB
Rapid - 16S	>20 ng	>20	>1	260/280 = 1.8 260/230 = 2.0-2.2	-	clean, target-specific, buffer: EB
Sanger Sequencing						
Full Service						
Plasmid	>500 ng	-	100 – 250	260/280 = 1.8 260/230 = 2.0-2.2	<5 kb	Buffer: 10 mM Tris/HCl or water Primer concentration 10µM
	>800 ng	-	150 - 600	260/280 = 1.8 260/230 = 2.0-2.2	5 kb – 15 kb	Buffer: 10 mM Tris/HCl or water Primer concentration 10µM
	>1,5 µg	-	>600	260/280 = 1.8 260/230 = 2.0-2.2	>15 kb	Buffer: 10 mM Tris/HCl or water Primer concentration 10µM
Full Service / Xpress Service						
PCR-product	max. 10 ng	-	<2,5	260/280 = 1.8 260/230 = 2.0-2.2	<100 bp	purified PCR products Primer concentration 10µM
	50 ng	-	< 5ng	260/280 = 1.8 260/230 = 2.0-2.2	100 bp – 1 kb	purified PCR products Primer concentration 10µM
	400 ng	-	20 - 50	260/280 = 1.8 260/230 = 2.0-2.2	>1 kb	purified PCR products Primer concentration 10µM

Sanger Sequencing

Application	Amount	Volume (µL)	Conc. (ng/µL)	Quality	Size (bp)	Comment
Xpert Service	-	-	-	-	-	Sample requirements as for Full Service
Pre-mixed Service						
Plasmid	300 – 600 ng	-	-	-	<10 kb	5 pmol Primer/reaction 7,5 µL total volume
	>700 ng	-	-	-	>10 kb	5 pmol Primer/reaction 7,5 µL total volume
PCR-products	<100 ng	-	-	-	100 bp – 1 kb	5 pmol Primer/reaction 7,5 µL total volume
	>100 ng	-	-	-	>1 kb	5 pmol Primer/reaction 7,5 µL total volume
Ready-to-load Service						
Plasmid, PCR-products	-	20	-	-	-	Purified sequencing reaction

Fragment Analysis

Ready-to-run	-	20	-	-	<600 bp	Fully prepared samples dissolved in formamide
Pre-pared	-	10	-	-	<600 bp	samples dissolved in formamide without length standard
STR analysis	50 ng	-	5	260/280 = 1.8 260/230 = 2.0-2.2	-	Buffer: 10 mM Tris/HCl, low TE or water