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

Illumina Short-Read Sequencing

Application	Amount	Volume (µL)	Conc. (ng/µL)	Quality	Size (bp)	Comment
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 concatenate)	50	> 200	RIN 8-10 260/280 = 2.0 260/230 = 2.2	-	DNase digested, buffer: RNase-free water up to 8-fold concatenation
	16S rRNA Amplicons	>500 ng - 3 µg (depends on size)	50	-	-	~ 1500 bp	Purified amplicons up to 12-fold concatenation (~ 19 Kb) Applicant must order Kinnex 16S Forward and reverse primers. From any oligo vendor, HPCL purification is recommended.
	Single-cell RNA	50 ng of 10x Chromium 3` or 5` single cell DNA.	25	-		500-1500 bp	Purified up to 16-fold concatenation 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

Oxford Nanopore Long-Read Sequencing

Application	Amount	Volume (µL)	Conc. (ng/µL)	Quality	Size (bp)	Comment
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

Application	Amount	Volume (µL)	Conc. (ng/µL)	Quality	Size (bp)	Comment
	400 ng	-	20 - 50	260/280 = 1.8 260/230 = 2.0-2.2	>1 kb	purified PCR products Primer concentration 10µM

Sanger Sequencing

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

Application	Amount	Volume (µL)	Conc. (ng/µL)	Quality	Size (bp)	Comment
STR analysis	50 ng	-	5	260/280 = 1.8 260/230 = 2.0-2.2	-	Buffer: 10 mM Tris/HCl, low TE or water