Illumina Short-Read Sequencing

Application	Amount	Volume (µL)	Conc. (ng/µL)	Quality	Size (bp)	Comment
Genome Sequencing						
Whole Genome - Low input (incl. PCR)	50 – 1000 ng	10 – 50	>2	high molecular for best results 260/280 ratio 1.8-2.0	-	FFPE possible with constraints in output quality
Whole Genome - PCR Free	1.25 – 2.5 µg	10 – 50	>25	high molecular for best results 260/280 ratio 1.8-2.0	-	standard is 350 bp insert size, for 550 bp insert size >2000 ng
Whole Exome Sequencing	> 50 ng	10	5	260/280 ratio 1.8-2.0	-	FFPE possible if amount based on qPCR measurement is sufficient
Gene Panel	600 ng	10	20	260/280 ratio 1.7-2.2 260/230 ratio 1.2	-	-
Amplicons	> 50 ng	10	5	260/280 ratio >1.5	-	less material possible on request
Microbiome						
Microbiome 16S	100 ng	10	10	260/280 ratio >1.5	-	less material possible on request
Microbiome Shotgun Metagenomics	50 ng	10	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
Transcriptome						
Whole Transcriptome – standard input	500 ng	10	50	260/280 ratio >2.0 RQN>=8; Δ<1 DNAse treated	-	-
Whole Transcriptome - low input	1 ng	10	<1	260/280 ratio >2.0 RQN>=8; Δ<1 DNAse treated	-	constraints in output quality possible if input amount is very low
Expression Profiling (mRNA) – standard input	500 ng	10	50	260/280 ratio >2.0 RQN>=8; Δ<1 DNAse treated	-	-
Expression Profiling (mRNA) – low input	1 ng	10	<1	260/280 ratio >2.0 RQN>=8; Δ<1 DNAse treated	-	constraints in output quality possible if input amount is very low
Small RNA Profiling (miRNA, lnc-RNA etc.)	50 ng	10	50	260/280 ratio >2.0 RQN>=8; Δ<1 DNAse treated	-	-
Sequencing of prepared NGS libraries	50 ng	10	5	No primer dimer residuals	-	Buffer: TRIS-HCL 10 mM

DNA-Microarray

Application	Amount	Volume (µL)	Conc. (ng/µL)	Quality	Size (bp)	Comment
Transcriptome Profiling – standard input	500 ng	10	50	260/280 ratio 1.8 – 2.0 260/230 ratio >2.0 DNAse treated RQN>=8; Δ<1	-	-
Transcriptome Profiling – low input	>0.5 ng	10	>0.05	260/280 ratio 1.8 – 2.0 260/230 ratio >2.0 DNAse treated RQN>=8; Δ<1	-	constraints in output quality possible if input amount is very low

10X Single-Cell RNA-Sequencing

Single-Cell Transcriptomics	1.000 – 30.000 cells /sample	50 µl	500-1.000 cells/µl	Vitality > 70 %	-	-
Spatial Transcriptomics	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

Application	Amount	Volume (µL)	Conc. (ng/µL)	Quality	Size (bp)	Comment
Transcriptome *(Iso-Seq)*	2 µg	20	100	RIN 8-10 260/280 = 2.0 260/230 = 2.2	-	DNase digested
Whole genome sequencing						
HiFi reads – standard protocol	15 µg	-	-	260/280 = 1.8 260/230 = 2.0-2.2	>50kb	HMW DNA, RNase digested
HiFi reads – Low Input	1 µg	-	-	260/280 = 1.8 260/230 = 2.0-2.2	>50kb	HMW DNA, RNase digested
HiFi reads – Ultra-Low Input	20 ng	-	-	-	>50kb	HMW DNA, RNase digested
CLR reads > 30 kb	8 µg	-	-	260/280 = 1.8 260/230 = 2.0-2.2	>50kb	HMW DNA
CLR reads > 15 kb	5 µg	-	-	260/280 = 1.8 260/230 = 2.0-2.2	>50kb	HMW DNA
CLR reads ~ 10 kb	1.5 µg	-	-	260/280 = 1.8 260/230 = 2.0-2.2	>20kb	-
Amplicon sequencing	500 ng – 3 µg (depends on size)	50	-	-	-	Clean, target-specific
Multiplexed Microbial	1 µg per sample	20-100	-	260/280 = 1.8 260/230 = 2.0-2.2	>20kb	-

Oxford Nanopore Long-Read Sequencing

Application	Amount	Volume (µL)	Conc. (ng/µL)	Quality	Size (bp)	Comment
Transcriptome						
Direct mRNA Sequencing	>500 ng polyA+	> 10	> 60	260/280 = 2.0 260/230 = 2.0-2.2	-	-
cDNA Sequencing	>100 ng polyA+	> 10	> 15	260/280 = 2.0 260/230 = 2.0-2.2	-	200 ng of already prepped cDNA can also be used as input
cDNA PCR Sequencing	>1 ng polyA+ or > 50 ng Total-RNA	> 10	> 1	260/280 = 2.0 260/230 = 2.0-2.2	-	-
Whole Genome Sequencing						
Ligation Sequencing	> 2 µg	10-50	> 40	260/280 = 1.8 260/230 = 2.0-2.2	> 30 kb	-
Native Barcoding	> 1 µg per sample	10-50	> 40	260/280 = 1.8 260/230 = 2.0-2.2	> 30 kb	-
Ultra Long Reads	20 µg	20	1000	260/280 = 1.8 260/230 = 2.0-2.2	-	-
Genome Rapid	> 400 ng	10	> 55	260/280 = 1.8 260/230 = 2.0-2.2	-	-
PCR Sequencing	> 100 ng	> 10 - 50	> 2	260/280 = 1.8 260/230 = 2.0-2.2	-	-

Oxford Nanopore Long-Read Sequencing

Application	Amount	Volume (µL)	Conc. (ng/µL)	Quality	Size (bp)	Comment
Amplicon Sequencing						
Amplicons by Ligation	> 1 µg per sample	10-50	> 40	260/280 = 1.8 260/230 = 2.0-2.2	-	-
16S	> 10 ng	> 10	> 1	260/280 = 1.8 260/230 = 2.0-2.2	-	-