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## Illumina NextSeq 2000 Sample Submission Form and Offer Acceptance

Fill in the order form completely! Don't forget your signature (otherwise no processing)! It is mandatory to completely fill in and sign this submission form. A printed and signed copy must be sent together with the samples. The project will not commence until the filled acceptance sheet has been returned.

Customer Information				
Name*:				
Company/Institution*:				
Institute/Department:				
Address*:				
VAT-ID*:				
Phone:				
Email*:				
StarSEQ Quotation No*:				
Purchase Order No*:				

Invoicing Information (if different from above)				
Name*:				
Company/Institution*:				
Institute/Department:				
Address*:				
VAT-ID*:				
Phone:				
Email*:				

## \*Required

For EU countries: Please provide the VAT-ID No. of your company. If this certificate is not present, the German tax of 19% will be charged. For non-EU countries: Send us an official confirmation of your entrepreneurial activity please.

Sample Type					
Genomic DNA		ChIP-Seq			
Total RNA		Genomic DNA (Exome Enrichment)			
mRNA		Small RNA			
cDNA		Plasmid DNA			
16s rDNA		Amplicon			
Amplicon (FFPE)		Ready to load library			
Tissue/Soil		Stool/Swab/Blood/Saliva			

Sample information				
Concentration:	Number of samples:			
Concentration Measured With:	Purification Method:			
Eluted In:	Gel Image/Bioanalyzer Data: Please attach or send by E-Mail			

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Requested Application					
Library Prep 🗆 gDNA/RNA Prep 🗆	Sequencing 🗌				
Library Prep Type:	Sequencing Type: NextSeq 2000 🗆				
gDNA/Amplicon	Single End Read				
mRNA (stranded)	Paired End Read 🔲				
small RNA	50 nt 🔲 100 nt 🗌 150 nt 🔲 300 nt				
Mate Pair 🗆	5 Mio Reads 🛛 10 Mio Reads 🗆				
ChIP-Seq/FFPE	25 Mio Reads 🗆 50 Mio Reads 🗆				
Exome Enrichment 🛛	75 Mio Reads 🗌 100 Mio Reads 🗌 other:				
Metagenome 🗆	400 Mio Reads (P2 Full Flow Cell, Single Read)				
DNA Nextera XT/NEBNext Ultra™ II FS □	800 Mio Reads (P2 Full Flow, Paired End Read)				
Total RNA (rRNA depletion necessary)	1000 Mio Reads (P3 Full Flow, Single Read)				
Cancer Panel	2000 Mio Reads (P3 Full Flow, Paired End Read) 🗆				
Size selection	Custom set-up needed 🛛				

Sample Information							
	Sample	Species	Concentration	Size (ready to	Volume	Index (ready	Total
	Name		(ng/µl)	load/ ChIP-	(μl)	to load	DNA/RNA
				Seq/FFPE/		libraries)	(µg)
				amplicons)			
Sample 1							
Sample 2							
Sample 3							
Sample 4							
Sample 5							
Sample 6							
Sample 7							
Sample 8							
Sample 9							
Sample 10							
Sample 11							
Sample 12							
Sample 13							
Sample 14							
Sample 15							
Sample 16							
Sample 17							
Sample 18							
Sample 19							
Sample 20							

For more samples please send a spread sheet by email. Sample names may only contain letters, numbers, underscores and minus signs.

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Additional Comments/Information Or Customization Of Library/Sequencing Required:

Sample provided	Application/ Library Prep Method	Minimum required amount	Recommended amount	Minimum Concentration	Minimum Volume
Genomic DNA	<i>de novol</i> resequencing	<i>10</i> ng*	250 ng	1 ng/µl	20 µl
Plasmids, cDNA, amplicons	<i>de novol</i> resequencing	<i>10</i> ng*	100 ng	1 ng/µl	20 µl
Genomic DNA	Exome Enrichment	200 ng*	500 ng	25 ng/µl	10 µl
Genomic DNA	Mate Pair	4 µg	5 µg	200 ng/µl	20 µl
ChIPseq	ChIPseq	<i>10</i> ng	100 ng	1 ng/µl	10 µl
Total RNA	RNA-seq/ stranded RNA-seq	20 ng	250 ng	2 ng/µl	10 µl
Total RNA (single cell / low input, RIN>7)	RNA-seq/ stranded RNA-seq	2 pg	1 ng	0,25 pg	16 µl
Total RNA + depletion RIN>7	RNA-seq/ stranded RNA-seq	input 100 ng	input 500 ng	2 ng/µl	10 µl
mRNA RIN>7	RNA-seq/ stranded RNA-seq	minimum amount of total RNA before mRNA isolation = 100 ng, delivered Volume must be 5 µl	maximum amount of total RNA before mRNA isolation = 4 μg, delivered Volume must be 5 μl	delivered Volume must be min. 5 μ	5 µl
rRNA depleted total RNA	RNA-seq/ stranded RNA-seq	minimum amount of total RNA before depletion = 100 μl delivered Volume must be min.5 μ	maximum amount of total RNA before depletion = 1 ng, delivered Volume must be min 5 μ	delivered Volume must be min. 5 μl	5 µl
Total RNA RIN>7	small RNA	100 ng	1000 ng	20 ng/µl	20 µl
Prepared small RNA	small RNA	10 ng	100 ng	0,2-15 ng/µl	5 µl
Ready to load libraries		10 nM (10 µl)	10 nM (10 µl)		10 µl

\* dependent on desired size range of library

All samples should be provided in nuclease free water or buffer (10 mM Tris-HCl, pH 8.5). **No EDTA!!!** 

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All samples should be free of contaminants that could inhibit enzymatic reactions. Precipitation with salt and EtOH is not suitable. Please use clean kit based preparation of samples. We recommend to use fluorometric-based methods for quantification (Qubit or PicoGreen) and 260/230 + 260/280 ratios to identify possible contaminants.

RNA samples should have an RNA Integrity Number (RIN) value greater or equal to 7. For low input total RNA a RIN value >8 is recommended. RNA sample shipment strictly has to occur on dry ice and should undergo DNase I treatment prior to submission (mandatory for rRNA Depletion).

For ready to load libraries or RNA samples, delivery of Bioanalyzer data or data from comparable technique is mandatory. As alternative StarSEQ can offer this service (see below).

Custom sequencing primer (read 1, index 1, index 2, read 2) must be delivered as 100  $\mu$ M dilution.

Genomic DNA (hmw) and RNA samples should not be degraded and are required to have the following OD ratios:

260/280 = 1,8 - 2,0

260/230 = 1,7 - 2,2

The use of too low or degraded starting material (RNA/DNA) can result in low yield or failure of the library preparation.

For custom or ready-to-load libraries do not use any indices beginning with two G bases (e.g. do not use Illumina index no. D504, N718, N705, RPI24, R724, R711, NR011, ND011). Ready-to-load libraries must be free of adapter dimers.

Custom libraries must be larger than 250 bp and less than 1000 bp. The fragment range of the ready-to-load libraries is not allowed to exceed 500 bp. StarSEQ will give no guarantee for sequencing of custom/customer prepared libraries. Failed runs or low output from poor custom libraries will be charged.

If the amount, concentration and/or quality of the starting material does not meet the requirements (see above) for the library preparation, StarSEQ will contact you to discuss how to proceed. If possible, StarSEQ will recommend additional pre-processing steps in order to optimize the sample quality. Size selection will be mandatory if sample quality or sample contamination affect enzymatic tagmentation or fragmentation resulting in large fragments. If additional services are requested, the following prices per sample apply:

- 1. RNA / DNA concentrating,  $\in 60$
- 2. RNA / DNA purification, € 80
- 3. RNase treatment, € 50
- 4. DNase treatment, € 50

5. Quality check of (replacement) samples on Bioanalyzer or Qiaxcel, € 30 (1-4 samples), € 25 (5-6 samples), € 20 (>7 samples)

- 6. Quantification of (replacement) samples on Qubit, € 20
- 7. Size selection, € 75
- 8. Run preparation with custom primers (read1, index 1, index 2, read 2) € 80

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The customer provided material is not infectious and not hazardous. The Data amount estimation relies on specifications of Illumina and may vary depending on the nature of starting material. For guality control and verification, StarSEQ will spike in up to 3% of PhiX to all runs. StarSEQ will give no guarantee for sequencing of custom/customer prepared libraries. Failed runs from poor custom libraries will be charged. For ready to load libraries, the customer will provide Bioanalyzer data or similar and sample names with index sequences as excel sheet. Indexing strategy must be discussed prior to project. StarSEQ will give no guarantee for successful library preparation from rRNA depleted material, all libraries will be charged if library preparation fail. StarSEQ will give no guarantee for successful library preparation from samples that did not fit our requirements. All libraries will be charged if library preparation fail due to missed sample requirements. For low diversity samples like amplicons it is necessary to co-load up to 30% PhiX in combination with low cluster density to provide the necessary diversity in the composition of the bases. In such cases the estimated data output is reduced in proportion to the content of PhiX and cluster density. Placing an order is possible only via our sample submission form.

All conditions defined in the sample submission form apply to this quotation. The sample submission form has to be filled in completely. Failed library preparation or sequencing attempts due to lack of information will be charged.

Turnaround time will be determined when StarSEQ has received the samples. Depending on the nature of starting material and according to Illumina specifications the data output can vary 5-10%. For difficult samples like amplicons or low quality samples, it is possible that the specifications cannot be reached.

Please note, that your samples and data will be stored under the appropriate conditions for 3 months after analysis. Hereafter the samples and data will be discarded if no other arrangement is made regarding shipment or storage of the samples. Please create one or more data backups immediately after receiving the data.

For Research Use Only – not for any clinical, diagnostic or therapeutic use in humans or animals.

With below signature I confirm above conditions and sample requirements (page 1-5) and order legally binding the quotation noted in this document. I also confirm that the provided material is not infectious and not hazardous.

## Placing of order and offer acceptance:

Name (capitals):

Signature/Stamp/Date