Dear researcher,

We offer a variety of preparative workflows and Next Generation Sequencing (NGS) technologies. Below we give an overview about available technologies and applications as well as practical information about sample submission.

Who can make use of our platforms?

Every group from the **University or University Clinics Cologne**, all groups from the Cluster of Excellence **CECAD** will be supported on our standard applications (see list below in bold). If other protocols provided by us or substantial changes to our protocols or new implementations are required, we decide individually whether and how we can help, please contact us! All innovative contributions both in lab work and bioinformatics should be assessed as a scientific collaboration.

All projects that are funded by the **NGS-calls of the DFG** will be supported in all matters confirmed on the West German Genome Center counseling reports.

All other national and international research groups are welcome to discuss their projects with us to set off a scientific collaboration if sequencing capacity allows. Please note that external prices will be charged. We will not accept requests in commercial or diagnostic settings.

What are the NGS protocols/applications we provide?

- **bold**: standards with guaranteed support and data quality and amount

Application	Protocol	Comments / Special Features
DNA		
PCR-free Genome	Illumina TruSeq, modified	
low-input Genome	Illumina Nextera DNA flex	e.g. single worm
Amplicon DeepSeq	Illumina TruSeq	e.g. Metagenomics/HLA
small Genome / long-range PCR products	Illumina Nextera / TruSeq or Oxford Nanopore	
Exome, gene panel	Agilent SureSelect: XT, QXT, HS,	different species, custom and standard, UMI for HS protocol, FFPE experience with modified protocol
ATAC	Illumina Nextera, modified	
HiC	inhouse (Illumina Mate-Pair or Agilent SureSelect HS)	HiC, easyHiC, HiChiP
RADseq	published protocol	
WGS long read	Oxford Nanopore	

Application	Protocol	Comments / Special Features
RNA		
mRNAseq	Illumina TruSeq stranded, NEB	
total RNAseq	Illumina TruSeq ribo zero (gold)	different depletion kits, dual transcriptomics
3´mRNAseq	Lexogen	
low-input RNAseq	Nugen (Nextera)	
3´scRNAseq	10x Genomics	
scRNAseq on Nuclei	10x Genomics	
full-length scRNAseq	Wafergen Takara	
Spatial Transcriptomics	10x Genomics, Visium	
circRNASeq		native cycles
circSeq	published protocol	artificial cycles for RNA editing analysis
transient RNASeq	4US, SLAMseq, TTseq	
Direct RNA or cDNA	Oxford Nanopore	for isoform detection
Epigenomics		
ChiP-seq	Illumina TruSeq, modified	
TADA (targeted DAMID)	published protocol	
miRNA	Illumina TruSeq or Lexogen	
MethylSeq	NEB	beta-test on cfDNA
WGBS	Illumina	
targeted BS	e.g. Illumina or Agilent	
RIP-seq, PARclip, eCLIP	published protocols	

How to submit samples?

Please ask us for the latest version of our sample sheets. First, always choose the application. All fields need to be filled, if it is not clear to you, what has to be chosen, please contact us. **"Sample names" should not have more than 10 characters and be exactly the same written on the tubes.** Please send us ahead this sheet electronically. After confirmation, samples can be submitted.

High quality data and requested data amount is guaranteed for standard applications where samples fulfill the following requirements:

Sample requirements for RNAseq and miRNAseq experiments

- min. 2µg Total RNA, concentration range 50–200ng/µL, solved in nuclease-free water
- constant volume per tube
- DNA free, no degradation, RIN > 7
- OD260/280 = 1.8-2.1 and OD260/230 > 1.5
- use clearly labeled 1.5mL Safe-Lock Tubes, sample names on the side and No. corresponding to the sheet on the cap.

Sample requirements for Genome Sequencing, PCR-free

- min. 3µg high molecular DNA, concentration range 50–100ng/µL, solved in 10 mM Tris-Cl, pH 8.5 (e.g. Qiagen Buffer EB)
- constant volume per tube
- no degradation, attach gel picture (50ng DNA on a 0.7% agarose gel)
- use clearly labeled 1.5mL Safe-Lock Tubes. sample names on the side and No. corresponding to the sheet on the cap.

Sample requirements for Amplicon sequencing

- min. 1µg column purified PCR product, concentration range 10–100ng/µL, solved in 10 mM Tris-Cl, pH 8.5 (e.g. Qiagen Buffer EB)
- constant volume per tube
- attach gel picture (50ng DNA on a 0.7% agarose gel)
- use clearly labeled 1.5mL Safe-Lock Tubes, sample names on the side and No. corresponding to the sheet on the cap.
- make sure that the read length chosen fits to the size of your amplicons

Sample requirements for ChIPseq

- at least 10ng ds ChIP DNA , solved in 10 mM Tris-Cl, pH 8.5 (e.g. Qiagen Buffer EB)
- constant volume of max. 50µL
- dsDNA fragments should be in a range of 300–500bp
- use clearly labeled 1,5mL Safe-Lock Tubes, sample names on the side and No. corresponding to the sheet on the cap.
- Please note that we will not do any QC steps with this kind of material to make full use of what you give us.

Sample requirements for WES (whole exome sequencing) or GPS (gene panel sequencing)

- min 2µg genomic DNA, concentration range 50–100ng/µL, solved in 10 mM Tris-Cl, pH 8.5 (e.g. Qiagen Buffer EB)
- constant volume per tube
- no degradation, attach gel picture (0.7% agarose)
- use clearly labeled 1.5mL Safe-Lock Tubes, sample names on the side and No. corresponding to the sheet on the cap.

Sample requirements for Single Cell RNA seq

- concentration range 700–1200 cells/µL
- viability > 75%
- medium: 1x PBS containing 0.04% BSA
- diameter < 40µm
- single cell suspension
- free of debris and cell aggregates
- scheduling of these experiments is critical – please contact us at least 4 weeks ahead of submitting samples for scRNAseq

In any case, please send us the sheet prior sending the samples and wait for us to reply. If you can't meet the criteria, we will probably have another protocol to support your project.

While still following strict measures taken in **regard of Covid-19**, we will re-open our NGS services to support your research on May 04, 2020.

Please note that all sample and sheet - handover will be done in a contact-free way in the entrance, ground floor at the CCG, where we provide boxes to place the samples. The entrance can be entered by simply pushing the green button in front of the building.

Please keep to the time frame 9am - 1pm and send us the sample sheet via Email (Ccg-rna@uni-koeln.de or Ccg-dna@uni-koeln.de) at least the day before, so we can clarify any open points.

We thank you for your understanding.

Contact

Most researchers, especially if they do one of our applications for the first time, will need some introduction, calculation of costs and project specific discussion. Don´t hesitate to contact us!

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