

Customer guidelines for Next-Generation Sequencing



General Information

The following document will guide you through the process of how to submit your project. Please go through the provided information carefully and do not hesitate to contact our staff if you have further questions. The contact details are located in the following document for either the Institute for Human Genetics or the Institute of Microbiology. All the best for your analysis!

Hazardous Materials

Before sending any material to the NCCT, it has to be checked for potentially posing a risk to laboratory personnel and if the material falls under a laboratory safety-level. Such materials have to be announced to the NCCT's associated project manager and need to be approved before shipment. Hazardous and safety-level materials have to be clearly indicated by labelling the corresponding samples. The omission of indication of hazardous materials with consequential health endangering effects is punishable by law. Please pay particular attention to the guidelines for hazardous materials of the NCCT Human genetics and NCCT Microbiology. You will submit your samples to either one and be provided with the according guidelines.

Genetically modified organisms

German Law requires the documentation of the generation, transport and usage of genetically modified organisms (GMO) which present a risk of proliferation. To comply with the governmental regulation, the NCCT needs to document all GMOs received, stored and manipulated in our laboratory which present a risk of proliferation. Fixed GMOs, which do not present a risk of proliferation, do not require detailed documentation. The documentation of GMO presenting a risk of proliferation is attached to this document as [ANNEX 1: documentation of GMOs](#).

Patent and ethical concerns

We do not take any responsibility concerning patent or ethical approval and assume that the user is authorised to request our services.

Format of transferred data

The sequencing facilities transfer by default FastQ files, the purpose of these files is to keep the information concerning sequencing quality as complete as possible. Access to instrument raw data (BCL files in the case of Illumina sequencing, FAST5 for Nanopore sequencing...) is generally not possible and requires special consulting.

For projects requiring access to raw data, experimental design and sequencing strategy need to be considered prior to the start of the project. Consulting will be performed to estimate costs related to the additional data transfer. We recommend using a single flow cell to ensure the feasibility of raw data transfer.

Batching and prioritising

All samples that are compared to one another should be run together through the different stages of processing. Please inform the associated project manager and indicate in the metadata-sheet which samples should be processed within one batch. If you submit many samples organised in different batches, you can indicate the priority of processing by the order in which your samples appear in the metadata-sheet. Without concrete and specific instructions, the NCCT is not responsible for any potential batch effect.

Nucleic Acid Isolation

We recommend sending extracted nucleic acids, especially if you have successfully established extraction methods for your materials. The success of extraction depends on compatibility of the extraction protocol and the starting material. We use a variety of commercial extraction kits and cannot guarantee best results for all kinds of different starting materials. All DNA and RNA samples (either sent by you or after isolation at our facility) will go through a standardised quality control protocol. This comprises a precise quantitation using a fluorescence method (such as Qubit), an assessment of the RNA/DNA quality (DIN/RIN on an electrophoresis capillary such as Bioanalyzer2100 or FragmentAnalyzer), and eventually an assessment of possible contaminants via photometry (Nanodrop). Preserving reagents, like RNAlater, should only be used if unavoidable and cannot replace appropriate handling. These reagents might interfere with our sample handling and have to be indicated in the metadata sheet.

Metadata sheet

All samples have to be documented in a metadata sheet, which has to be sent with the samples as a printout and also sent in digital form. Depending on the application, different metadata sheets can be acquired via www.ncct.life or upon request at ncct@med.uni-tuebingen.de. It should also be indicated, if samples have been treated with DNase or RNase if they are supplemented with preserving reagents, such as RNAlater.

Sample labelling, choice of container and transport conditions

All tubes and plates have to be labelled clearly with a sample ID, date and the customer's name. Keep the labelling as simple as possible and only use letters, numbers, dashes and underscores. For batches smaller than 20 samples, we recommend the use of nuclease-free, Safe-Lock, DNA-free, PCR-clean, LoBind 1.5 ml tubes. We do not handle tubes smaller than 1.5 ml as storage and labelling is problematic. For batches larger than 20 samples, use 96- or 384-well LoBind-plates. We require at least 15 µl volume of the samples of which at least 5 µl are needed for quality control measurements.

Please dilute high concentrated samples to volumes above 15 µl with the buffer used for sample elution. Tubes and plates and water (for elution) should not be autoclaved.

Double sealing of the samples has to be performed to avoid potential leak. This is commonly done using a zip bag, seal bag or 2 tubes.

RNA and DNA for short-read sequencing should be shipped on dry ice. DNA for long-read sequencing can be sent at 4°C. DNA for long-read sequencing, samples which have already been frozen and samples meant for epigenetic analysis, should be shipped on dry ice.

Couriers and shipment conditions

The samples can be brought to the laboratory directly. In this case, propose a time slot to the laboratory using the ticket system to make sure a coworker will be available to make the sample entry.

Rigid outer packaging is needed for transport; the packaging needs to be labelled with proper shipping name, if possible printed from an electronic document. Shipper (or consignee identification) is also needed. Avoid reusing commercial packages as they might be confusing for the laboratory team, indeed there is a risk that samples packaged in a carton box from goods which are normally stored at room temperature (such as cartons containing gloves) might not be unpacked immediately.

We recommend selecting a courier with a warranty of delivery time such as TNT, UPS, GLH, Fedex, or similar services. In case of unique samples, sensitive to temperature, a custom courier such as world courier is recommended as they allow personalised tracking solution, but are more expensive. Shipment costs need to be covered by the user, using the invoicing courier ID of the user.

Samples sent on dry ice have to fulfil specific requirements. The recommended quantity of dry ice depends on the size of the package and of the ambient temperature. Couriers like UPS recommend 5 kg per day and other couriers like world courier have a refilling service for dry ice.

For international shipment

When declaring the samples to the tax office, consider if the samples have a commercial value. If there is no clear IP, most of the biological material do not have a commercial value (good of 0.1 \$). If the samples have a commercial value, 19% VAT applies to all samples coming in Germany. Usually biological samples have the import reference: 3001 2010. Please validate that it applies to your samples (sender responsibility).

ANNEX 1: documentation of GMOs

Formblatt für Core Facilities zur Aufzeichnung von GVO der Risikogruppe 1, die von einem anderen Betreiber kommen (nicht-fixierte GVO)

Risikobewertung des gentechnisch veränderten Organismus

(Für jeden GVO ist ein separates Formblatt auszufüllen)

Kundenname: _____

Auftrags Nr.: _____

Kontakt E-Mail: _____

Bitte senden Sie uns (*Core Facility*) das vollständig ausgefüllte Formular **vor der Zusendung** Ihrer Proben zu, damit wir die Sicherheitsstufe Ihres GVO vorab überprüfen können. Wir können **nur mit vollständig ausgefülltem und unterzeichnetem Formular** den Service ausführen.

1) Spenderorganismus (Spender ist der Organismus, aus dem das zu übertragende Nukleinsäurefragment ursprünglich stammt, ggf. sind mehrere Spenderorganismen anzugeben):

Bezeichnung (z. B. *Mensch, Maus, Qualle*):

Risikogruppe des Spenderorganismus: RG 1 ☐ RG 2 ☐ RG 3 ☐ RG 4 ☐

Einstufung erfolgte gemäß: ZKBS-Liste¹ ☐ TRBA-Listen² ☐ eigene Einstufung ☐

2) Informationsgehalt der klonierten Nukleinsäure (Kurzbeschreibung der Funktion des klonierten Nukleinsäureabschnittes bzw. Gen/Genfragmentes):

Bezeichnung (z.B. *humanes Insulin-Gen, Green Fluorescent Protein (GFP), handelt es sich um ein Onkogen?*)

3) Empfängerorganismus:

Bezeichnung (z. B. *E. coli DH10B*):

Risikogruppe des Empfängerorganismus: RG 1 ☐ RG 2 ☐ RG 3 ☐ RG 4 ☐

Einstufung erfolgte gemäß: ZKBS-Liste¹ ☐ TRBA-Listen² ☐ eigene Einstufung ☐

4) Vektor (auch bei Standardvektoren genaue Bezeichnung angeben; falls kein Standardvektor lt. [Vektor-Liste der ZKBS](#), bitte VEKTORKARTE - soweit verfügbar - anfügen, siehe:

Standard-Vektor (nach ZKBS): ja ☐ nein ☐

Bezeichnung (z. B. *pcDNA3*):

5) Gentechnisch veränderter Organismus

Bezeichnung des GVO (z. B. *lentiviral transduzierte Zelllinie xy*):

Risikogruppe des GVO: RG 1 ☐ RG 2 ☐

Begründung für die Einstufung (zwingend erforderlich):

(z. B. *die Zelllinie ist nachweislich frei von den zur Transduktion verwendeten lentiviralen Viruspartikeln und wird deshalb in die Risikogruppe 1 eingestuft, Gene ohne Gefährdungspotenzial*).

Name und Unterschrift Auftraggeber/-in:

(Datum / Name in Blockschrift / Unterschrift)

Name und Unterschrift Projektleiter/-in:

(Datum / Name in Blockschrift / Unterschrift)

Nach Abschluss der Arbeiten:

☐ Gentechnisch veränderte Organismen wurden in das externe Labor zurückgebracht.

Datum

Name

Unterschrift Auftraggeber/-in

☐ Gentechnisch veränderte Organismen wurden vernichtet.

Datum

Name

Unterschrift Projektleiter/-in

¹ZKBS: Datenbank zu sicherheitsbewerteten Organismen

<https://zag.bvl.bund.de/organismen/index.jsf?dswid=8714&dsrid=969>

²Technische Regeln für Biologische Arbeitsstoffe (z. B. TRBA 460 Pilze, TRBA 462 Viren, TRBA 464 Parasiten, TRBA 466 Bakterien, TRBA 468 Zelllinien)

<https://www.baua.de/DE/Angebote/Rechtstexte-und-Technische-Regeln/Regelwerk/TRBA/TRBA.html>

Zusammensetzung der gentechnisch veränderten Organismen (GVO):

Anlage zur gentechnischen Arbeit (Az. bzw. Projekt-Nr.):

[illegible]

¹ z.B. Transkriptionsfaktor, Onkogen, Toxin, Gen kodiert für das Protein ...

² Sind die oben genannten Spender- und Empfängerorganismen oder Vektoren in den einschlägigen Listen (Organismen- oder Vektorliste, z.Zt. unter <http://194.95.226.234/GENTEC/ZKBS/ZKBS.HTM>) nicht genannt, so ist aussagekräftige Literatur beizufügen.

