Customer guidelines for Next-Generation Sequencing

NCCT
NGS Competence Center Tübingen

General Information

Please carefully review the following document that outlines the process for project submission. It provides comprehensive instructions to guide you through the submission procedure. If you have any additional questions or require further assistance, please don't hesitate to reach out to our staff. All the best for your analysis!

Hazardous Materials

Prior sending any material to the NCCT, it is important to ensure that they do not pose any risks to laboratory personnel and comply with the appropriate laboratory safety level. Laboratory safety level has to be announced to the NCCT's associated project manager and need to be approved before shipment. In order to maintain safety standards, it is essential to clearly communicate any hazardous materials at the sample level by using the metadatasheet provided on our website (www.ncct.life). Please note that failure to indicate hazardous materials, which may result in health risks, is considered a violation of the law.

Genetically modified organisms

In accordance with German law, it is mandatory to document the generation, transportation, and utilization of genetically modified organisms (GMOs) that pose a risk of proliferation. To ensure compliance with governmental regulations, the NCCT is required to maintain thorough documentation for all GMOs received, stored, and manipulated within our laboratory if they present a risk of proliferation. Fixed GMOs, which do not present a risk of proliferation, do not require detailed documentation. The documentation of GMOs presenting a risk of proliferation is provided as an attachment to this document ANNEX 1: documentation of GMOs .

Patent and ethical concerns

We would like to clarify that our organization does not bear any responsibility regarding patent or ethical approvals for the requested services. We assume that the user who is seeking our services is authorized to do so and holds the necessary approvals and authorizations from relevant entities. It is the user's responsibility to ensure compliance with all applicable patent and ethical requirements.

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. We recommend elution in nuclease-free water, do not send samples in DEPC-treated water (for safety and toxicity reason).

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.

General sample quantity and quality

Please do not send less than 15 µl, even if you have high concentrations. In this case, you can dilute the samples accordingly. We require 5µl of each sample only for quality control (QC) measurements.

If you treat your samples with RNAse, please declare this visibly on the sample sheet. As an RNA-lab, we have to take special precautions with RNAse treated samples.

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).

Regarding packaging

Ensure that all tubes are clearly labeled with a sample ID, date, and the name of the sender. To prevent potential leaks, it is necessary to double-seal the samples using methods such as zip bags, seal bags, or two tubes. We highly recommend using nuclease-free, DNA-free, PCR-clean SafeLock 2 ml tubes (avoid autoclaving). **The use of 2 ml tubes is crucial for tissue and cell extraction as lysis is performed using metal beads**, which cannot be done in conical tubes. For large sample batches,

consider using 96-well low-bind plates. If required, samples should be shipped on dry ice, and the package must be appropriately labeled. It is important to note that preserving reagents like RNAlater should only be used if absolutely necessary and cannot replace proper handling. For RNA/DNA isolation from blood, please send the original blood collection tubes, either PAXgene RNA or EDTA tubes. The responsibility for the samples lies with you until they have been delivered to c.ATG.

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-Liste[1] ☐ TRBA-Listen[2] ☐ 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-Liste[1] ☐ TRBA-Listen[2] ☐ 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).

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_____
_____
_____
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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

[1]ZKBS: Datenbank zu sicherheitsbewerteten Organismen
https://zag.bvl.bund.de/organismen/index.jsf?dswid=8714&dsrid=969

[2]Technische 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.): .....

Spenderorganismus (Organismus, aus dem die verwendete Nukleinsäure ursprünglich stammt)		**Insert** (übertragener Nukleinsäureabschnitt)		**Vektor**	**Empfängerorganismus**		**Gentechnisch veränderter Organismus (GVO)**		**Weitere Infos**
Name	RG	Abk.	Vollständiger Name, Funktion[1], Gefährdungspotenzial		Name	RG	Name	RG	Anhang Nr.[2]

[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.