Customer guidelines for Next-Generation Sequencing

NCCT
NGS Competence Center Tübingen

General Information v.2

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 no presenting a risk of proliferation, do not require a 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.

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 standardized 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 https://github.com/qbicsoftware/metadata-doc 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 and choice of container

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 should not be autoclaved.

Couriers and shipment conditions

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.

We recommend selecting a courier with a warranty of delivery within 72 hours 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 personalized tracking solution, but are more expensive.

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).

RNA and DNA for short-read sequencing should be shipped on dry ice. DNA for long-read sequencing can be sent at 4°C or if the sample has already been frozen, or is meant for epigenetic analysis, it should be shipped on dry ice.

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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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.