VBCF-ProTech
Facility Usage Policy

This policy for the Vienna Biocenter Core Facilities GmbH (VBCF) Protein Technologies Facility (VBCF-ProTech) constitutes a part of the collaboration agreement in addition to the "General Cooperation Conditions" (GCC). The latest version of this document is available from the facility's website http://www.vbcf.ac.at/facilities/protein-technologies. Upon publication, the latest version automatically comes into effect.

General Policy

To start any new project, please contact the head of the VBCF-PROTECH or the person responsible for the service area of interest to discuss the possibilities available and instructions on how to order service. Users are encouraged to have some initial information ready, such as previous attempts, papers that show results similar to those the user would like to obtain, and - last but not least - a clear idea of what one would like to see and what question one would like to answer.

Note that for any type of service **approval of the order by the group leader is required in electronic form before** service can begin.

VBCF-PROTECH provides full service (cloning, protein expression, purification, characterization …; "service projects"), and also allows direct usage of some instrumentation by trained users ("instrument usage", see below). VBCF-PROTECH staff, the user, and the user's supervisor choose one of these approaches or a combination of both in agreement with each other, depending on the nature of your experiment (technical complexity, number of samples etc.), the availability of facility staff, and financial considerations.

Use of PROTECH services and infrastructure is restricted to material that is classified as **safety level S1** (no infectious or hazardous material).

Any utilization of the PROTECH Facility (service projects and usage of instrumentation) requires authorization by the person responsible for the respective cost center (usually the group leader).

For training and service projects, users on the Vienna BioCenter (academic institutions and companies) have priority. Other than that, users turning in their requests first will be served first. Exceptions may be made for urgent experiments (e.g. manuscript resubmissions).

Service fees and prices are specified in the price lists for each type of service. The VBCF-PROTECH reserves the right to update prices at any time other than after a specific service project has already been started.

Molecular Cloning Services

We offer a cloning service into vectors for single protein expression in either *E. coli*, BEVS, or HEK293 cells, and into vectors for multiple protein expression in BEVS. In addition we offer cloning into a variety of vectors for tagging with fluorescent proteins for expression in mammalian cell lines, etc. For a list of available vectors please contact the facility. Please note that we cannot provide users with vectors unless the proper Materials Transfer Agreement or License is in place. In some cases, a fee for sharing vectors is charged in order to cover costs.

Template material for the molecular cloning service must be in the form of a purified plasmid with the target sequence **confirmed by sequencing** (target DNA sequence and sequencing files in ab1/abi format **must** be sent along with the order form).

We do not take any responsibility for mistakes arising from corrupted material handed over by the user (e.g. mutated template). In such cases the full price will be charged for any service completed using corrupted material.

When cloning is finished, the user will be notified and, if ordered, protein production service will start. The DNA material will be stored for 5 years and be available for further processing (such as cloning into a compatible vector with a different tag or for a different expression system). Any shipping costs resulting from sending samples to off-campus users must be covered by the user.

Recombinant Protein Production Services

We offer recombinant protein production services in *E. coli*, insect cells using the baculovirus system (BEVS), S2 cells, and HEK293 cells.

We cannot guarantee that a particular target gene will be successfully expressed using any of these systems. Users must be aware of the risk of negative results. We also take no responsibility for errors resulting from incorrect design/preparation of expression plasmids/viruses provided by users.

Recombinant protein production services start either with cloning of the gene carried out by VBCF-PROTECH, or with a user-provided plasmid.

- For *E. coli* expression, plasmids must be IPTG or arabinose-inducible (pET or pBAD vector series, or any vector with a T7-lac promoter).
- For BEVS, transfer plasmids must be compatible with transposition in *E. coli* (pFastBac or Gateway vectors).
- For HEK293 cells, plasmids must contain a CMV or other strong mammalian promoter (pcDNA, etc).

Any material provided to the VBCF-PROTECH has to be sequence-verified and of good quality. We do not take any responsibility for negative results arising from low quality or errors in the DNA templates or plasmids given to our facility. In such cases the full price will be charged for any service completed using corrupted material.

Once any ordered expression is finished, we kindly ask the user to pick up the cell material as soon as possible.

We will store the V0 and other viral stocks from BEVS for 1 year after their creation unless arranged otherwise. Users will be contacted before any material is discarded. Academic users are also free to pick up their viruses for their own use. Any shipping costs resulting from sending samples to off-campus users must be covered by the user.

According to our licensing agreement with Geneva Biotech, industrial users can only be provided with insect cell pellets (no bacmids or viruses may be distributed unless the user also has a license with Geneva Biotech).

Protein Purification Services

Protein purification services are only available to users ordering protein production services, and are performed either as a full service or in combination with user training.

Protein purification projects must be discussed with the head of the VBCF-PROTECH in order to evaluate the potential for the success of the purification (i.e., what type of protein is it, does a protocol already exist, etc).

Protein purification can be a lengthy and uncertain process, and many proteins will aggregate or precipitate in purified form. In addition, many published purification protocols are not reliable and such protocols can be difficult or impossible to reproduce. Additional optimization may be necessary even when starting from a published protocol. Users must be aware of this before ordering purification services. We will attempt to identify conditions resulting in properly folded and soluble protein before proceeding to large-scale purification in order to save time, costs, and material. For certain proteins, purification within the scope of our facility may not be possible.

If you require a very specialized buffer or additive for your purification, you must provide the VBCF-PROTECH with an appropriate stock solution.

Before large-scale purification can be carried out, a successful small-scale or test-scale purification combined with analysis via analytical size exclusion chromatography must have been performed, to verify that the protein is soluble and monodisperse, and can be successfully purified.

Protein Characterization Services

We offer protein characterization via a variety of techniques (see the ProTech facility webpage for an up-to-date list). Additional techniques are available through our partner facilities, the Biomolecular Interactions and Crystallization facility at CEITEC, Brno, Czech Republic and the Biomolecular and Cellular Analysis at BOKU (links can be found on the ProTech facility webpage). Please contact the head of the VBCF-PROTECH for more information regarding these techniques.

For DLS, MST, ITC, and CD trained users can access the instrumentation themselves (please see “infrastructure usage”).

Any material provided to the VBCF-PROTECH must be of good quality. If a protein sample is generated by the user, we ask that you evaluate the monodispersity/homogeneity of your sample (via size exclusion chromatography and/or light scattering) and have accurately determined the concentration before requesting characterization services. We do not take any responsibility for negative results arising from low quality or errors present in the material given to our facility. For assistance with evaluation of the quality of your sample, please contact us. Additional characterization of user-provided samples necessary to perform the requested service may result in additional costs.

Users will be informed when experiments carried out for them by the VBCF-PROTECH are completed and whether any material is remaining. Users are kindly asked to pick up any remaining material promptly. Any shipping costs resulting from returning samples to off-campus users must be covered by the user.

Primary data generated from protein characterization services will be stored for 5 years and then deleted. Users are responsible for long-term storage of primary data generated for them by the VBCF-PROTECH.

CRISPR/Cas9 Services

We offer consulting, DNA, RNA, and protein reagent generation, electroporation, generation of *A. thaliana* CRISPR-modified lines, and can undertake more complex collaboration projects in the area of genome engineering.

Template material for the reagent generation services must be in the form of a purified plasmid with the target sequence **confirmed by sequencing** (target DNA sequence and sequencing files in ab1/abi format **must** be sent along with the order form). We take no responsibility for errors due to incorrect design/preparation of gRNA templates or other materials provided by the user.

We take no responsibility for mistakes arising from any corrupted material handed over by the user (e.g. mutated template), including *A. thaliana* seeds or other plant material. In such cases the full price will be charged for any service completed using corrupted material.

In case of HDR templates construction the user is responsible with providing proper and sequence-verfied DNA templates. The user must confirm and agree on the proposed design of the HDR template. We take no responsiblity for downstream experimental problems, such as lack of activity or silencing of potential promoters, that might arise from introduction of mutations and insertions necessary for HDR template design. All such mutations/deviations from the template sequence will be communicated to the user.

Due to the inherent uncertainty in the *in silico* design of gRNA sequences, we cannot guarantee that gRNA's designed by the facility will be active. Users must be aware of the risk of negative results. Users are strongly encouraged to design experiments that incorporate testing of more than one gRNA sequence for each target. If the gRNA design is required from VBCF-PROTECH the user is responsible to provide the exact target DNA sequence of the cell line or organism of choice in case it might be different from the available genomic sequences.

Users will be informed when experiments carried out for them by the VBCF-PROTECH are completed and whether any material is remaining or whether material must be picked up. Users are kindly asked to pick up any material promptly.

DNA material will be stored for 5 years and be available for further processing (such as re-transcription, re-transformation, or protein production). Primary data generated from

CRISPR/Cas9 services will be stored for 5 years and then deleted. Users are responsible for long-term storage of primary data generated for them by the VBCF-PROTECH.

For Cas9 and Cas9 fusions, mutants and versions production, the general rules listed under the „Recombinant Protein Production Services“ section are applied. The user is responsible for the final design of novel Cas9 fusions.

Electroporation

The Neon electroporation system used by VBCF-PROTECH can be accessed by users only under supervision or after being trained by VBCF-PROTECH staff. Users are charged a fee to cover costs of electroporation tips and staff working time. Users are responsible for proper handling of their cell cultures. We take no responsibility for loss or contamination of cell cultures due to user error during electroporation or when the cell cultures are not handled according to our standard protocols. In case of electroporation failures that do not arise from VBCF-PROTECH staff errors we are not responsible for any material loss. See also the Instrument Usage section for further guidelines on instrument usage.

Instrument Usage

Training

User access to VBCF-PROTECH instrumentation is allowed for specific instruments, including the Chirascan Plus CD spectrometer, the Monolith NT.115 and NT.LabelFree instruments, the Aekta Purifiers, the Neon electroporation system, the PEAQ-ITC, and the DLS plate reader. Work in the PROTECH Facility is allowed exclusively for trained personnel.

Access to VBCF-PROTECH instrumentation is allowed only after user training by VBCF-PROTECH staff. **Users are absolutely not allowed to train other users!!** Doing so will result in loss of access to instrumentation by both users. To schedule training please contact the facility.

If a user has not used instrumentation for a period of more than one year, he/she should see the VBCF-PROTECH staff for an update on usage and – if required – retraining.

During training, users will also be made familiar with safety regulations. If training is not successful or if the user does not comply with the rules of the facility, in particular safety rules, VBCF-PROTECH staff can ban individual users temporarily or permanently from the utilization of instrumentation or the facility.

Biosafety and Lab Safety

General rules for biosafety and lab safety are available in a separate document provided by the VBCF safety officer. Use of VBCF-PROTECH services and infrastructure is restricted to material that is classified as **safety level S1** (no infectious or hazardous material).

Instrument Booking

Most instrumentation is available to trained users during regular working hours (Mon-Fri, 8-18). For usage outside of those hours, please consult the VBCF-PROTECH staff.

Instruments must be booked in advance using the online booking system (https://ideaelanweb.vbcf.ac.at/vbcf/). Users must log usage in the provided electronic or paper logbooks and record any VBCF-PROTECH consumables (affinity columns, CD cuvettes, MST capillaries) in the online booking system.

Cancellations must be made at least 24 hours in advance. Users will be charged for the amount of time the instrument is booked, not the actual time they use the instrument.

VBCF-PROTECH reserves the right to cancel bookings when instrument maintenance or repair is necessary.

No instrument may be booked for more than 3 days in a row. Exceptions can be made for short term external visits or urgent experiments (e.g., for revision of a manuscript). No restrictions for booking instruments apply to VBCF-PROTECH staff for carrying out projects or for maintenance.

If instruments become unavailable, VBCF-PROTECH staff will inform all affected users at the earliest possibility. VBCF-PROTECH staff reserves the right to cancel bookings on short notice if instrument maintenance needs to be performed. Preventive maintenance will be scheduled to minimize impact on usage.

In addition to the booking system, there is a logbook for each VBCF-PROTECH instrument. Users are obliged to enter date and time, their name and affiliation before starting to work and sign out once they are done. If anything noteworthy happens, e.g. the instrument is not performing as well as usual, a comment in the log should be left. Users who repeatedly do not log their usage will be banned from using the instrument.

It is self-evident that all instrumentation should be used with utmost care, that resources should be used wisely, that users check back with facility staff immediately if anything is unclear or if any problems are experienced with the instrument, and that the instructions laid down in the lab protocols and given by VBCF-PROTECH staff are followed accurately.

User Samples and Reagents

All samples and reagents that are brought to the VBCF-PROTECH by users must be clearly labeled with a designation of the compound and the name of the user. For reasons of safety and cleanliness, unlabeled samples will be discarded by VBCF-PROTECH staff without advance warning.

During periods of active work, VBCF-PROTECH users are welcome to store their own samples and reagents in designated areas of the facility. These areas have to be emptied, however, when the user finishes work. VBCF-PROTECH staff will discard samples and reagents should users not comply with requests to clean up.

Borrowing Items

Users are expected to bring their own pipettes, buffers, and special reagents. Tips, tubes, and other common laboratory consumables will be supplied. For specialized applications items may be purchased from or checked out of the facility (MST capillaries, CD cuvettes, purification columns).

If borrowed items are not returned, are broken or damaged, or are not properly cleaned the user will be charged for the cost of cleaning or replacement. Users who borrow VBCF-PROTECH CD cuvettes will be automatically charged a cleaning fee.

General lab instruments and reagents belonging to the VBCF-PROTECH are not for general use and should not be used by external users!

Data Storage

Raw and processed data generated on VBCF-PROTECH instruments will be stored for 5 years and then deleted. It is the responsibility of users to transfer their own data after their experiments are finished.

Communication

Publications

If users present data obtained with support of the VBCF-PROTECH (publications or poster presentations), you **must** acknowledge the PROTECH and the "Vienna Biocenter Core Facilities". The VBCF receives public funding, and citing the facility in the acknowledgements is the only way we can track the impact of our services and justify our funding. We would appreciate receiving a copy of all publications for which VBCF-PROTECH infrastructure was used.

If users need help with preparing a manuscript using data generated via VBCF-PROTECH services (e.g. presentation of characterization data or description of the methodology), please contact VBCF-PROTECH staff.

Good scientific practice demands that members of the Facility who have contributed intellectually to a publication will be considered as co-authors.

Mailing lists

To request service or training, please email peggy.stolt@vbcf.ac.at or protech@vbcf.ac.at

If you would like to join the VBCF-PROTECH mailing list, please contact Peggy Stolt-Bergner: peggy.stolt@vbcf.ac.at

Feedback and Evaluation

Users and group leaders are encouraged to give feedback about the performance of the facility or suggestions for improvement at any time to the head of VBCF-PROTECH. A VBCF user survey is conducted every 2 years and all users are encouraged to participate.