IMBA Stem Cell Core Facility Service Terms & Conditions

We use well-established protocols which we adapt to our service pipelines. However, a lot of projects can be challenging due to technical and/or biological reasons we cannot control, and that may cause the service to be paused or stopped entirely. Our goal is to make this process transparent and help you prepare before requesting one of our services. Here you will find information on the ISCCF conditions, as well as considerations for your project planning. We kindly ask users to read and agree to the ISCCF Service Terms & Conditions before requesting our services.

General

1. The IMBA Stem Cell Core Facility provides service for a fee. Please review costs before requesting a service.

a. Internal users (IMBA): Pay for consumables only.
b. Academics within Austria: Pay for consumables, IMBA services and rent/utilities.
c. External Academics: Pay for consumables, IMBA services, rent/utilities, and personnel cost rate.

3. To ensure transparency, the facility will be providing updates for each step of the procedure and will ask the users to confirm that we can proceed to the next step.

4. When users choose to provide us with reagents for genome targeting (cell lines, gRNAs, donors, primers, enzymes or other) they are fully responsible for the design, preparation, quality and quantity of those reagents.

5. When a service project cannot be completed for technical and/or biological reasons a 50% charge applies. Repetitions will be treated as new request.

6. Customers should acknowledge the Stem Cell Core Facility for generation of lines/reagents that are included in publications and presentations.

Human IPS Reprogramming

1. ISCCF offer consulting and non-integrating reprogramming technologies to generate induced pluripotent stem cells (iPSC) using the Sendai Virus System.

2. IMBA must comply with existing ethics laws. Therefore, all patient material for iPS derivation must have received ethics approval prior to starting the service project.

3. We perform reprogramming routinely with high rates of success; however, the starting material or other factors may cause sometimes reprogramming to fail.

ISCCF, IMBA-Institute of Molecular Biotechnology
Dr. Bohr-Gasse 3
1030 Vienna
Austria
Version 2.0 from 22.07.2020

4. The facility hands over to the user 3 independent feeder-free reprogrammed clones tested automatically for: Pluripotency, SNP genotyping, STR profiling and mycoplasma. A 3-lineage differentiation assay can be performed upon request.

Genome Editing in Human and Mouse Stem Cells

1. ISCCF offers consulting on project strategy and generation of genome-edited lines.

2. The facility cannot predict genome targeting success of a specific gene locus and therefore cannot guarantee delivery of clonal edited lines.

3. The user provides ISCCF with reagents for genome targeting (cell lines, gRNAs, donors, primers, enzymes or other) and is fully responsible for the design, preparation, quality and quantity of those reagents.

4. The facility provides clonal cell lines validated via Sanger sequencing on the DNA level only for the requested modification (e.g. indels, point mutation, reporter etc). Once results confirm the modification, the facility is not responsible for the gene behavior beyond the DNA level, e.g. mRNA, protein or other biological contexts.

5. The facility cannot guarantee that after targeting, the line will be suitable for downstream applications (e.g. differentiation, blastocyst injection, screening, other). Please consider the following when planning to request a genome editing service. Targeting success depends on many factors such as:

a. Cell line type
b. Genomic locus to target
c. Type of modification
d. Delivery method of targeting reagents
e. Targeting regents (Cas9 enzymes, gRNAs, donors)

Cell line

1. Cell line type plays key role in determining overall genome editing success.
Between species: Generally, mouse ES cells are easier to manipulate with CRISPR/Cas than human ES and IPS cells. Within species: There is big variability even within species in terms of suitability for genome targeting; some lines are robust and others totally unsuitable for manipulation. Cell line origin and background therefore may affect the genome editing process itself as well as time and cost of the procedure.

2. If the facility is requested to target a previously uncharacterized human or mouse stem cell line, it will run diagnostic tests to determine suitability of the line for targeting. Diagnostic tests include growth & expansion, transfection efficiency with control plasmids, clonality and editing efficiency on a pool level; specifically, for human stem cells genomic stability (SNP) and pluripotency; for mouse stem cells, cell culture optimization, i.e. usage of specific FCS batches. These extra steps will increase the time and cost of the targeting procedure. The user may choose to perform some or all the tests in their lab, if they wish to save on cost and time.

ISCCF, IMBA-Institute of Molecular Biotechnology
Dr. Bohr-Gasse 3
1030 Vienna
Austria
Version 2.0 from 22.07.2020

Genomic locus

1. Efficiency targeting of a specific locus depends on local chromatin environment, DNA sequence, biological function/essentiality of the gene.

2. Investigate as much as possible about the specific biology of the gene and how its manipulation may affect cell fitness after targeting. When essential genes are targeted for example, growth defects may affect severely overall cell fitness and genomic stability. In most cases, full knock-out of essential genes is not feasible and it will result in WT/KO clones.

3. The target gene region should be sequenced prior to gRNA or donor design in the cell line of choice. Presence of SNPs or indels might inhibit or block completely gRNA activity and cause mistakes during donor design.

Type of modification desired

1. Knock-Outs: 'knock-out' generation relies on introducing a frame-shift within an exon of protein coding genes resulting in a truncated and most likely degraded protein. In some cases, indels form as deletions or insertions of triplicate nucleotides and therefore will not result in a frameshift therefore protein level or function remain intact. This means that a representative population of cells will never have a perfect 100% knock-out efficiency.

2. Knock-Ins: 'knock-ins' cover all modifications based on a co-delivered template introducing specific point mutations, tags or large DNA fragments including genes.

a. Knock-in generation is generally much less efficient than gene knock-out

b. There is no universal, verified method that allows for improvement of efficiency. If a homozygous insertion is required, in most cases modification of each allele seems to be statistically independent. That means that if e.g. insertion efficiency is 10 %, biallelic insertion will occur in 1 % of cases.

c. Knock-in projects are often time consuming and expensive, mostly due to the need to screen many clones (usually >200). When planning a knock-in, it is important to consider whether homozygous modification is necessary and what cell line should be used for it.

d. If possible, we recommend using excisable fluorescent reporters or antibiotic resistance cassettes to facilitate screening for proper clones.

e. There is no consensus on what makes the best repair template. Long or short, single or double-stranded DNA templates have all been successfully used for knock-in generation. While dsDNA seems to be more stable, it increases the risk of unspecific integration at a random location in a genome. Most important, often only fragments of the repair template are integrated, making them virtually impossible to detect with methods other than full genome sequencing. It is also important to consider the transfection efficiency of a cell line of choice and to plan the size of repair template accordingly.

ISCCF, IMBA-Institute of Molecular Biotechnology
Dr. Bohr-Gasse 3
1030 Vienna
Austria
Version 2.0 from 22.07.2020

Delivery method of targeting reagents

Delivery methods of CRISPR/Cas reagents rely usually on electroporation and lipofection. There is no universally preferred method as efficient delivery of reagents will depend on cell fitness, toxicity and survival after transfection. We offer a choice of Amaxa nucleofector and Lipofectamin as standard transfection methods.

Targeting regents

High transfection efficiency is a prerequisite for efficient genetic modification.

1. Cas9 Protein vs. plasmid. Cas9: gRNA complexes, mostly due to the small size, can be efficiently introduced into most cell lines. Plasmid DNA, especially large constructs, may be hard to introduce into some particular cell lines due to their size or because some cell lines are refractory to DNA uptake. We recommend testing the efficiency of DNA and/or Cas9: gRNA uptake.

2. gRNA: To date, there is no reliable way to predict gRNA activity. Published algorithms are inconsistent and do not provide a guarantee for choosing an active gRNA sequence. We recommend testing at least 4 gRNAs per target before deciding on one.

3. Donor constructs: Efficiency of incorporation depends on many factors and the type of modification required. For single nucleotide changes, ssODNs coupled with CAS9 RNP complexes seems to offer higher efficiency rates than DNA based reagents.

4. Repair templates: There is no consensus on what makes the best repair template. Long or short, single or double-stranded DNA templates have all been successfully used for knock-in generation. While dsDNA seems to be more stable, it increases the risk of unspecific integration at a random location in a genome. Most important, often only fragments of the repair template are integrated, making them virtually impossible to detect with methods other than full genome sequencing. It is also important to consider the transfection efficiency of a cell line of choice and to plan the size of repair template accordingly

ISCCF, IMBA-Institute of Molecular Biotechnology
Dr. Bohr-Gasse 3
1030 Vienna
Austria
Version 2.0 from 22.07.2020