**GENETAGUS**

Thank you for approaching GENETAGUS. If you are interested in a CRISPR screen or a heavily customizable project, please do not use this form and contact us directly. If you are looking for a knockout, knockin or a deletion, please proceed as follows:

1) DOWNLOAD THE FILE;

2) COMPLETE THE FIELDS BELOW;

3) SEND THE FILE TO genetagus@egasmoniz.edu.pt and we will be in touch within 24 hours.

Please fill in this table the best way you can. **If you don’t know the details we ask, don’t waste any time searching for information we can find easily**. We are mostly interested in avoiding communication mistakes and getting any additional information that you may have that is impossible to find elsewhere (for instance, any tricks to keep your cell line happy, a splice form you found but did not publish that could escape a knockout strategy, a published knockout strategy in some obscure or old publication that you would like to mimic,  etc.).

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| --- |
| **Contact Information** |
| First Name:       Last Name:     Company/Institution Name:        |
| Address:        |
| Phone:      Email:      **TIN - Taxpayer Identification Number of your institution (**essential for the invoice**):** |
| **How did you find us?** *Please check the box here and throughout the document****Google* search** [ ] **Group email sent by us** [ ] **Word-of-mouth** [ ] **From a former client\*** [ ]  **Please type the name of the client:** **\*This information is important for our referral rewards.** |
| **YOUR PROJECT**  |
| **PROJECT DESCRITION**Please describe your project in this box. You may be succinct about the science, but be as precise as possible about the genomic edition. Add links to the gene, a type of edition you may want to mimic, etc. If you have specific requests about the editing (HR-based versus base editor-based, etc.), time constraints or specific concerns, mention them here.*Please note that the project can be CRISPR knockouts, deletions, knockins, CRISPR-based gene activation/repression, CRISPR-based screens, stably transfected/transduced cell lines and others.*  |
| **PREFERRED CELL LINE**  |
| **NAME OF THE CELL LINE YOU WILL SHIP****Insert name + ATCC link or ref if possible:** **Number of allelic copies (related to ploidy):** **If you know the karyotype of the cell line and the chromosome of your target gene:**  |
| Relevant papers and any useful information not found in the literature. Please include complete information about the culture medium and any relevant details to handle the cells:  |
| Is there a selectable marker in the cell line of choice. Which one? The cell may have been engineered to have a puromycin resistant gene, express GFP, etc., which could be relevant for the CRISPR approach. |
| Here we refer to modifications (knockins, knockouts, stable transfectants, etc.) not described in the link to the cell line: |
| **Detailed culture conditions, including how to culture, split and freeze the cells.** Please mention how you handle the cells. Send us protocols as detailed as possible: |
| Additional non-trivial tips on how to handle the cells: |
| **CRISPR PROJECT: SINGLE GENE EDIT** [ ]  |
| **STARTING PRICE:****Isolated edited clones (5000 €)** [ ] **Clones in bulk cultures (2600 €)** [ ] **“Do it yourself” package (1850 €, the client has to purchase the reagents)**[ ] **Strategy design only (300 €, within 3 days)** [ ] **ABOUT THE EDIT:****Knockin SNP or small tag (add 500 € for isolated or bulk cultures)** [ ] **Knockin large insert (1 allele, e.g. GFP ) (add 1500 € for isolated clones or bulk cultures)** [ ] **Deletion** **(add 1000 € for isolated clones and 500 € for bulk cultures)** [ ] **EXTRA FEES:****iPSC or any cell line requiring an expensive medium (add 400 € for bulk cultures and 2000 € for clones per guide used)** [ ]  |
| **Extra fees for the number of targeted alleles in the isolated clone package (there is no extra fee for the bulk cultures):** | **For knockouts, deletions, SNPs, and short inserts (e.g. a small tag), the starting price is for the targeting of two alleles (sufficient for a KO in a diploid cell). Add 1500 € per extra allele (e.g., triploid cell) for any of these modifications.** [ ] **For large insert knockin the starting price is for a single allele (as in a diploid cell heterozygous for the insert). Add 1500 € per extra allele para (e.g., homozygous diploid cell).** [ ]  |
| **TARGETED GENE OR REGION** |
| **Gene or genome coordinates** *For genes, please use a link describing your gene in at least one of the following databases:*[NCBI:](https://www.ncbi.nlm.nih.gov/refseq/rsg/browse/)     [UCSC genome browser gateway](https://genome-euro.ucsc.edu/cgi-bin/hgGateway?redirect=manual&source=genome.ucsc.edu):     [UNIPROT](https://www.uniprot.org/uniprot/P10107):       |
| **KNOCKOUT OR DELETION (Ignore this section if you are looking for a knockin)** [ ]  |
| Please mention the **most critical domain** of the protein, the **isoforms** and **any report describing the knockout you plan to make** (even if from a different species and using different technology). **PLEASE BE CLEAR ABOUT THE ISOFORM(S) YOU WANT TO KNOCKOUT**. **By default, we target all isoforms**:  |
| Is the knockout expected to affect cell growth/viability? **No** [ ]  **Yes** [ ]  **Unknown** [ ] If yes, please give details (e.g., a reference) here:  |
| Do you also want +/- (het) clones besides the knockout (-/-) clones? **No** [ ]  **Yes (add 300 €)** [ ]  |
| **KNOCKIN (Ignore this section if you are looking for a knockout or deletion)** [ ]  |
| **Type of knockin** | **Point mutation or small insertion (*e.g.* a FLAG tag of loxP)** [ ] **Large insertion (*e.g.* GFP)** [ ]  |
| **Genotype of knockin**  | **Homozygous** [ ]  **Heterozygous** [ ]  |
| **For protein fusions, specify the fusion partner from this menu:** Choose an item.**If you want to insert a different insert, please write the protein sequence here:**  |
| **STARTING ATG. Please notice that if you do not tick one of these boxes we will by default keep the starting ATG (most people agree that there is no strong reason to remove it). In the case of the fusion partner gene added to the C-terminus of the gene, the starting ATG: will** **be kept** [ ]  **will be removed** [ ]  |
| **Position of added protein****Fused to the N-terminus of the target protein:** [ ] **Fused to the C-terminus of the target protein:** [ ]  |
| **Protein linker: for an overview, check this** [**review**](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3726540/)**. Please select your linker using the dropdown menu (**Choose an item.**) or write the protein sequence here:** |
| **FOR knockin projects: can the PAM site or the spacer sequence in the template be altered?** To avoid retargeting the allele after the introduction of the right edit, and also to facilitate the genotyping of the clones, a common strategy is to mutate the PAM sequence or the spacer of the template, so that Cas9 will not cut the edited allele. **This is done by introducing silent mutations that will not change the protein sequence and respecting codon usage as much as possible**. If for some reason you prefer not to introduce these additional changes, please be aware that the efficiency of targeting may be affected. | **YES** [ ] **NO** [ ]  |
| **FINAL IMPORTANT DECISIONS ABOUT YOUR SINGLE-GENE CRISPR PROJECT****Please check the section of our page describing the EXTRAS. If you don’t check any box, we will apply the default option** |
| **Type of Cas9** **By default, we use a commercial conventional Cas9**. If you want an engineered Cas9 that reduces the number of off-targets, please select the option “Hifi” Cas9. | **The default option (conventional Cas9)** [ ] **“Hifi” Cas9\*(add 500 €)** [ ] **\*Click** [**here**](https://www.nature.com/articles/s41591-018-0137-0.epdf?author_access_token=Wtec9cfK50gLQz-7uvlGYtRgN0jAjWel9jnR3ZoTv0Ntfhjswnf1sPQd5fRljUHKhqyrYd0Updi1lStjhZQgICItLQHDbZozzZ27Xw6WUHl8po4Hb3tsX_-7SoWgOuAeSXYmThfioUTRNa7myzh5hg%3D%3D) **to know more about this protein.** |
| **Wild-type controls****By default, we will provide 2 control clones that were exposed to Cas9+sRNA but did not acquire the mutation.** Essentially, these clones are identified when we genotype the collection of clones for the presence of the mutation. Since the method is not 100% efficient, there will always be clones that were not edited in the target region but may have been edited elsewhere in the genome (off-targets). If you prefer another wild-type control, please select it. | **The default option (no extra control)** [ ] **Controls from a parallel mock transfection (add 500 €)** [ ] **Controls from a parallel transfection with only Cas9 (no guide) (add 500 €)** [ ]  |
| **Extra guide****By default, we test more than one guide RNA but the edited clones come from cells transfected with the same guide RNA**. Including an extra guide may be helpful because the best control for off-target effects is the confirmation that the phenotype is the same for clones edited with different guide RNAs. | **The default option (no extra guide)** [ ] **Use extra guide (add 3000 € for clones and 400 € for bulk cultures)** [ ]  |
| **Confirmation of the knockout****By default, we confirm that the two alleles acquired frameshift mutations**. Further confirmation at the RNA and/or protein level can be provided. | **The default option (no extra confirmation)** [ ] **RT-qPCR (add 300 €)** [ ] **Western blot\* (add 300 €)** [ ] **\*You have to provide the antibody** |
| **CRISPR PROJECT: GENE ACTIVATION OR REPRESSION** [ ]  |
| ***Details will be discussed over a zoom meeting.*** |
| **CRISPR PROJECT: CRISPR-BASED SCREEN** [ ]  |
| ***Details will be discussed over a zoom meeting.*** |
| **STABLE TRANSFECTION (with plasmids)/ TRANSDUCTION (with viruses)** [ ]  |
| ***Details will be discussed over a zoom meeting.*** |
| **PLASMID BUILDING** [ ]  |
| ***Details will be discussed over a zoom meeting.*** |