

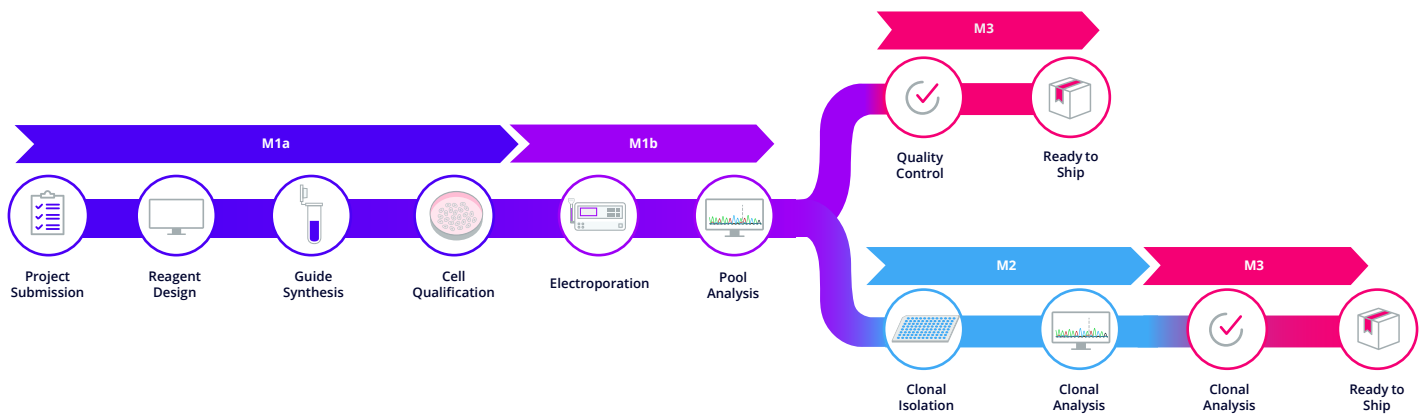


# Engineered Cells–Milestone Definitions

Developed by EditCo

## Introduction

There are many milestones that a cell line will pass through while on the EditCo platform. Below you can find detailed information of the work that is performed in each milestone of an Engineered Cells order.



## Milestone 1a: Design and Synthesis of CRISPR Components

This milestone includes cell on-boarding and reagent ordering, including guides and primers. Guides will be synthesized onsite by Synthego and shipped to Editco as chemically modified synthetic single guide RNA. If this is for a Knock-in, the HDR donor will also be designed and ordered.

For customer supplied cell lines, we will have one of the vials sent to IDEXX for QC testing. Upon receiving passing results, we will thaw another vial for cell expansion and banking.

For customer supplied induced pluripotent stem cell projects with added karyotyping analysis, the provided cell pellets will be sent out to our third party partner for analysis. If the karyotype test reveals no abnormalities, we will thaw another vial of cells for expansion and banking

## Milestone 1b: Transfection and Creation of the Pool

In this milestone, we will schedule and perform transfection on the cell pool. Your specific guide RNAs are complexed together with spCas9 to form a ribonucleoprotein (RNP). RNPs are then delivered to the cells via the optimized electroporation setting.

**Note:** if we have not optimized your cells yet, we will use a positive control sgRNA to perform transfection optimization, and this will take an additional ~2 weeks.



To confirm a successful editing, we use PCR, Next Generation Sequencing (NGS) or Sanger sequencing depending upon the requested edit and our ICE analysis tool (if applicable) to choose the pool with the highest editing efficiency for the next step. Should an intermediate pool deliverable be included in your clone order, we will supply a QC document which shares:

- Knockouts: editing efficiency (%), NGS sequence alignment graphs or ICE results (if applicable)
- Small Knock-ins (<100 bp): knock-in efficiency (%)
- Large Knock-ins (>100 bp): confirmation of knock-in success

## Milestone 2: Single Cell Clonal Isolation (\*not applicable for pool line items)

Single-cell cloning and initial clonal genotyping are the main components of this milestone. We use a single cell printer to ensure that each population originates and grows from a single cell. Each well seeded is imaged every 2-3 days and rigorously tracked to ensure the population is truly clonal and only the progeny of a single cell. We do not use any selection agents to enrich or select for clonal populations.

We closely monitor the growth of these cells before moving to the hitpicking stage. From here we will choose the best population for initial clonal genotyping with NGS/Sanger sequencing to confirm that the edit is present and that we have clones of your desired zygosity.

**Note:** Edited Cell Pool orders will skip Milestone 2 and go straight from Milestone 1b to Milestone 3.

## Milestone 3: Final Expansion & QC

Lastly, we proceed with final expansion, sequence verification, and final QC. The selected clones or pools will be cryopreserved. Media will be tested for mycoplasma and we will isolate gDNA one last time for QC genotyping by NGS/Sanger sequencing. We will contact the customer at this stage to confirm shipping information and availability to receive their cells. Please note the order will typically not ship until the shipping address and availability is confirmed.

**Note:** For induced pluripotent stem cell projects with requested pluripotency and karyotyping services, samples are sent to our third party partner for final assessment.

## Additional Information

For an up-to-date list of all protocols and other resources, please visit this [link](#).

For technical assistance, contact our Scientific Support Team at [technicalsupport@editco.bio](mailto:technicalsupport@editco.bio).

For common FAQs, please visit this [link](#).