CELL & PROTEIN PRODUCTION

Evotec’s wealth of expertise covers all principles including cell line generation, small- or large-scale transient transfection, frozen cell technologies and protein expression from small-scale proof-of-concept studies through batch production for HTS, counter screening activities as well as structural biology applications. For clients looking to outsource the management and supply of their own cell collections, Evotec offers a cell bank management service, establishing, propagating and distributing thousands of cell lines between various locations.

In 2015 the offering for reagents has undergone a rapid expansion with new facilities opening in Princeton, NJ, on the East Coast of the US, to service the North American market, and the acquisition of the Sanofi site in Toulouse, France. In Princeton the team offers rapid cycle times of the highest quality in cell and protein production with a focus on membrane proteins. In Toulouse, the team brings new capabilities in large scale microorganism or cell culture processes as well as the relevant purification steps to provide larger amounts of high grade quality proteins batches. It also incorporates scientists with research, development and industrial expertise in the biotherapeutics field (from recombinant hormones, enzymes to antibody production). The team is expert in adapting laboratory scale results to large scale equipment including 20L to 450L bioreactors for bacteria or yeast, 100L wave system for mammalian or insect cells and all the related separation techniques in a controlled environment. Relevant in-process and final controls are elaborated and run to document quality prior to batch release.

CELL LINE GENERATION AND BANKING

From recombinant cells which have been either generated by stable transfection or viral transduction, monoclonal cell lines are isolated and screened for their functional expression of the target protein. After a first broad screening on expression level using immuno fluorescence labelling, Western blot, or RT-PCR, usually a secondary screening of selected clones by a cell-based assay is applied.

There are numerous reasons why a target may not be stably expressed in mammalian cells and a transient approach may be more appropriate for cell-based screening. To address this problem, Evotec routinely uses a variety of transfection technologies from lipid based to viral mediated delivery to achieve transient expression.

Particularly the MaxCyte electroporation platform offers access to large batches of up to 10 billion cells, a cost-effective and efficient transfection process. Transiently transfected or virus transduced cells can be provided as assay-ready frozen instant cells, which are functionally expressing the target instantly after thawing.

The availability of cells is a critical bottleneck in screening campaigns. Frozen instant cells, which can be assayed without prior cultivation have become a valid and frequently used alternative to cells from a continuous growing culture.

Evotec’s cell culture unit was one of the first suppliers of frozen instant cells for global pharmaceutical research. Cells are:

- Expanded under controlled culture conditions to large bulk quantities
- Harvested applying a gentle method which does not affect surface receptors
- Prepared to a density which corresponds to the requirements of your assay, the cells are dispensed into vials using an automated filling device. This fast processing of the cells significantly decreases the time from harvest, which is critical for maintaining the quality of frozen instant cells
- Frozen in a controlled rate freezer and stored in the vapour phase of a N2 tank until they are shipped.

For your ligand binding assays or in vitro transporter activity assays, we offer cell membranes in bulk in two different purity grades. All batches of cells and cell-based products are carefully controlled for their quality before they are shipped to you. Frozen instant cells are checked not only for viability but also for their ability to adhere shortly after thawing. A good "fitness" of the cells is often correlated with fully functional response. Finally, the assay performance of the frozen cells is compared to cells from a continuous growing culture considering Z’ S/B and EC50.
Evotec offers professional management and maintenance of client cell banks encompassing the addition of new cell lines and shipment of assay ready cells and vials to the client’s research centres. A dedicated team with access to the necessary bioinformatics, quality control processes and infrastructure can additionally provide support in the sourcing and maintenance of disease relevant cell banks such as oncology. The databases covering all aspects of the respective cell line such as growth rates, morphology and genetic confirmation are available over a web-based client portal providing clients with the essential access to all necessary information.

**RECOMBINANT PROTEIN PRODUCTION**

Protein production lies at the heart of drug discovery. Evotec’s recombinant protein production facilities provide comprehensive coverage of expression hosts, purification techniques and analysis tools and delivers a unique solution for the efficient delivery of proteins in a timely fashion. Our capabilities can be accessed flexibly, either as standalone units or as part of an integrated drug discovery programme.

All proteins are prepared with end use in mind, including the delivery of high-quality proteins for bio-assay and biophysical applications.

- **Labelled proteins for NMR and Mass Spectroscopy**
- **Yields and quality suitable for Isothermal Calorimetry, Differential Scanning Fluorimetry and Microscale Thermophoresis**
- **High-throughput protein purification and construct triage to deliver crystal grade material for macromolecular X-ray crystallography**

The experienced team of scientists will determine optimal host for expression and yield and develop suitable protocols for purification using a suite of techniques, including semi-automated expression analysis and purification.

- **Specific expertise in the team covers the common protein families and also extends into the more demanding areas of macromolecular complexes (protein:protein and protein:DNA) and membrane proteins.**
- **The team has unparalleled experience in labelling of proteins for NMR work including ubiquitous 15N, 13C and 2H in E. coli and yeast and side chain specific labelling in insect expression hosts.**

In addition to purification of proteins by SOPs or according to literature precedents, Evotec has extended methods commonly employed in structural genomic facilities so that the team can perform high-throughput (“HTP”) expression screening in E. coli, insect and mammalian hosts to determine optimal conditions for production ahead of parallel purification or large scale production runs. Large scale expressions can be carried out in parallel, either in shake flasks or in WAVE cellbag bioreactors.
Evotec offers labelling of biological relevant molecules and reagents for use in ELISA, FACS, FCS, plate readers and other fluorescence based detection systems. Our service helps clients to improve their research results in all areas of biological and pharmacological assays, as well as clinical diagnostics and analytics. Our expertise covers the labelling of:

- Small organic molecules e.g. hormones, neurotransmitters
- Peptides and chemokines (as enzyme substrates, GPCR ligands, etc.)
- Proteins including antibodies
- Site-specific mutagenesis of proteins (for site-specific labelling)
- Glycoproteins

Evotec’s labelling chemistry team has acquired a comprehensive expertise having successfully designed and labelled components for more than 150 different assays representing a broad range of assay classes. For labelling we use the optimally suited dyes and tags for your application, such as:

- Proprietary dyes (EVOblue™ family)
- Commercially available fluorescent dyes and the in-licensed MR121 dye
- Non-fluorescent markers and probes like affinity tags, biotin, reporter enzymes, etc.

With a range of ÄKTA purification modules (from Purifiers to Pilots) and HTP methods suitable for cost-effective parallelisation, Evotec has the capacity to deliver significant quantities of proteins in parallel using a variety of purification schemes (see figure above).

All proteins pass stringent in-process quality controls, including Mass Spectroscopy, Dynamic Light Scattering, Differential Scanning Fluorimetry, Activity Testing and NMR, before final release to the end user. In summary, by tailoring the purification schemes to the problem to be solved and using appropriate input from scientists on the projects, Evotec delivers the target protein optimised for the end use.