Fermentation and Protein Biomanufacturing

Drawing on more than 15 years of contract manufacturing experience, LuinaBio scientists have the expertise, training and experience to ensure a successful outcome to any biomanufacturing project.

In the past, we have manufactured recombinant proteins, whole cell vaccines, viral vaccines, purified plant extracts, pro-biotics and synthetic molecules for pre-clinical, phase 1 to 3 human clinical trials, and commercial veterinary products for clients both within Australia and internationally in the United States, New Zealand and Asia.

Utilising our TGA/APVMA licensed facilities operating to international cGMP standards, as a Contract Manufacturing Organisation (CMO), we can provide comprehensive manufacturing solutions for both biological and small molecule drugs.

In regards to biological molecules, Pharma Synth has an expertise in the production and purification of biopharmaceuticals (also called "biologics") from the fermentation of bacterial and yeast cell lines. Protein or carbohydrate biological molecules can be produced that way.

The first protein biologics were developed by inserting the gene encoding the desired protein into an *Escherichia coli (E.coli*) bacterial host system. Insulin, growth hormone, and Neupogen (G-CSF or granulocyte colony stimulating factor) are today produced in this manner. The advantage of *E. coli* production is that proteins can be made using a well characterized production system that is relatively cost effective and easy to scale-up through fermentation of the fast growing cells in bioreactors.

Protein therapeutics production and purification using the system also *E.coli* system has an extensive regulatory and commercial track record. However using current *E.coli* production technology, not all proteins can be produced because of mis-folding of the protein molecular backbone or the inability of the host cellular systems to modify the protein background structure by adding the necessary carbohydrates and other functional groups that contribute to the activity of the macromolecule (“post-translation modifications”). Without these post-translational modification steps, many proteins are unable to fold into their three-dimensional shape, which determines how they interact with other proteins for biological activity. Therefore many complex protein macromolecules that require extensively post-translational modification for activity, such as human monoclonal antibodies, cannot currently be properly produced in *E. coli* and must be produced in phylogenically higher Eukaryotic organisms such as yeast, fungi, mammalian and plant cells.

LuinaBio can also use *Saccharomyces cerevisiae*, a species of yeast, in fermentation to produce biopharmaceuticals. Worldwide, it is perhaps the most useful yeast, having been instrumental to winemaking, baking and brewing since ancient times. The advantages of using Saccharomyces cerevisiae (S. cerevisiae) as a cell factory for the production of biopharmaceuticals is that there is therefore a tremendous amount of information available about this organism through databases, sequenced genomes, and an extensive toolbox for

molecular modification, which provides a knowledge base for further engineering of this organism.

Advantages of (*S. cerevisiae*) are that this eukaryotic model system enables the production and proper folding of many human proteins that would not be properly processed by *E.coli*. Furthermore, in *S. cerevisiae*, unlike in *E.coli*, the biopharmaceutical proteins can be secreted to the extracellular medium and this facilitates subsequent purification. A further advantage is that in many cases yeast can perform proper post-translational modifications of the protein, including proteolytic processing of signal peptides, disulfide bond formation, subunit assembly, acylation and glycosylation. One of the limitations with the use of yeast is, however, that it performs high-mannose type N-glycosylation. This confers a short half-life of the modified protein in vivo, which then can have a reduced efficacy for some therapeutic use.

LuinaBio also offers our clients the ability to produce biopharmaceuticals using *Pichia pastoris* (*P. pastoris*), a robust yeast expression system that produces high levels of recombinant proteins. The Pichia system is stable, durable and cost-effective. Pichia grows on simple media and secretes low amounts of endogenous protein, making it easier to recover and purify the desired recombinant protein from the cell supernatant.

Pichia has two main advantages over *S. cerevisiae* in laboratory and industrial settings. Firstly, Pichia is a methylotroph, meaning it can grow with the simple alcohol metahnol as its only source of energy — a system that is cheap to set up and maintain. Additionally, Pichia can grow in media containing only one carbon source and one nitrogen source. Secondly, Pichia can grow to very high cell densities, and under ideal conditions can multiply to the point where the cell suspension is practically a paste — a great advantage when trying to produce large quantities of protein without expensive equipment.

However it is well known that a number of proteins require chaperonins for proper folding. Thus, Pichia is unable to produce a number of proteins for which the host lacks the appropriate chaperones. Additionally Pichia has been reported to produce hyperglycosylations of a number of proteins, which makes it unsuitable for the production of some molecules such as those having applications in structural biology. However if you are concerned about the latter problem, recently a group has managed to reengineer Pichia to produce a strain that produces complex terminally sialylated glycoproteins that are more similar to that found in humans.

In regards to fermentation and related services, briefly LuinaBio can undertake for our clients:

- Master and working cell bank preparation, validation and storage
- Fermentation and subsequent downstream purification
- Up and downscale fermentation optimisation
- And downstream processing optimisation and development

Pharma Synth can provide batches of GMP material produced in a range of microbial expression systems for pre-clinical studies and also for clinical Phase 1, 2 and 3 clinical trials and the market. LuinaBio's microbial manufacturing is underpinned by fermentation capabilities up to 500L scale.

LuinaBio's fermentation vessels range from 5L to 500L production and can be operated in numerous modes (e.g., batch, fed batch and perfusion). We are able to offer a full suite of manufacturing both whole cell preparations, recombinant products from microbial hosts, and naturally produced cellular products such as carbohydrates. Processing can be undertaken under GMP or non GMP as needed.

Contact Us

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