



PROCESS DEVELOPMENT INFORMATION

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NUMEROUS ADVANTAGES OF USING RABBITS

- ✓ Rapid and easy generation of transgenic rabbits (20 transgenic founders for each project in less than 2 months, generation time: 5-6 months).
- ✓ Short time-to-market and flexible scale-up (liter-scale daily production of milk in less than one year).
- ✓ High level of recombinant protein in milk (1 to 10 g of recombinant protein / litre).
- ✓ Low cost of breeding & production (30 to 60% compared to equivalent production using CHO cells).
- ✓ Correct post-translational modifications (glycosylation, gamma-carboxylation, structural conformation, cleavage, metal ion insertion, etc.).
- ✓ Suitable for complex and difficult-to-express proteins (antibodies, plasma proteins, hormones, viral like particles, etc.)
- ✓ No known prion disease in rabbit.

RABBITS MEET THE REGULATION

- ✓ Safety Validation similar to other mammalian expression systems (CHO, BHK, etc.)
- ✓ Excellent Reliability & Reproducibility of the process.
- ✓ GAP, GLP and GMP process
- ✓ Stable expression and inheritance of the transgene.
- ✓ High quality product (e.g., no protease contaminants in milk).
- ✓ No prion clearance validation required.

Main documents containing definitions and guidelines for the safe production of pharmaceuticals from transgenic animals issued by Center for Biologics Evaluation and Research (FDA) and European Committee for Proprietary Medicinal Products (EMEA).

RABBITS DELIVER KILOGRAM QUANTITIES OF PROTEIN

For a typical project (400 transgenic female s), each rabbit delivers:

- ✓ Up to 150 ml of milk/rabbit/day of lactation (i.e. 10-15 liters /year/female).
- ✓ 1 to 10 g of recombinant protein per liter of milk.

Kilogram quantities of purified protein per year (assuming a 40% recovery during the Downstream Process).



PROJECT MANAGEMENT

A Project Development Committee (PDC) will be in charge of the organization of the Production Process Development and will meet as often as necessary. It will include one or more representative(s) nominated by the Sponsor's Company and a Project Manager designated by BioProtein Technologies, with the heads of Quality Assurance (QA), Molecular Biology, Cellular Expression, Embryology, and R&D Purification teams.

MOLECULAR BIOLOGY



BioProtein Technologies has developed QA Procedures in order to provide the Sponsor's Company with the best guarantees concerning each step of the expression vector preparation, from the DNA sequence identity at the receipt, to the subcloned recombinant DNA fragment used in the transgenesis process (double strand sequencing on three independent clones).

In parallel to the transgenesis experiments, a transfection study in mammalian and/or mammary cells will be performed in order to rapidly validate the transgenic expression vector containing the cDNA or gene of interest (analytical assays: secretion, expected apparent molecular weight, post-translational modifications, etc.)

TRANSGENESIS

The *in vivo* produced embryos are collected from New Zealand female rabbits and their pronuclei are micro-injected with the purified recombinant DNA insert (containing no relevant genetic sequences such as antibiotic resistance gene or origin of replication).



One-cell cultivated embryos are re-implanted into recipient rabbits by surgical procedure (under general anesthesia). Parturition naturally occurs 29-31 days following embryo transfer.

During the embryogenesis process, the micro-injected recombinant DNA randomly integrates into the genome. Potential transgenic rabbits are screened for the presence of this exogenous DNA via ear biopsy of 10 days-old rabbits followed by a PCR analysis. Animals positively identified are then termed "transgenic founders or F0".

RECOMBINANT PROTEIN PRODUCTION IN MILK

Sexually mature F0 rabbits (4 months for females and 5 months for males) are bred with non-transgenic animals (artificial insemination procedure) in order to obtain heterozygous F1 offspring (pregnancy is monitored by echography).



Parturited F0 females provide on average 100-150 ml of milk. Rabbits are mechanically milked in a project-dedicated milking room. Aliquots of milk are analyzed for the presence of the recombinant protein. Purity, identity (ELISA, biological activity), and quantification of the protein are performed in order to select the best transgenic producers. Aliquots of crude milk will be shipped to our partner, Q-One Biotech Ltd. (Glasgow, Scotland) for biosafety testing.

LARGE-SCALE PRODUCTION

Transgenic F1 progeny is identified via tissue biopsy followed by PCR analysis. Sexually mature transgenic F1 females are then inseminated with non-transgenic sperm. Their milk is mechanically collected and the recombinant protein is characterized in order to select the best offspring lines for large scale production and to develop the purification process strategy (GLP, pre-GMP, GMP).

In parallel, sperm from F1 transgenic males is collected and cryo-preserved in liquid nitrogen, as recommended by the FDA Guidelines and the European Regulation. This sperm will constitute a **Master Sperm Bank (MSB)** and will be used to artificially inseminate non-transgenic females to generate the second filial offspring (F2).

Sperm from F2 transgenic males will be recovered and cryo-preserved in liquid nitrogen, to set up a **Working Sperm Bank (WSB)**. For 15 to 20 years, this bank will be used to generate transgenic F3 females which will produce industrial amounts of recombinant proteins in their milk.



By courtesy of Grimaud Frères Selection

Animal manipulation and housing are performed under Good Agricultural Practices (GAP) in accordance with European and US Regulations. Rabbits are bred under veterinary control in a confined environment to ensure minimal risk of zoonotic infections.

A 120 m² (1300 sqft) room allows BioProtein Technologies to breed 130 females and their young rabbits. Three rooms like the one besides are sufficient to breed 400 rabbits, and allow the large-scale production of recombinant protein.

DOWNSTREAM PROCESSING

A first protocol (expression analysis) is the definition of a convenient and rapid frame process to screen and purify the recombinant protein of interest in the milk of F0 transgenic females.

A second protocol (large-scale purification process) is to develop a customized purification process by using specific sorbent support for the efficient capture and purification from large quantities of milk containing the recombinant protein.

Milk will be collected, clarified (fat and caseins removal) and stored directly into sterile flexible plastic bags at -20°C under GMP conditions before to be shipped to Eurogentec (Liège, Belgium), our DSP partner. The purification process will be specifically designed for each recombinant protein with a minimal target purity of 95-99% in a convenient way, with limited purification steps. No matter the process chosen, the purification process will be performed taking account of future GMP production constraints (including additional polishing steps).



All fractions will be analyzed for purity by SDS-PAGE. The study will be completed with a determination of host protein content and endotoxin contamination. If necessary, a Validation Master Plan can be established. The optimal conditions for the purification process will be defined during the 'Purification Development Programme'. The filling and vialing should be outsourced to a suitable Contract Manufacturing Organization (CMO). The product will be released as sterile bulk.

A fully GMP compliant Batch Record will be provided with a Certificate of Release signed by the Qualified Person. These documents will certify that the production of the batch complies with the requirements of the GMP and the specifications defined in the Process Document, and that the batch can be used for administration in humans.

BIOSAFETY TESTING & PROCESS VALIDATION

The virus clearance validation, as well as the biosafety testing will be outsourced to our privileged partner, Q-One Biotech (Glasgow, Scotland). Q-One Biotech has extensive experience in the design and implementation of biosafety testing and process validation studies for a wide range of innovative biotechnology products.

The main viruses that could infect rabbits or their milk have been identified and appropriate tests are in place to ensure a safe product. Biosafety testing will be performed for every batch, in accordance with US and EU regulations. Crude milk is tested for bacteria, mycoplasma and viruses. Appropriate virus clearance validation studies will be performed on the purification process in accordance with the relevant guidelines.





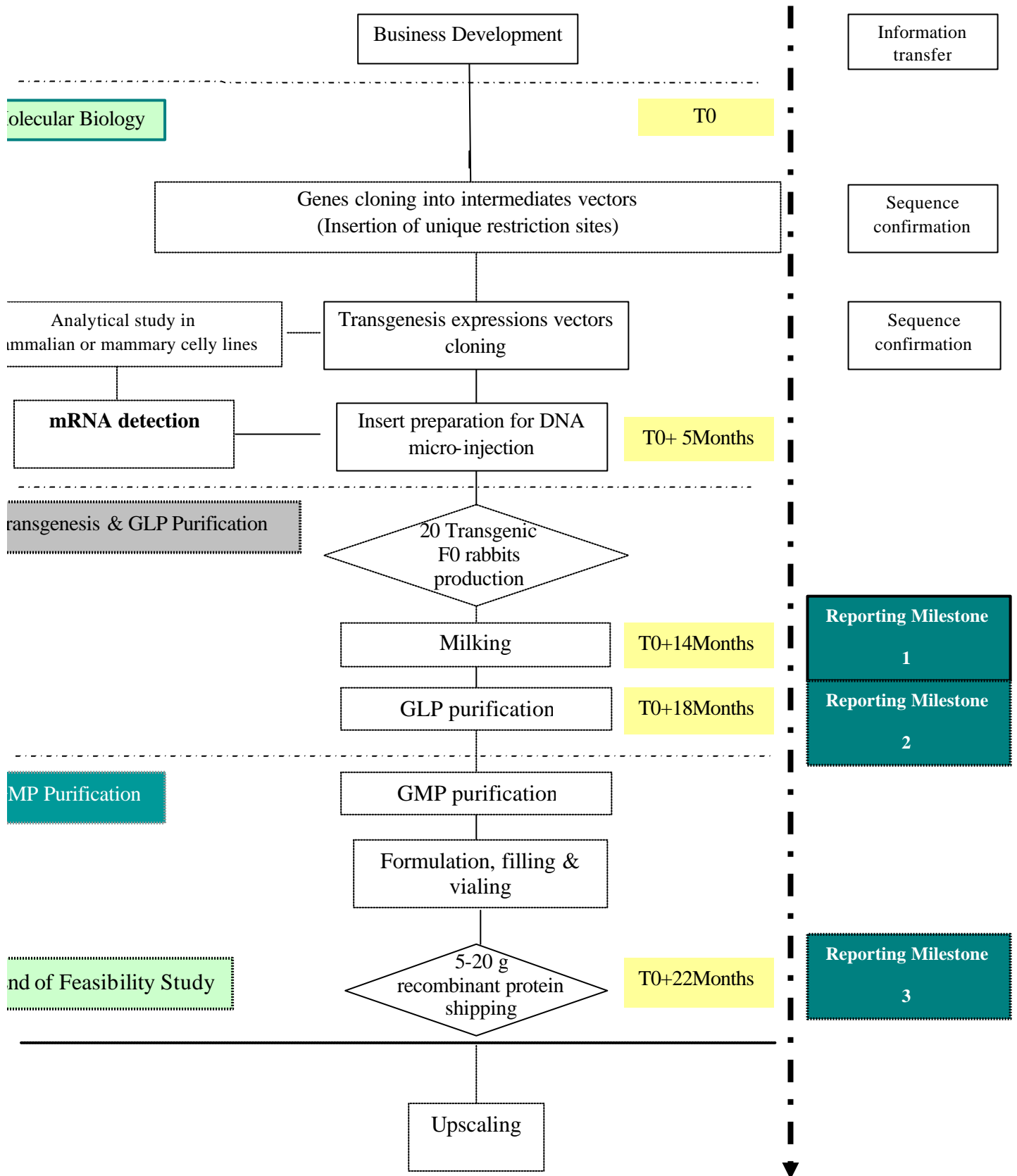
WORKING WITH BIOPROTEIN TECHNOLOGIES

BioProtein Technologies offers its expertise to pharmaceutical or biotechnology companies who want to develop therapeutic proteins in the milk of transgenic rabbits. The Company welcomes the opportunity of being your partner for transgenic production.

Based on BioProtein Technologies' experience, the production and purification scenario will be developed for each project as follows:

- A first milestone will include the **generation of 20 transgenic founder rabbits**. The animals will be selected for their efficiency to produce the protein of interest and milked in order to provide our customers with crude milk batches.
- A second milestone will be the development and optimisation of a few-step purification process for scouting the production level of the sought-after protein produced in the transgenic rabbits' milk. **GLP batches** will be provided to our customers, allowing them to start preclinical and toxicological studies.
- A third milestone will include the beginning of the large-scale production of the protein and its direct capture in compliance with cGMP regulation. A customized purification process will be developed for the capture and purification from large quantities of milk containing the recombinant protein with a target of 95-98% purity. No matter of the process chosen, the purification process will be performed taking account of future cGMP production constraints. **GMP batches** will be provided to our customers, in order to start their first clinical studies.

These scenarios could be customized according to your needs. For example, at one end of the spectrum, we may produce finished bulk drug. At the other, we may provide partially purified or clarified intermediate in order that you can control the final production and release of the product utilizing your internal expertise and resources. The program described above will be performed within the framework of a feasibility study.





FEASIBILITY STUDY

BioProtein Technologies basically proposes to its customers to make a feasibility study. This will give BioProtein technologies' partners the opportunity to evaluate the efficiency of the technology compared to other expression systems. In order to minimize the cost-risks taken by its customers, BioProtein Technologies has developed cut-out feasibility studies defining milestones and success fee payments.

All the data given below are presented as information and are not contractual.

THREE OPTIONS ARE PROPOSED

➤ From genes / cDNA to **20 founder rabbits** and **Milk batches** (Total duration: 14 months)

• Upfront payment	=	€200.000
• Generation of 20 founders	=	€150.000
• First milk batches delivery	=	€150.000

➤ From milk to **GLP batches** of recombinant proteins (+ 4 months)

• Delivery of 0.5 to 5 g	=	€300.000
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➤ From milk to **GMP batches** of recombinant proteins (+ 4 months)

• Delivery of 5 to 20 g	=	€700.000
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Once the feasibility study is achieved, BioProtein Technologies will have selected the most performing transgenic rabbit line and established a validated Master Sperm Bank and Working Sperm Bank.

In case of success with clinical trials and upon request of its customers, a kilogram scale production would be performed within a relatively short timeframe (6 to 12 months).