



# The Future *of* Transgenics

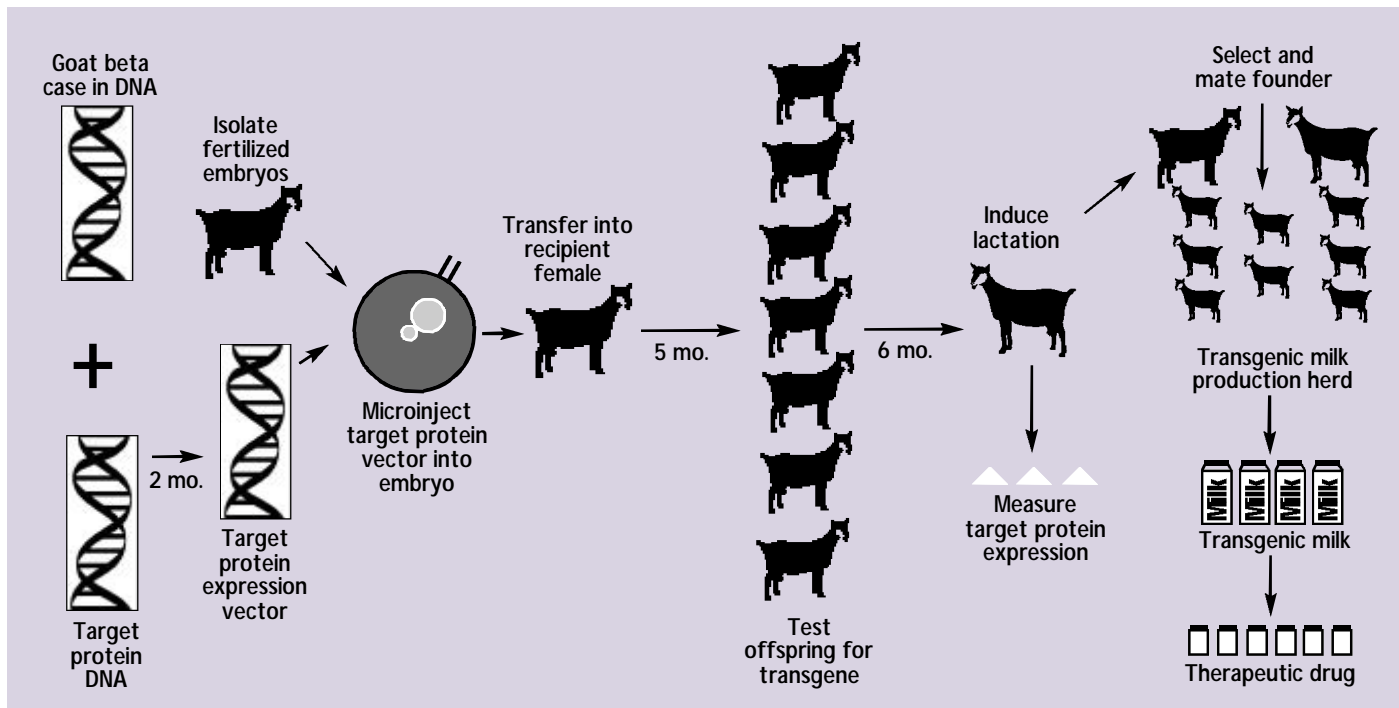
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## Introduction

The use of transgenics as a technology for producing recombinant proteins has made remarkable strides in the past few years.<sup>1</sup> A number of companies (e.g. Genzyme Transgenics Corp., PPL Therapeutics and Pharming B.V.) are actively engaged in preclinical and clinical trials with therapeutic proteins derived from the milk of transgenic animals (e.g., anti-thrombin III,<sup>2</sup> monoclonal antibodies,<sup>3</sup> factor VIII, alpha-1-proteinase inhibitor, alpha-glucosidase, etc.). With that in mind, this article focuses initially on the science behind transgenics, explores the unique issues surrounding the technology, and touches upon what the next few years may have to offer.

The production of the first transgenic farm animals was documented in 1985.<sup>4</sup> The demonstration of biopharmaceutical production by these animals followed shortly thereafter. Although gene expression and heterologous protein production is possible in many different tissues and fluids of the animal (e.g., blood, urine, semen, etc.), this article will highlight transgenic milk production as it is at present the most feasible and furthest along in development and regulatory process.

Figure 1: Flow Diagram for Transgenic Production



### Why Use Transgenics

Transgenics initially was recognized as a novel platform for the production of recombinant products for a number of reasons. First, the ability to produce significantly greater amounts of protein with a higher expression level and volume output than traditional culture systems made transgenics a worthy technology for investigation. Second, the ability to express novel proteins due to the unique nature of the mammary gland for production of complex molecules was identified. Lastly, the potential for a significant reduction in the cost per unit protein was envisioned due to the animal being the true “bioreactor,” requiring fewer inputs (raw materials) and less complex monitoring and support systems than a traditional recombinant cell culture system.

### The Science Behind the Technology

Producing the initial founder transgenic animal is the first critical step and involves integrating an engineered segment of DNA into a host cell. This DNA segment, called a construct, typically contains an upstream mammary

specific promoter sequence, the coding sequence for the gene of interest, and usually a downstream regulatory sequence. Traditionally, microinjection (direct injection into the pronucleus of a one-cell embryo) and subsequent transfer of the embryo to a surrogate female animal resulting in the birth of transgenic offspring, has been the mainstay for transgenic founder development.<sup>5</sup> However, newer techniques for integrating the construct have seen remarkable advances in the past few years, including retroviral transfer, fetal cell/somatic cell nuclear transfer,<sup>6</sup> and sperm-mediated transfer.

Regardless of the technique used, the predominant regulatory requirement is stable integration of the genetic sequence without any alteration. Once this requirement is demonstrated and traditional outbreeding has begun, the next step is raw product recovery during lactation. An illustration of the overall process is found in **Figure 1**. Although this article highlights production in goats, additional species (mice, rabbits, chickens (eggs), sheep, cows, etc.) are being explored commercially.

### Initial Quality and Regulatory Compliance

Compliance with quality assurance practices and regulatory guidelines begins at the level of construct development. Good documentation practices and the use of appropriate laboratory notebooks are essential. This same level of compliance also applies at the next stage when the construct is inserted into a host cell (e.g., a fertilized one-cell embryo) and subsequently transferred to a recipient animal. Proper documentation tracks the path from embryo microinjection to the birth of the transgenic founder animal. This level of control is the same as that of a traditional cell culture-based recombinant protein production system.

### The Use of Transgenic Animals for Recombinant Protein Production

The use of transgenic animals for production does, however, add a new layer of quality and regulatory controls not needed in cell culture-based production. A sound understanding of the health and physiology of the species

is essential. Unlike cell culture production (with specific production starts, duration and stops), a transgenic animal, such as a goat, may live and produce for up to seven to 10 years. With cell culture production, each batch is made from a unique initiation or fermentation run. With transgenics, a production animal is bred and lactates annually. Additionally, each animal experiences its own physiological changes and various environments throughout its life (e.g., nursery, general housing, milking parlor, etc.) as it develops, gives birth, and ultimately lactates.

Predominantly, ruminants (cows, goats and sheep) are being used due to their high volume of milk production, which in and of itself brings along certain regulatory requirements. Careful attention to sourcing of animals plays a key role in assuring disease freedom and affects the future testing program. Sourcing animals from Transmissible Spongiform Encephalopathy-free countries, such as New Zealand or Australia, has significant benefits when dealing with FDA and European regulatory agencies.<sup>7</sup> Regardless of the animal source, appropriate quarantine procedures, both external (per federal and state regulations) and internal (on-site for further monitoring and testing) should be practiced.

Strict control of an animal or a herd starts at the level of identification. Reliable and permanent identification is available in many forms (ear tags, ear tattoos, external electronic transponders, subcutaneous electronic transponders, etc.) and it is prudent to use redundant systems. Another aspect of control is establishing and maintaining a closed herd. It is well documented in the veterinary arena that a closed herd results in a significant reduction of disease transfer by limiting animal introduction into a herd and protecting animals in the production environment.

Establishing a healthy, well-monitored herd starts with a comprehensive preven-

tative herd health plan. This plan should consider the species being used, environmental requirements or restraints, housing standards, vaccination regimes, internal and external parasite control, and obviously, continuous veterinary care. Establishing a biosecurity program is another critical component of maintaining a healthy herd. Consideration for biosecurity from external (employee, visitor and vehicle flow, and access, etc.) and internal sources (husbandry practices, pest control, animal isolation and quarantine procedures, etc.) should be incorporated.

Developing a disease monitoring and testing program for transgenic animals requires considerable diligence. First, a complete list of every potential disease (parasitic, bacterial, fungal, viral) of that species must be developed. Second, this list needs to be refined by knowledge of those diseases that are the most possible or probable due to geographic location or husbandry or milking practices, the most harmful to either goats or humans (zoonotic), and whether the disease is treatable or fatal. Third, a specific understanding of which diseases can be passed into the raw source material (i.e., the milk) needs to be investigated. Lastly, this list must be constantly updated with new information about the disease or the detection methodology. For each disease identified, a reliable test methodology needs to be used and implemented at an appropriate frequency. Veterinary expertise in the species of question should be sought when working through a disease testing program. Regulatory guidance in this area<sup>8</sup> recommends the use of expert veterinary panels in the development, review and approval of a testing program for transgenic animals.

#### Initial Milk Production, Testing, Product Recovery and Characterization

Production of small quantities of material early in the project is advanta-

geous for starting the biochemical characterization of the molecule. Early milk collection can be accomplished through normal breeding and lactation or through hormonal induction of lactation when the animal is young. Typically this material is used for determining biological activity, measuring concentration of expression, amino acid sequencing, carbohydrate analysis and identifying contaminants. This information is necessary for any recombinantly produced product. Unique to transgenics however, this product characterization also should be done for each transgenic animal at different stages of lactation and during different lactations to ensure consistency of the product throughout its production.

It is at the point of milk collection that the quality and regulatory controls transition to traditional current Good Manufacturing Practices (cGMP) practices to ultimately ensure product safety, purity, potency and efficacy. Prior to the collection of milk for processing, a number of practices, procedures, documentation and equipment-related functions need to be in place. First, performance and documentation of personnel training, specifically relating to goat handling and milking, need to be completed. Second, process documents must be in place to ensure that key steps in the collection process are identified and meet the required standards established by quality assurance. At this point, production and batch records need to be established, specific to the transgenic recombinant production operation. Lastly, equipment used in the collection and storage of source material needs the appropriate operation, calibration and validation documentation.

Testing the animal prior to collection is an additional control in transgenic production. Pre-screening the animal's health (e.g., general health, mammary gland condition, body temperature) and the initial milk for evidence of sub-clinical or clinical mastitis (e.g., gross

milk appearance for mastitis, California Mastitis Testing (CMT)) is recommended. Once the animal meets the criteria for these initial “by-the-doe” tests, milking can begin. Milk can be collected individually from each animal at each milking and maintained until other testing criteria have been met.

If an animal is in poor health, has evidence of mastitis, or does not pass any of the established animal or milk tests, it must be removed from the production system. There needs to be a well documented process for the removal and re-introduction of an animal from production that ultimately is controlled by quality assurance. Whether the animal has additional testing performed, is treated for a veterinary condition, or is permanently removed from production needs to be controlled, documented and handled on an individual animal basis. Veterinary treatments given to a lactating animal need to be documented so that any potential possibility for a residue in milk is identified. An appropriate withdrawal time should be adhered to with follow-up testing for clearance to ensure milk safety.

At some point, pooling of milk is usually desired for processing. Pooling can happen immediately if milk is kept fresh in a liquid state. Alternatively, if milk is frozen for storage, it can be done when thawing individual collections. Additional testing, such as for endogenous and adventitious agents (e.g., bacteria, viruses, etc.) can be performed on this pool. Specific testing should be developed with agency guidance depending upon the transgenic system being used.

### Initial Product Purification

Once the milk has been collected, purification of this source material again follows the traditional recombinant protein production requirements. Because the source material is unique, a significant development phase is needed for the initial process steps and should be

developed in parallel with the first lactations. During process development, variations in the processing scale need to be considered to address the increasing milk volumes collected during herd scale-up. Whether the milk is collected individually or in bulk and whether the milk is processed fresh or frozen are a few of the issues that need to be addressed.

Downstream purification needs to be able to ultimately produce a very safe, pure and reproducible end-product. Validation of the downstream process is required, as for traditional recombinant protein production. Unique to transgenics, however, is the need to address removal or inactivation of species-specific endogenous and adventitious viruses and prions. Studies need to be geared toward addressing the viruses/prions of concern and incorporated into the viral validation studies.

### Preclinical and Clinical Studies

Once appropriate quality and regulatory controls are in place, the purification process developed, and product biochemical characterization well under way, then, as for any product, preclinical studies are necessary. The preclinical plan is based on the product and its intended use, not the transgenic origin of the product. Route of administration, dose, frequency and duration are the traditional parameters that need to be defined. To assess product safety and efficacy, both *in vitro* studies and *in vivo* animal models should be considered. At this stage, the primary goal is to understand the mechanism of action, the efficacy and toxicology of the recombinant product.

If the initial preclinical studies are favorable, then development proceeds along the traditional path with clinical trials initiated. At this point, the process for downstream purification is further developed and validation nearly completed. The product should be well-characterized and assay development

should be near completion by the time late-stage clinical trials are initiated. It is imperative that at each stage of preclinical and clinical development with a new technology that regulatory guidance and review of data be sought to ensure that the direction will be acceptable to the agencies and that ultimately safety is achieved and efficacy proven.

### Initial Regulatory Guidance

Prior to specific regulatory guidance for transgenic production, already existing documents for recumbent technology were applicable. Specifically, the 21 Code of Federal Regulations (CFR) parts 58, 210, 211, 600 and 680 apply.

Regulatory guidance for transgenics has been developing over the past few years from a number of agencies. In the US, FDA is the primary regulatory body providing guidance; however, FDA’s Center for Veterinary Medicine (CVM), US Department of Agriculture-Animal and Plant Health Inspection Service-Veterinary Service (USDA-APHIS-VS) and Animal Care (USDA-APHIS-AC), and state and local agencies also have specific oversight. Additionally, accreditation from organizations such as the Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC International) is looked upon favorably by the regulatory agencies as a high standard for research laboratory animal care facilities to achieve.

Specific regulatory guidance began for this technology with FDA’s issuance of “Points to Consider in the Manufacture and Testing of Therapeutic Products for Human Use Derived from Transgenic Animals” in 1995. This document set the initial standards for how companies working with this technology should strive for product approval and outlined five main points:

#### ■ Generation and characterization of

**the transgene construct** to include construct design, regulatory sequences, gene coding sequence, method of production, natural protein expression and function.

■ **Creation and characterization of the founder animal and its propagation** to include history and health of donor and recipient animals, method of transgene integration, methods for founder analysis, determination of transgene stability and expression.

■ **Maintenance of transgenic animals and production herds** to include proof for continuous transgenic animal production, development of master and working transgenic banks, methods for generation and selection criteria for production herds, monitoring of herd health encompassing housing, feeding, removal and re-introduction, and animal disposal.

■ **Purification and characterization of the transgenic product** to include recovery methods, definition of lots, endogenous and adventitious agent screening/testing, product analysis and purity, biochemical characterization, and product release.

■ **Preclinical safety evaluation** to include consideration for route of administration, *in vitro* studies, and *in vivo* animal studies.

This document made a significant effort in highlighting and, where possible, detailing the areas that need to be addressed specifically for transgenic recombinant protein production. However, there were still a number of unaddressed or vaguely defined issues that led to differences in interpretation and a lack of agreement as to the best approaches to go forward.

The USDA-APHIS-VS input provided information on animal disease testing and control/eradication. Specifically, for diseases within the US, standardized testing and reporting is

required and ultimately animal disposition falls under their jurisdiction. For animal import/export, USDA has complete authority for establishing animal disease testing criteria and quarantine programs by the exporting nation and has the ultimate decision on the release of animals into the US. Specific disease detection within the US also triggers the involvement of the USDA-APHIS-VS, which has final say on the disposition of the animal, the herd and farm of origin, and can lead to restriction of animal use or movement and potentially, ultimate animal destruction.

The USDA-APHIS-AC oversight deals with the implementation of the Animal Welfare Act (AWA). In this role, USDA is responsible for ensuring animal health and welfare in licensed research facilities. Under the AWA, USDA is charged with ensuring that registered and licensed facilities abide by the AWA, which addresses areas such as animal environments, housing, feeding, lighting, veterinary care, personnel training, Institutional Animal Care and Use Committee (IACUC) formation and function, scientific protocol review, and occupational health and safety. These regulations can be found in 9 CFR parts 1-3.

### Initial European Regulatory Guidance

Early on, European regulatory agencies relied on existing documents pertaining to recombinant technology and production, as there were many similarities. Initial guidance from the European Medicines Evaluation Agency (EMA) came through the Committee for Proprietary Medicinal Products (CPMP) document on the use of transgenic animals for producing human medicines.<sup>9</sup> This document was similar to FDA's guidance document on transgenics. Again, the focus was on the transgene, the animals, husbandry, source material, purification and charac-

terization, etc. The CPMP document on transgenics is now under revision.

More recent regulatory guidance for recombinant technology has come from the International Conference for Harmonization (ICH). Although no specific guidance has been given governing products derived from transgenic animals, the guidance documents that have been published or are under development do provide the fundamentals for recombinant production technology that should be applied, at a minimum, to transgenics.

### Summary

Regardless of the scientific milestones and clinical trial data already generated, a number of questions still abound for the quality, regulatory, and approval process in the arena of transgenic technology. These questions include: acceptable animal husbandry practices, acceptable animal health and testing programs, the fact that it is a new type of animal-derived product, the ability to show purity/potency/efficacy through validated purification processes in pre-clinical and clinical programs, and the willingness of regulatory agencies to accept this technology as an acceptable form of recombinant protein production. Dealing with the number of areas that have not been addressed or clearly outlined has led to a standard of "best practices" that continue to be developed. Additionally, due to the wide range of applications for this technology, there is a lack of uniformity of standards within the industry and uncertainty as to exactly what the regulatory agencies will require. Approval of the first product from this technology is greatly anticipated, as it will demonstrate the capability for transgenic recombinant product production.

However, what is clear, as evidenced by the number of companies with regulatory and clinical milestone achievements, is that moving a transgenic product through both the FDA and

**European approval process is possible and ongoing. For the most part, the technology is being met with enthusiasm and optimism. Based on publicly available information, the first transgenic product is expected on the market in one to two years. The number of products in the development pipeline is expanding significantly and thereafter, one can expect to see a number of transgenically derived products on the market over the next several years.**

NOTES:

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9. *Guideline on the Use of Transgenic Animals in the Manufacture of Biological Medicinal Products for Human Use*, Committee for Proprietary Medicinal Products (CPMP), 1995; III/3612/93.

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