Target Selection and Validation with Transgenic Targeting and *In Vitro* Embryonic Stem Cell Differentiation — The PolyGene Advantage

*a report by*

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**Introduction**

Fuelled by the genomics revolution, pharmaceutical and biotechnology companies are blessed with an unprecedented wealth of potential drug targets. Current therapies predominantly act at the receptor, hormone, ion channel, nuclear receptor, various factor and DNA levels, i.e. on approximately 500 drug targets known and dealt with today. With additional insight gained from different fields of research in the post-genome era, the number of tractable targets is likely to increase significantly, and a prioritised subset of orphan receptors having the greatest therapeutic potential alone, could factor down to as many as 300 candidates.

With skyrocketing costs of drug development, the existence of interesting scientific hypotheses is insufficient to support the levels of commitment required to bring new drugs to market. Potential targets need to be validated beyond findings such as those based on differential messenger ribonucleic acid (mRNA) expression patterns. Modulation of target level and/or activity in cell culture and animals must be shown to significantly ameliorate the disease phenotype. Hence, one of the most significant challenges (particularly with respect to target validation) is the provision of models truly predictive of the disease under investigation.

With the on-going identification of genes, gene products and biochemical pathways potentially involved in the susceptibility to (and/or progression of) human diseases, a key question is how to select the appropriate intervention points amenable to pharmaceutical development.

Irrespective of whether a systems or a molecular approach is chosen (starting from patients or animal models and from clinical or cell samples, respectively), the drug discovery process entails validating targets in both cell and animal models. Ultimately, transgenic and knock-out mice remain the option of choice in target selection and validation because they represent the best available model for investigating the complex interactions that underlie pathophysiological responses.

This article presents two solutions to significantly enhance the current molecular validation toolbox — Transgenic Targeting in mice and embryonic stem (ES) cell differentiation, designed to minimise failure at later stages by allowing more reliable selection and validation of just the most promising candidates early in the drug development process.

**Transgenic Targeting in Mice**

Transgenic Targeting is a procedure in which transgenes are inserted into specific and predetermined genomic loci (e.g. via Cre/lox recombination). A model mouse, in which a lox-flanked marker is placed under control of a given promoter, is generated and the transgene expression pattern thoroughly assessed. Generating further mouse lines by inserting transgenes (replacing the marker) via recombinase mediate cassette exchange reproducibly yields identical expression patterns.

The UBI-mouse, which is now available, is the first in a series of Transgenic Targeting models. In the UBI-mouse, transgenes are specifically placed under control of the β-actin promoter. The born heterozygous animals are phenotypically normal and the cassette exchange procedure reliably yields strong, constitutive and ubiquitous transgene expression (see Figure 1). The following benefits apply to this mouse model:

- Predictability — transgenes are specifically introduced at predetermined genomic loci, warranting defined and precharacterised expression patterns (full documentation on the expected level and pattern of expression is available before project initiation).
- Cost-efficiency — a single mouse line is needed for any one transgene. Since experiments are performed with fewer mouse lines, mouse housing and manpower costs are reduced significantly.
- Speed — all required vectors and genetic screening procedures (polymerase chain reaction, Southern,
primers, probes, controls) are pre-established, thus reducing mouse generation time.

- Flexibility – additional new mutations are easily introduced (truly comparable mouse lines with further transgenes can be established, as required).

- Control – insertional effects (e.g. attribution of phenotypes to gene disruption as a consequence of fortuitous transgene integration) can be excluded.

Offsetting the major drawbacks of conventional transgenics, Transgenic Targeting is ideal for such applications as the following:

- Gain of function experiments – since the procedure is swifter, fully reproducible and reliable with respect to expression pattern (particularly noteworthy for ubiquitous or inducible expression).

- Parallelisation – evaluating a series of targets (one mouse line being sufficient for each transgene).

- Line comparison – be it for judging the effects of various single nucleotide polymorphisms (SNPs), different oncogenes or a series of small interfering RNA (siRNA) molecules.

Exchanging the lox-flanked marker sequence is feasible as well via co-injection (in oocytes) as via co-electroporation (in ES cells) of new floxed-transgene-harbouring constructs with Cre-protein and thus, different procedural strategies (as outlined in Figure 2) have evolved.

Establishing ES cell lines before transgenic mice are generated allows early validation steps to be performed in cell culture (including \textit{in vitro} differentiation), in turn providing important information for decision-making in the target selection process.

ES cells, under defined culture conditions, have the capacity of differentiating into so-called embryoid bodies (in many aspects similar to 11.5-day embryos), and further specifically into a great variety of cell types, including any kind of neuronal cell, haematopoietic or epithelial cells, beating cardiac myocytes, smooth or skeletal myocytes, adipocytes, chondrocytes, etc.

With the broad range of differentiation markers now available, ES cell investigations can be monitored at both the mRNA and the protein level. Applications include the following:

- Testing drugs with unknown cell specificity – embryoid bodies are the ideal model system to screen for anti-angiogenic factors, as well as to test proliferative, cytotoxic or embryo-toxic potentials of compounds such as cytokines, growth or differentiation factors.

- Isolating cell types for pharmacological studies – \textit{in vitro} differentiation is a reliable procedure to establish and purify given cell types such as cardiomyocytes in large quantities for subsequent \textit{in vitro} testing.

\textbf{ES Cell \textit{In Vivo} Differentiation}

Establishing ES cell lines before transgenic mice are generated allows early validation steps to be performed in cell culture (including \textit{in vitro} differentiation), in turn providing important information for decision-making in the target selection process.
Dealing with a newly identified gene or regulatory (e.g. promoter) element – this technique (for example complemented by immunofluorescence microscopy) allows you to assess spatial and temporal regulation of expression (in which cell types and at which stages of embryonic development).

Having established a new knock-out or knock-in ES cell line – in possible anticipation of developmental abnormalities or embryonic lethality, in vitro differentiation is a rapid and reliable method for comparing differentiability of wild-type and genetically modified ES cells.

Summary and Prospects

Reproducibly yielding predictable transgene expression patterns, Transgenic Targeting is the most reliable procedure for mouse transgenesis. The technique is perfectly suited for large-scale target evaluations by knock-in. Moreover, as an effective cellular delivery technique, Transgenic Targeting is the solution of choice for overcoming the limitations of target knock-down (e.g. siRNA) strategies. Combined with in vivo ES cell differentiation for precocious target selection, Transgenic Targeting saves considerable time and money by astute target validation in the drug development process.

A first Transgenic Targeting model, for strong, constitutive and ubiquitous expression, is now available at PolyGene, Inc. Launch of models for tissue-specific (neuron-, pancreas- and adipocyte-) expression is anticipated for the second or third quarter of 2004, and another model, for inducible transgene expression, is currently under development.

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