



X-MAN™ and GENESIS™ FAQ

1. What are X-MAN cell models?

X-MAN cell models are controlled pairs (**M**utant **A**nd **N**ormal) of human, or other mammalian cell lines, that have defined genetic mutations (X), knocked-in or knocked-out; and which accurately model the disease causing mutations found in cancer patients.

2. What is GENESIS?

GENESIS is a virally-mediated (Adeno-Associated Virus) homologous recombination technology that enables the first efficient means to perform gene-targeting in somatic human cell-types. GENESIS has a level of efficiency that is orders of magnitude greater than previous plasmid-based technologies that are typically used to create transgenic mice from embryonic stem-cells, but are poorly efficient in somatic cell types.

3. How do you make X-MAN cell lines?

GENESIS targets the gene of interest at its endogenous locus; allowing for the first time the accurate modelling of disease causing mutations (knock-ins or knock-outs) and single nucleotide polymorphisms (SNPs) in human somatic cell-lines. GENESIS also permits the definitive study of gene function or protein activity via highly specific targeted knock-outs of the whole protein or discrete protein domains, respectively.

The steps involved are:-

1. Define the mutation to be inserted, or the gene to be knocked-out
2. Engineer an AAV virus with homology to the target gene, carrying a defined mutation
3. Infect the cell line of interest
4. Clonally select individual cell lines and PCR screen for the correct recombination event
5. Confirm the mutant cell line at the genomic level and perform biochemical characterisation for gain/loss-of-function prior to shipping the cell line

4. What are the features of X-MAN cell lines that make them different from other cell lines?

The most important feature of X-MAN cell-lines is that DNA-modifications are always made within the endogenous gene; closely recapitulating the genetic events that lead to a specific disease. The second important feature is that a matched 'isogenic' normal cell-line is also provided; containing a wild-type version of that gene. This enables the definitive and controlled study of a chosen genetic alteration on a cell's function and the search for novel pharmaceutical agents that selectively target it.

5. Why do we need to create X-MAN cell lines?

Targeted drug discovery aims to better target the root causes of a disease. In the case of cancer, there are 100's of cancer genes now known and very few cell-based models that either; a) carry your mutant gene or genes of interest, or b) provide a matched normal control line that enables the study your gene or genes of interest away from other confounding genetic variations. GENESIS allows the engineering of any genetic variation into any endogenous target locus to accurately recreate any target-patient genotype of interest.

6. How many X-MAN cell lines are there?

To date there are over 150 X-MAN cell lines in our Discovery 1, Discovery 2 and Researcher panels. These have been assembled by Horizon through in-house development programs and collaborative research groups using this gene-targeting technology over the last 15-years. Collectively, these cell lines are being marketed under the X-MAN brand.



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7. What is the difference between Discovery 1, Discovery 2 and Researcher X-MAN lines?

Discovery 1 lines are 'single-mutant' gene containing cell-lines - These encompass both 'gain-of-function' models (generated via the knock-in of oncogenic mutant alleles) and 'loss-of-function' models (generated via the knock-out of pre-existing oncogenic mutant alleles or tumour suppressor genes). Discovery 2 lines are 'double-mutant' cell-lines - These are currently 'gain-of-function' models generated via the double knock-in of two different oncogenic cancer genes. X-MAN lines can either be in a normal parental background, or be created from a cancer cell line with an established mutant/normal genotype. X-MAN Researcher cell-lines are cancer cell lines (mainly colon) with a specific cancer-associated gene knocked-out to study its gene-function.

8. Can X-MAN cell-lines model diseases other than cancer?

Any genetic variation can be introduced using the GENESIS system, so any genetic disease or predisposition can be modeled using X-MAN cell-lines. Horizon will be addressing this need for genetically defined disease models in other therapeutic areas in the coming months and years.

9. What are AAV-Targeting Vectors?

AAV-targeting vectors are engineered AAV-viruses containing a single-stranded DNA 'replacement' genome that is substantially homologous to the target gene of interest. DNA-variations incorporated into this AAV-homology construct enable the efficient introduction of specific mutations or gene-deletions into any chosen genomic locus; not just within a defined location on chromosome 19, which represents the natural integration site for wild-type AAV. Engineered AAV-vectors do not have any viral genes, and thus, no viral genes are co-inserted into the target cell's genome during the homologous recombination process.

10. Why are AAV vectors better at gene targeting than other methods?

The homologous recombination machinery is essentially shut off in somatic cells. However, AAV-vectors uniquely deliver their targeting constructs in the form of a single-stranded DNA-species, which seems to reactivate this process in some way – perhaps via mimicking a cross-over event or damaged DNA. While the mechanism is not well understood, AAV-vectors are consistently more efficient than double-stranded plasmid-based vectors. Moreover, AAV-vectors elicit precise alterations within their target genes, without introducing confounding 'side-modifications' that are inherent with other gene-targeting techniques (see 'Zinc Fingers').

11. Are AAV viruses pathogenic?

Wild-type Adeno-Associated viruses (AAV) are small non-pathogenic DNA viruses that infect humans and other vertebrate species without causing a disease. Wild-type AAV produce a very mild immune response and this property, combined with their ability to integrate into a specific site on chromosome 19, has led AAV to be proposed as safe vehicles to perform gene-therapy. Our engineered AAV-vectors do not have any viral genes, and thus lack the ability to replicate and no viral genes are incorporated into the final targeted genomic site.

12. Do any viral 'sequences' remain in the cells after causing the desired mutation(s)?

While there are no viral genes in our AAV-targeting constructs, there are two non-coding viral ITR-sites that flank the AAV-vector. This allows the vector to be packaged into a viral capsid when it is introduced into a specialised packaging cell-line (where all replication and capsid genes provided in trans). Other elements in the vector are: a) DNA homologous to the target human gene in question, b) a commonly used heterologous selection marker e.g., NEO, and c) two lox-P recombination sites to enable the subsequent removal of the selection marker. In our experience, the non-coding ITR sequences are not integrated into the final targeted genomic locus due to their position significantly distal to the site of homologous recombination.

13. Will GENESIS knockout or modify a gene in my cell line of interest?

Yes. GENESIS has been used to knockout many genes (from tumour suppressor genes such as p53, PTEN and BRCA) or perform knock-ins of activated mutant oncogenes (such as K-Ras, PI3K and EGFR) in a range of cell-lines (both human and mouse). The tropism of AAV is wide with respect to tissue type and species of cell. The only major requirement is that the cell-line grows continuously in culture conditions. There is no limit to the number of rounds GENESIS can be used. Sequential gene-targeting enables either both alleles of a target endogenous locus to be modified, or the building of multiple disease genotypes within one target cell.

14. Should I expect off-target effects with GENESIS?

GENESIS does not use any 'priming' DNA-cleavage methodology; homologous recombination is mediated solely via its approximate 2kb of homology to the target locus. Potential concerns with random double strand-breaks are therefore removed. The process of homologous recombination is still competing, however, with non-homologous recombination; even using our highly efficient AAV-vectors. For this reason, all our cell-lines go through a clonal selection process; and Southern Blotting confirms that in each resulting clone, genomic integrations are always single events using homology-directed AAV-vectors.



15. How does GENESIS differ from other gene insertion/knockout technologies?

This can be best summarized in the table below:

Technique	Gene Knock-in	Gene Knockout
GENESIS	Targeted insertions or modifications are created within endogenous genes; and so are subject to: a) the correct gene-regulation mechanisms; and b) accurately reflect the disease events found in real patients. GENESIS can introduce subtle point mutations, SNPs as well as small insertions with high efficiency. Moreover, many peer reviewed studies have shown that GENESIS does not introduce any confounding off-target genomic events.	Gene knockouts are at the endogenous locus, and thus are definitive, stable and patient relevant. No confounding off-target effects are elicited at other genomic loci. It requires a 2-step process: 1. Generate a heterozygous KO 2. Generate a bi-allelic knockout by targeting the second allele This process can therefore generate 3 genotypes (+/+; +/- and -/-); enabling therefore the analysis of haplo-insufficient gene function.
Plasmid based homologous recombination	Insertion is at the endogenous locus and has all the above benefits, but it is very inefficient. It also requires a promoterless drug selection strategy entailing bespoke construct generation	Deletion is at endogenous locus and has all the above benefits, but it is inefficient. It also requires a promoterless drug selection strategy that entails bespoke construct generation
Flp-in	This is an efficient technique that allows the directed insertion of 'ectopic' transgenes at a single pre-defined genomic locus (via the integration of a FLP recombinase site). This is not a technique for modifying any endogenous locus. Transgenes will usually be under the control of an exogenous promoter, or a partially defined promoter-unit in the incorrect genomic location. Their expression will therefore not be under the same genomic and epigenetic regulation as the endogenous loci, which limits the utility of these systems for studying gene-function. They are however, good for eliciting rapid and stable exogenous gene expression.	Not applicable
Zinc-Finger Nucleases (ZFNs)	ZFNs have been reported to achieve high rates of genetic knock-outs within a target endogenous gene. If ZFNs are co-delivered with a transgene construct homologous to the target gene, genetic knock-in's or insertions can also be achieved. However, few peer-reviewed publications exemplifying this application can be found and user feedback is mixed regarding the efficiency of the latter. One worrying aspect is the potential for ZFNs to produce off-target double-strand breaks which may also lead to random off-target gene insertions, deletions and wider genomic instability; confounding the resulting genotype. Whole genome sequencing would also be required as a standard characterization step to determine whether such off-target genomic alterations are present in any resulting cell-line.	ZFNs are sequence-directed endonucleases which enable the rapid and highly efficient (up to 90% in a bulk cell population) disruption of both alleles of a target gene. However, user-defined or patient-relevant loss-of-function alterations may be more difficult to achieve. Off-target deletions or insertions elsewhere in the genome cannot also be controlled for or readily defined. The speed advantage of obtaining a biallelic KO in one step is also partially mitigated if one needs to derive a clonal cell-line study gene function in a homogenous cell-population. ZFN are likely more selective than RNAi, however, for high-throughput functional genomic studies.

16. How long does it take to make X-MAN models?

Currently it takes 12 weeks to make a human cell-line pair with a single knock-in of a desired mutant gene. To make a human cell-line pair with the knock-out of both target alleles currently takes 16 to 20 weeks.



Key Publications relating to X-MAN cell lines and GENESIS:

Use of isogenic human cancer cells for high-throughput screening and drug discovery
[Nat Biotechnol.](#) 2001 Oct; 19(10): 940-5

Targeted transgene insertion into human chromosomes by AAV vectors
[Nat Biotechnol.](#) 2002 Jul; 20 (7):735-8

Mutational analysis of the tyrosine kinome in colorectal cancers
[Science.](#) 2003 May 9; 300 (5621): 949

Facile methods for generating human somatic cell gene knockouts using AAV
[Nucleic Acids Res.](#) 2004 Jan 2; 32(1): e3

Mutational analysis of the tyrosine phosphatome in colorectal cancers
[Science.](#) 2004 May 21; 304 (5674): 1164-6

Improved methods for the generation of human gene knockout and knock-in cell lines
[Nucleic Acids Res.](#) 2005 Oct 7; 33(18): e158

Somatic mutation of EGFR domain and treatment with gefitinib in colorectal cancer
[Ann Oncol.](#) 2005 Nov; 16(11): 1848-9. Epub 2005 Jul 12

Kinase mutations in cancer: chinks in the enemy's armour?
[Curr Opin Oncol.](#) 2006 Jan; 18(1): 69-76

Oncogenic activation of the RAS/RAF signalling pathway impairs the response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies
[Cancer Res.](#) 2007 Mar 15; 67(6): 2643-8

Genetic targeting of the kinase activity of the Met receptor in cancer cells
[Proc Natl Acad Sci U S A.](#) 2007 Jul 3; 104(27): 11412-7. Epub 2007 Jun 26

Knock-in of Mutant K-ras in Nontumorigenic Human Epithelial Cells as a New Model for Studying K-ras-Mediated Transformation
[Cancer Res.](#) 2007; 67: (18). September 15, 2007

Knock-in of oncogenic Kras does not transform mouse somatic cells but triggers a transcriptional response that classifies cancers
[Cancer Res.](#) 2007 Sep 15; 67(18): 8468-76

Wild-Type BRAF Is Required for Response to Panitumumab or Cetuximab in Metastatic Colorectal Cancer
[Journal of Clinical Oncology](#) Published Ahead of Print on November 10, 2008 as 10.1200/JCO.2008.18.0786

Replacement of Normal with Mutant Alleles in the Genome of Normal Human Cells Unveils Mutation-Specific Drug Responses

[Proc Natl Acad Sci U S A](#) December 30, 2008 vol. 105, no. 52

Knock-in of Mutant PI3KCA Activates Multiple Oncogenic Pathways

[Proc Natl Acad Sci U S A](#) Printed early at www.pnas.org/cgi/doi/10.1073/pnas.0813351106

PIK3CA Mutations in Colorectal Cancer Are Associated with Clinical Resistance to EGFR-Targeted Monoclonal Antibodies

[Cancer Res.](#) 2009; 69: (5). March 1, 2009

A Panel of Isogenic Human Cancer Cells Suggests a Therapeutic Approach for Cancers with Inactivated p53
[Proc Natl Acad Sci U S A](#) Printed early at www.pnas.org/cgi/doi/10.1073/pnas.0813333106

