Altogen Labs

Provider of Preclinical Research Services (GLP/non-GLP) for Drug Discovery
Efficacy and Pharm/Tox IND contract research studies (clients worldwide)
100+ Xenograft Models (validated in-house) and IND-enabling Toxicology studies
100% IP belongs to client, experienced IACUC-regulated barrier facility

Generation of Stable Cell Lines

Contact us: **info@altogenlabs.com** | Read more at **AltogenLabs.com**
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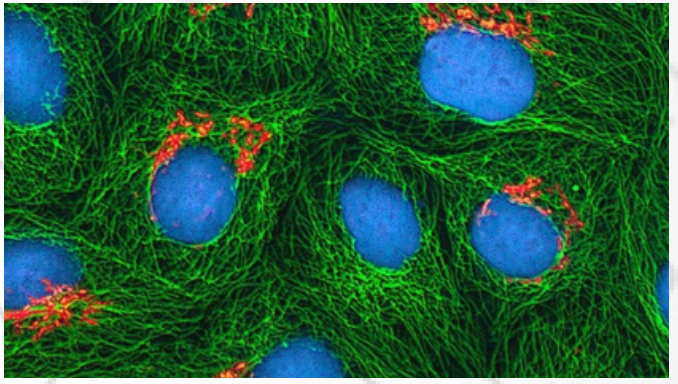
Transfected genetic material

Transfected genetic material can be expressed in the target cells either transiently or permanently, depending on the methods utilized and the experimental questions being investigated. Stable transfected cell lines are developed in order to analyze the long-term impact of altered gene or protein expression.

Altogen Labs provides services for the following type of cell lines:

1. Reporter stable cell lines (luciferase, GFP, RFP, YFP)
2. Protein overexpressing cell lines
3. Knockdown (RNAi) cell lines
4. Tetracycline-inducible cell lines



GFP-expressing HeLa cells
src: tumblr.com

Transient Transfection

- Transient transfection is used most commonly to analyze the short-term impact of altered gene or protein expression. Plasmid DNA (pDNA), messenger RNA (mRNA), short interfering RNA (siRNA), and microRNA (miRNA) are introduced and gene products are expressed in the target cells. However, the nucleic acids do not integrate into the host cell genome.

- Therefore, gene product expression is transient and typically results in high expression levels that persist for 24-72 hours when RNA is transfected, or 48-96 hours following DNA transfection.

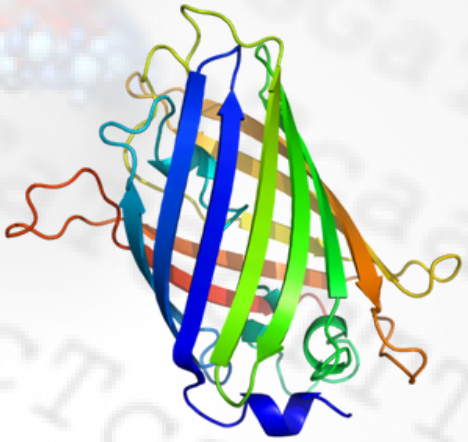
Stable Transfection

- Conversely, stable transfected cell lines are developed in order to analyze the long-term impact of altered gene or protein expression. In a subpopulation of transfected cells, the transfected genetic material will integrate into the genome. In order to create stable cell lines, the gene of interest along with a selectable marker is introduced in to the cell.

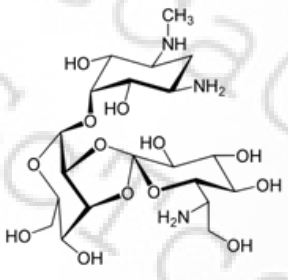
- The growth of transfected cells, in the presence of a selecting agent, will enable the subpopulation of cells in which the exogenous genetic material has been incorporated into the genome to persist while the remaining cells undergo selection.

Stable Cell Line Selection

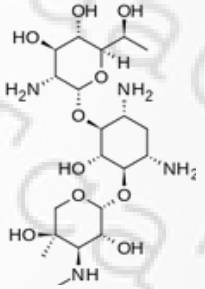
Typically, antibiotic resistance or fluorescent reporter gene markers are incorporated into the plasmid DNA construct to facilitate selection process. These selection markers can be co-expressed on the same vector or independently expressed on two separate vectors. The selection process facilitates the selection of the most efficient expressers or silencers of the gene of interest.



Green-fluorescent protein



Hygromycin B

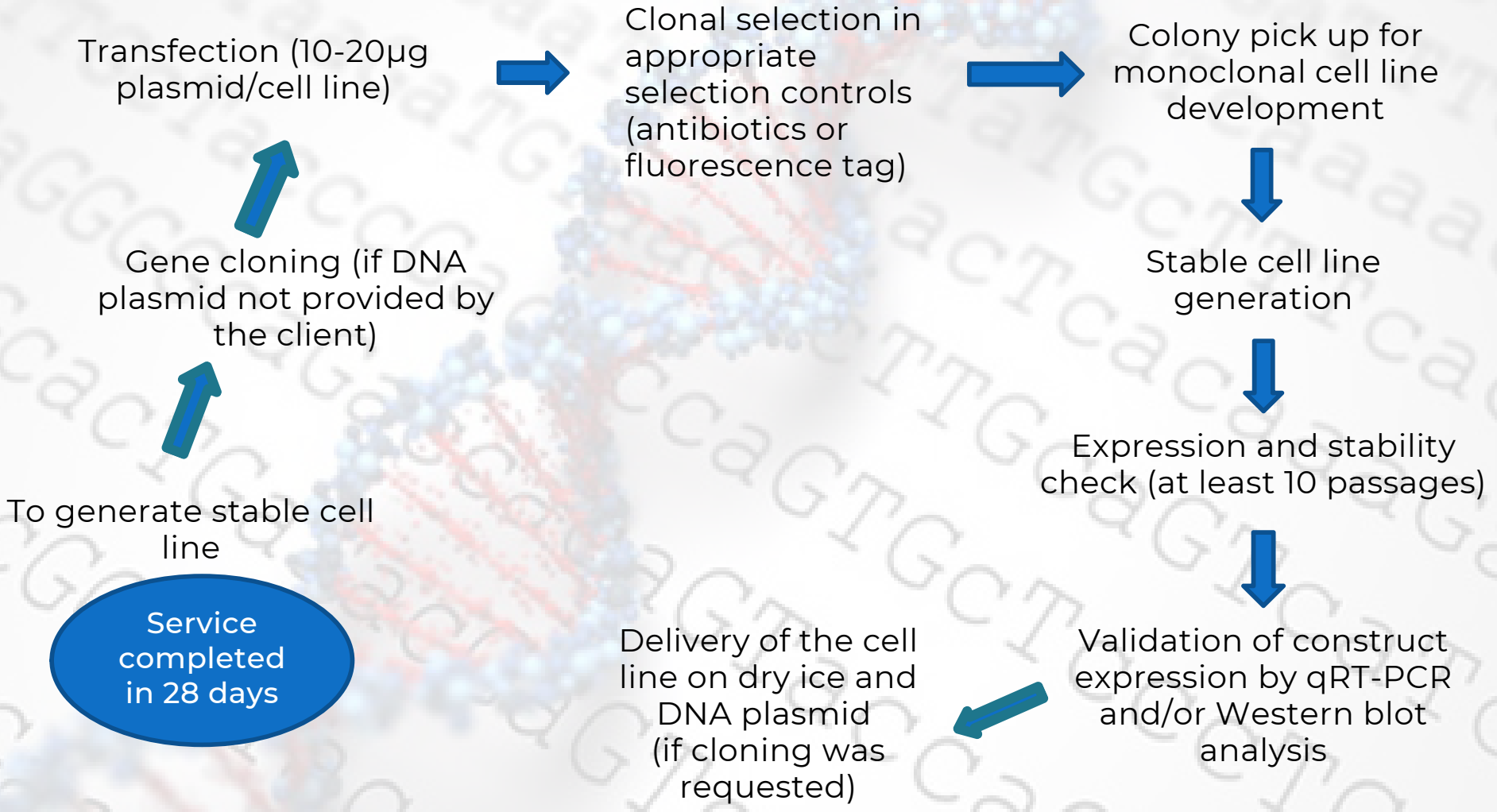


G418

Geneticin

For example, Geneticin, also known as G418 sulfate, is commonly used for the selection of mammalian, plant, or yeast cells. Hygromycin B is an aminoglycosidic antibiotic that can inhibit protein synthesis by disrupting translocation and promoting mistranslation of the 80S ribosome. Hygromycin B can be utilized in dual-selection experiments since its mode of action is different from Geneticin.

Stable Cell Line Work Flow



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Mammalian Stable Cell Line Development

Hybridoma mammalian cell lines/hybrid cells produced from the fusion of normal cells with myeloma tumor cells.

Characteristics:

- Constitutively produce specific antibodies from the primary lymphocytes
- Immortalization
- Mammalian post-translational modification and protein folding that are crucial in the production of antibodies, recombinant proteins, viral-subunit proteins, and vectors for gene therapy

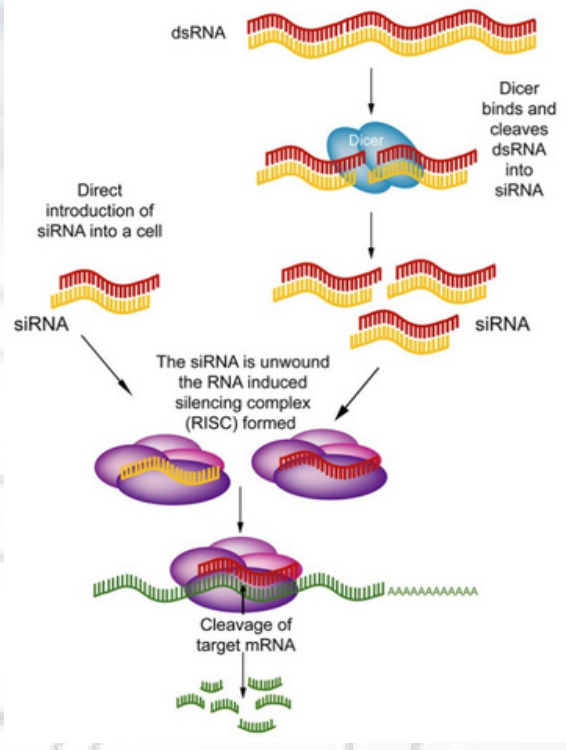
Non-hybridoma stable cell lines may be produced with integrated plasmids in the genome that also produce gene-specific over-expression of desired proteins. Importance: Gene therapy, new compound screening, and therapeutic drug research

Stable RNAi Cell Line Generation

- The mechanism of RNAi is based on the sequence-specific degradation of host mRNA through the cytoplasmic delivery of double-stranded RNA (dsRNA which are typically 21-28 nucleotides in length) identical to the target sequence.

- Targeted gene silencing is achieved by the transfection of small interfering RNA (siRNA), which are bound by the RISC complex used as guides for destruction of the mRNA containing the complementary sequence. RNAi induced gene silencing can be achieved by stable engineering of a cell line expressing the siRNA.

- RNAi expressing cell lines allow for functional studies of genes, gene target discovery, validation of gene targets, assay development, and screening of drug compounds.



Gene Silencing via RNA Interference

Tetracycline Inducible Cell Lines

RNAi gene knockdown experiments using transient/stable over-expression are not viable when manipulations of gene expression result in cell growth/proliferation defects or unwanted cell differentiation. Consequently, researchers have adapted the Tetracycline repressor protein (TetR), taken from *E. coli*, to generate very efficient and tight regulatory systems to express cDNAs in mammalian cells.

TetR has been modified to either:

- Block initiation of transcription by binding to the Tet-operator (TO) in the promoter region upon addition of tetracycline (termed Tet-off system)
- Bind to the TO in the absence of tetracycline (termed Tet-on system).

Tetracycline Inducible Cell Lines

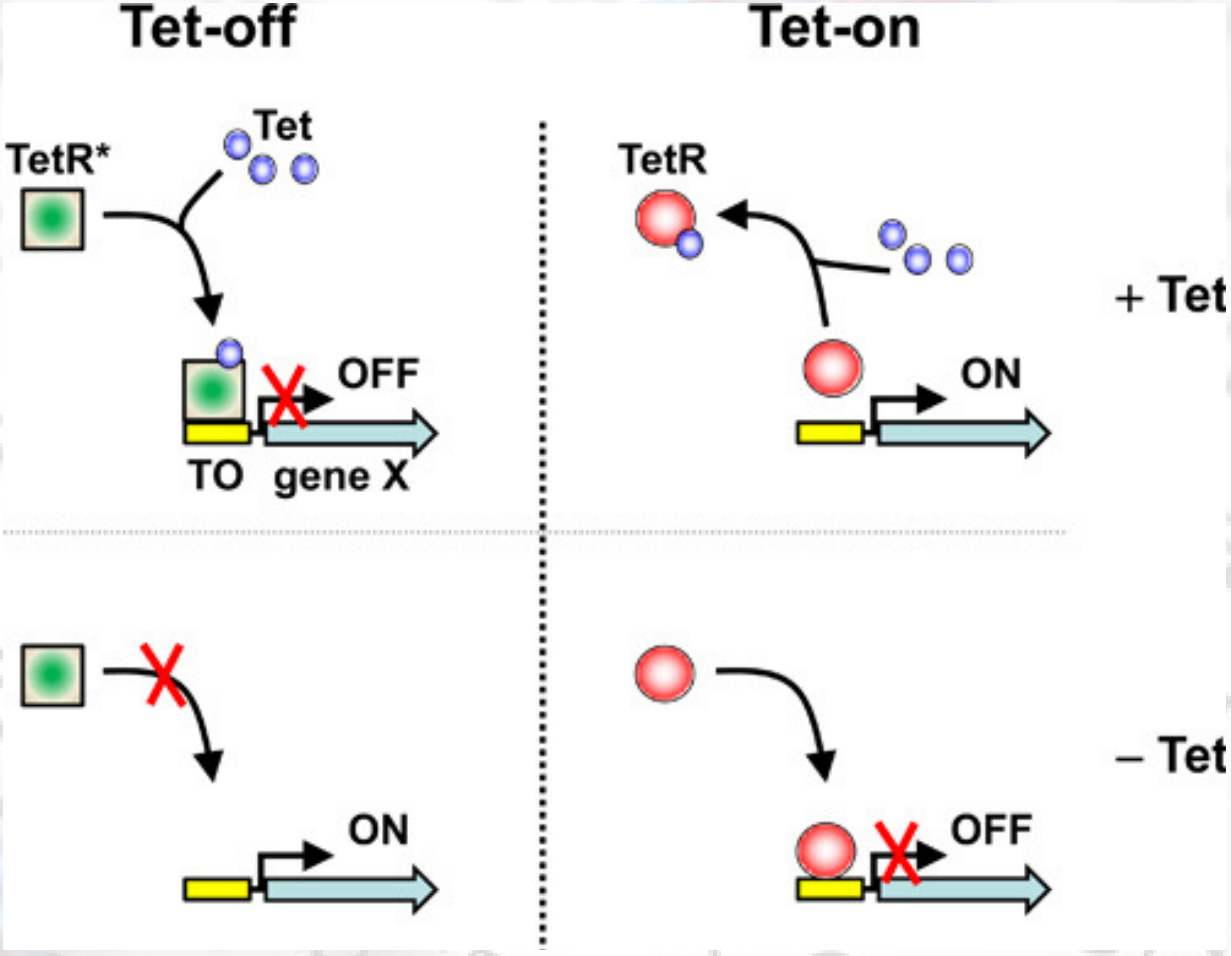
The Tet-off system requires the continuous presence of tetracycline (which has a half-life of about 24 hr in tissue cell culture medium). Hence the Tet-on system has been more extensively optimized, resulting in the development of very tight and efficient regulation of expression.

Positive Selection Markers

- Positive selection markers make stable cell line generation possible and effective. They can be delivered in different ways, depending on the project.

- Cells differ in their susceptibility to certain markers such as G418 but marker selection is determined with the project and relevant cells in mind.

Tetracycline Inducible Cell Lines



Source: Gomez-Martinez et al., 2013

Stable Cell Line Services

We have optimized our stable cell services to be delivered in 28 days.

- In-house cell lines built on the Tet On/Off system
- More than 150 cancer cell lines available
- Highly efficient gene delivery technologies
- We have extensive cell culture experience and expertise, as well as specific optimized protocols for efficient cell line generation
- Customizable reporter gene expression systems
- Cost effective and time managed delivery (Duration: 28 Days)

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Contact Us

In addition to cell line development services, Altogen Labs provides following preclinical research services:

- Cell banking
- RNA interference services
- Cell-based assay development and antibody production
- Vector construction, cloning and recombinant construct expression
- Xenograft animal services
- Pharmacology and toxicology studies

Altogen Labs offers a full spectrum of pre-clinical research services in a GLP-compliant environment

Contact us to discuss details, timeline estimates, and price!

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