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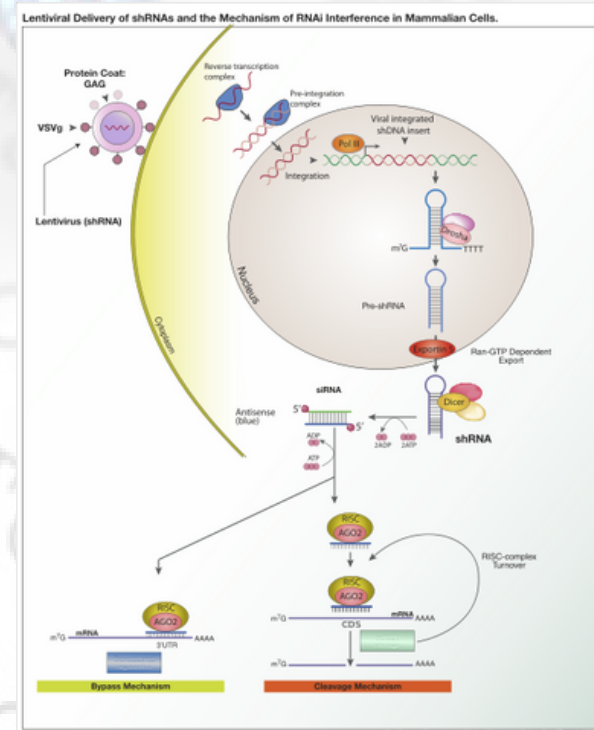
Provider of Preclinical Research Services (GLP/non-GLP) for Drug Discovery
Efficacy and Pharm/Tox IND contract research studies (clients worldwide)
100+ Xenograft Models (validated in-house) and IND-enabling Toxicology studies
100% IP belongs to client, experienced IACUC-regulated barrier facility

RNA Interference (RNAi)

Contact us: info@altogenlabs.com | Read more at AltogenLabs.com
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RNAi

- RNA Interference (RNAi) technology has made it possible to effectively regulate gene expression post-transcriptionally.
- The ability of RNAi to knock down target genes enables scientists in identification and validation of drug targets as well as in the discovery of cell-signaling pathways that are vital to organism survival and viral defense.



RNA interference pathway
www.wikipedia.org

How Does It Work?

- RNAi is a cellular process in which gene expression is reduced in a sequence-specific manner following the expression of short-hairpin RNA (shRNA) within the cell. Transfection of a plasmid DNA construct containing shRNA and an antibiotic-resistant gene induces the knockdown gene expression of the gene of interest and enables selection and isolation of successfully transfected cells. Cells with a prolonged ability to proliferate in the presence of a selecting agent (antibiotic) are capable to initiate a stable RNAi gene knockdown cell line. This important research tool has been vital to the studying of mammalian gene function and gene targeting for potential therapeutic treatments.

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Applications

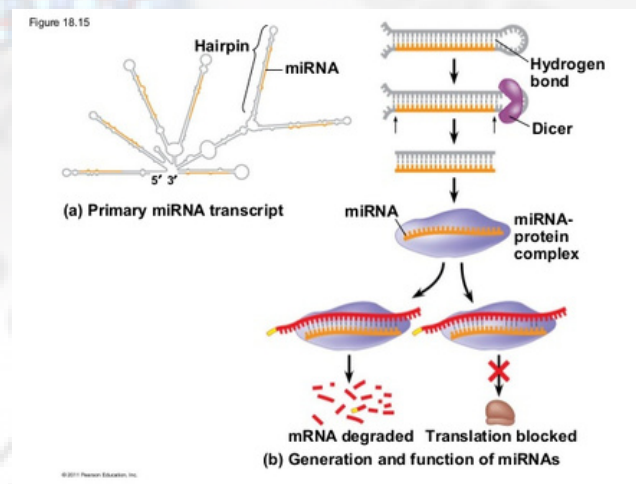
- RNA interference (RNAi) is a powerful experimental method for specific silencing of genes, and is now an important technology for the elucidation of gene function, as well as gene involvement in specific biological processes.
- RNAi screening technology (high-throughput siRNA and microRNA library screens) accelerates the identification of novel genes and their functions, and accordingly, the discovery of new gene signaling pathways.

RNA, miRNA, and siRNA

- Ribonucleic acid (RNA) is a biopolymer central to various biological processes including coding, decoding, regulation, and expression of genes. RNA and DNA are nucleic acids, and, along with proteins and carbohydrates, constitute the three major macromolecules essential for all known forms of life.
- Two types of small ribonucleic acid (RNA) molecules – microRNA (miRNA) and small interfering RNA (siRNA) – are central to RNA interference.

RNA, miRNA, and siRNA

- A miRNA molecule is single-stranded and 22 nucleotides long; it binds to a complementary segment of RNA to create a double-stranded molecule. Both of these molecules may occur naturally in the cell as well as being artificially introduced.



miRNA regulation of gene expression

<https://www.slideshare.net/kindarspirit/18-regulation-of-gene-expression>

Quality Services

- At Altogen Labs, our scientists apply advanced biotechnological techniques to develop new RNAi products and RNAi services.
- We are committed to offer the **highest quality RNAi services** for scientific research at the lowest price. Altogen Labs' life science research services include complete A-to-Z RNAi gene silencing services including gene targeting, siRNA synthesis, functional *in vitro* validation, siRNA encapsulation, *in vivo* siRNA protection and tissue-targeted delivery (mouse and rat models).

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Screening Services

- We also perform RNAi library-based high-throughput screening, which expedites finding corresponding genes for particular phenotypes, accelerating the process of scientific discovery.
- Also available: custom siRNA libraries, RNAi manufacturing, quality control validation, and cell-based assay development services.

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Cloning Services

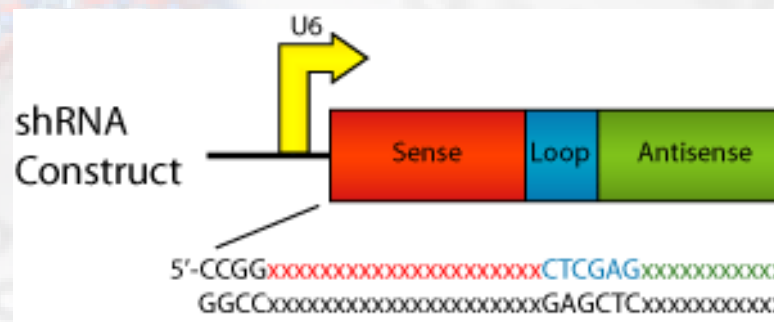
- Altogen Labs offers molecular cloning and subcloning services, DNA sequencing, alignment, and plasmid construction. Customers are welcome to send Altogen Labs the Gene ID/Genbank/Swiss-Prot number, the gene/protein sequence, or a plasmid containing a gene of interest to start the project.
- This technology gives scientists the ability to silence genes in a reliable manner and has the potential for future drug development for such diseases as HIV and cancer.

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PCR Cloning DNA Vector Construction (shRNA)

- Short hairpin RNA (shRNA) refers to a sequence of RNA that has the ability to silence genes in a sequence-specific manner via RNA interference (RNAi). Through mechanisms of mRNA cleavage when in complex with the RISC complex, shRNA can be used to knockdown the expression of target proteins.



DNA construct expressing a shRNA
addgene.org

In Vivo siRNA Delivery and Tissue Targeting (In Vivo RNAi)

- Two basic methods are adapted for RNAi use *in vivo*: administration of siRNA or miRNA and administration of plasmid DNA and viral vectors, which *in vivo* express short hairpin RNAs (shRNAs) that are subsequently processed into active siRNA.
- One of the key challenges faced when using RNAi technology is the successful delivery of a stable and functional siRNA or microRNA molecule to the target tissue. Altogen Labs has developed methods to prevent siRNA degradation in serum (including both chemical modification and encapsulation of siRNA), increasing delivery efficiency to the target organ, and producing strong tissue-targeted functional effects.

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Modifications

- Chemical modifications of siRNA or miRNA are performed to increase their stability in vivo. These chemical modifications sustain the potency, efficacy and specificity of the siRNA molecules and increase their overall stability in the dynamic environment of a living organism. Standard modifications include 2'MOE, FANA, 2'-Fluoro, LNA, and encapsulation into liposomes.

siRNA Transfection and RNAi Cell-based Library Screening

- In the pursuit of understanding gene functionality, RNAi library screening has become a valuable research tool used to study gene function and associated signaling pathways. RNAi screening has allowed researchers to study the functionality of genes in a cell, both individually and in combinations of up to thousands of genes at a time, by temporarily silencing their expression.
- Altogen Labs offers siRNA and miRNA library screening, primary (high-throughput) and secondary cell-based assay development, and validation. **At Altogen Labs we have developed and validated highly efficient and optimized delivery systems for over 80 cell lines and over 20 primary cell cultures to provide high throughput shRNA, siRNA and miRNA library screening.**

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Custom Services

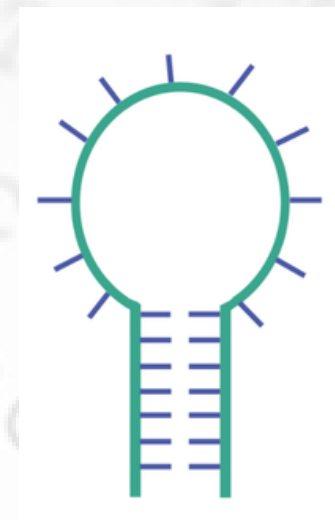
- Custom RNAi services include: siRNA Transfection, RNAi cell-based library screening, siRNA/shRNA design, synthesis, in vitro validation, qRT-PCR test of target mRNA knockdown, development of RNAi cell lines, mRNA reduction in tissues, siRNA targeting, chemical modification, cell transfection optimization, siRNA and microRNA genome-wide library screening, RNAi assay development.
- Altogen Labs offers transient and stable RNAi transfection services based on the client's individual needs.

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Generation of Tet-inducible RNAi Gene Knockdown Cell Lines

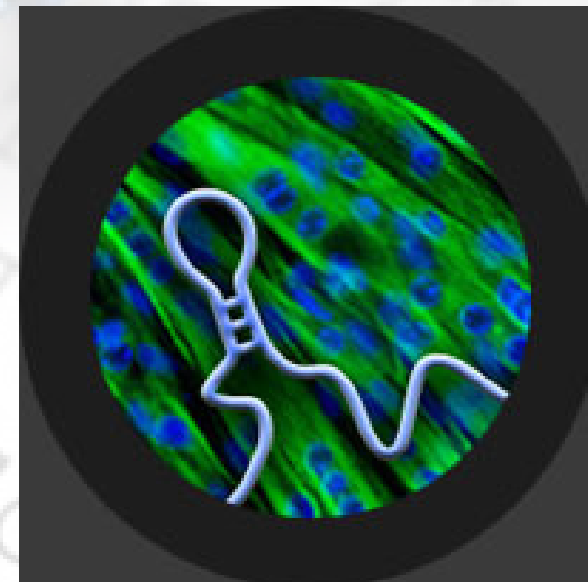
- Altogen Labs offers the development of inducible RNAi stable cell lines expressing shRNA constructs that target genes of interest. The generation of tetracycline-inducible RNAi gene knockdown cell lines allows researchers to tightly control short hairpin RNA (shRNA) expression. The client is only required to provide the gene ID of the target
- Standard services include transfection of plasmid DNA (at least 20 µg) into a cell line of interest, drug selection of clonal cells, generation of stable cells, validation of functional shRNA construct expression, and RNAi gene silencing by real-time qRT-PCR (reduction of mRNA expression) and/or Western blot (reduction of protein expression). Final cell lines are delivered to the client as cryovials on dry ice.



Stem loop structure of an shRNA

RNAi Screen Pathway Analysis: siRNA and microRNA

- Genome-wide library screening produces a comprehensive set of genes involved in cell signaling pathways, and enables secondary screens for more detailed pathway analysis and characterization.
- With over 30,000 genes in the human genome, simultaneous RNAi library screening of several thousands genes requires robust statistical pipeline and pathway analysis.



www.broadinstitute.org

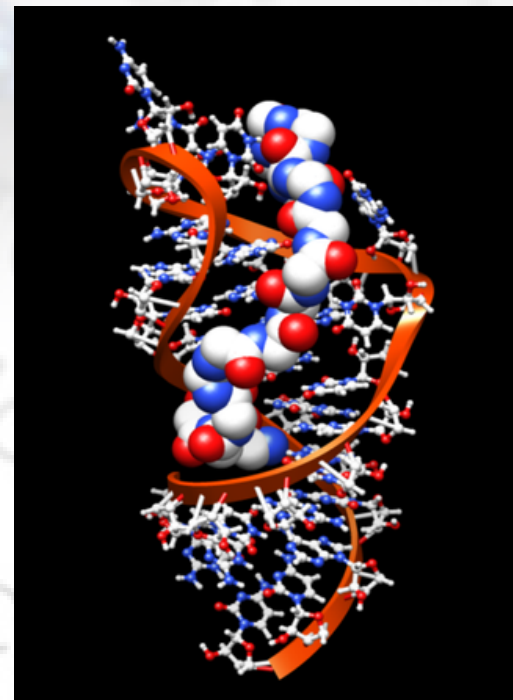
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RNAi Manufacturing and CRO Services

- Sequence-specific gene silencing (RNAi) through the use of siRNA and shRNA has become a vital experimental tool in the study of gene function and genetic involvement in disease and cell signaling pathways.
- At Altogen Labs, we provide complete siRNA design, synthesis, and chemical modification services, as well as service for preparing DNA templates and synthesizing hairpin shRNAs. Purification of the resulting shRNAs and preparation for transfection into cultured cells are offered as well.



RNA aptamer bound to a peptide

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RNAi Manufacturing and CRO Services

- mRNA Amplification
 - Altogen Labs uses the T7 promoter (tagged polydT) primer for priming first strand cDNA synthesis. Double-stranded cDNA is used as a template for *in vitro* transcription. In addition to standard ribonucleotides, company scientists also incorporate modified nucleotides (e.g., aminoallyl-UTP) to produce labeled RNA probes. The resulting ribonucleotides can then be used to amplify the original mRNA more than a million fold. RNA is also purified and prepared for all additional applications.
- Gene Knockdown by shRNAs
 - Altogen Labs offers gene knockdown services and quantitation of shRNA-induced RNAi *in vitro* and *in vivo*. Our standard service include shRNA design, construction, and sequence confirmation of the shRNA vectors, synthesis of the SYBR-labeled qPCR primers for quantitation of target mRNA expression, transient shRNA transfection, and gene expression analysis by qPCR at 24, 48, and 72 hr post-transfection with associated Western blot analysis at 48 and 72 hr timepoints.

Altogen Labs guarantees 70% or more inhibition of target gene expression.

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PCR Cloning DNA Vector Construction (shRNA)

- At Altogen Labs, we specialize in the shRNA design and synthesis of shRNA-encoding vector constructs as well as the purification of shRNA encoding plasmid DNA and transfection of shRNA-expressing constructs into cultured cells. Altogen Labs offers a complete service for preparing DNA templates and synthesizing hairpin shRNAs.
- In order to successfully knockdown genes, it is imperative that multiple vector constructs are designed to target various regions of the gene of interest. There are several criteria to be considered: knockdown efficacy, stability, longevity of RNAi effect, etc.

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Available Vectors

- Altogen Labs offers a library of commercially available vectors for specific applications (shRNA designed to target gene of interest, vectors encoding antibiotic resistance gene for selection, etc.)

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High-throughput Screens

- Altogen Labs provides genome-wide siRNA and miRNA (microRNA) high-throughput screens (HTS) for RNAi research. RNAi HTS technology enables researchers to simultaneously screen thousands of loss-of-function genes and identify the association of genes with their corresponding biological phenotypes.

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Recap of Services

Altogen Labs provides a number of RNAi services:

RNAi assay development, in vivo siRNA synthesis, encapsulation, and administration, siRNA transient and shRNA stable transfection, RNAi cell-based library screening, siRNA/shRNA design, synthesis, in vitro validation, qRT-PCR test of target mRNA knockdown, development of RNAi cell lines, mRNA reduction in tissues, siRNA targeting, chemical modification, cell transfection optimization, siRNA and microRNA genome-wide library screening.

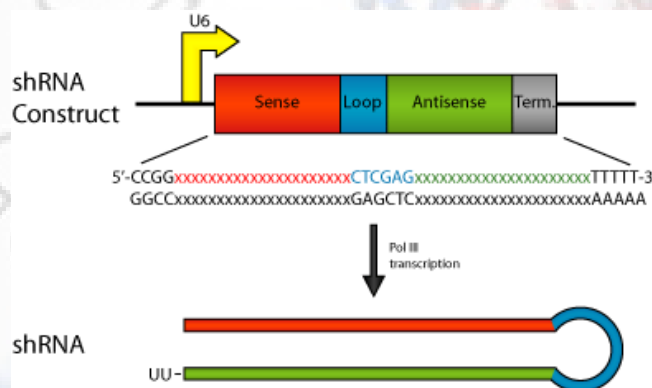
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Quantitation sh/siRNA-induced Knockdown by qRT-PCR and WB

- Altogen's shRNA knockdown services includes: Design, construction, and cloning of shRNA into commercially available vectors, design and synthesis of the SYBR-labeled qPCR primers for quantitation of target gene expression, shRNA transfection into the cell line supplied by the customer. Gene expression analysis is performed by qPCR at 24, 48, and 72 hr post-transfection. **Altogen Labs guarantees 80% or more inhibition of target gene expression.**



A DNA vector construct is transcribed to produce a pre-RNA that is edited to produce a mature shRNA

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Genome-wide siRNA and microRNA High-throughput Screens

- At Altogen Labs, we focus on gene target discovery to identify potential targets for therapeutic treatment and compound inhibition as well as target novel proteins involved in disease pathways. While working with Altogen Labs, you can be confident that comprehensive analysis of data collected during genome-wide HTS will be fully examined and validated to determine the most promising candidates.

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Stable RNAi Cell Line Generation: Stable Gene Knockdown

- Altogen Labs offers generation of stable RNAi cell lines expressing shRNAs targeting genes of interest. Such stable RNAi cell lines can be a tremendously powerful research tool, however, the generation of RNAi cell lines and primary cells can be very laborious, expensive, and time-consuming to produce. The distinction of a stable cell line is that the exogenous genetic material (in this case shRNA) has been fully incorporated into the genome of the cell resulting in permanent expression of the vector (plasmid DNA construct).
- Altogen Labs offers the generation of stable knockdown (RNAi) cell lines based on individual client requirements and project specifications. Validation of gene silencing is performed using real-time qRT-PCR (mRNA expression level) or Western blot (protein expression level).

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Stable RNAi Cell Line Generation: Stable Gene Knockdown

Standard services include:

- **Cloning and subcloning (vector construction)**
 - Plasmid DNAs encoding antibiotic-resistant genes
 - Synthesis and cloning of shRNA constructs targeting genes of interest into plasmid DNAs
 - Final plasmid DNA amplification, and Maxi-prep purification
- **Stable Cell Line Development**
 - Transfection of plasmid DNA into the cell line of choice
 - Drug selection
 - Generation of stable cells
- **Validation**
 - Validation of shRNA construct expression
 - Validation RNAi gene silencing by real-time qRT-PCR and/or Western blot

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Transient (siRNA) and Stable (shRNA) Transfection Services

- Transfection is the process of introducing purified nucleic acids (DNA or RNA) into a eukaryotic cell via viral or nonviral methods. Through the process of transfection, genes can be manipulated to be overexpressed (by DNA plasmid constructs) or silenced based on sequence-specific targeting of genes (RNAi techniques). This application allows researchers to analyze gene function, determine disease pathways and identify potential drug targets.
- Altogen Labs offers transfection services for the delivery of siRNA, microRNA, shRNA, and plasmid DNA into the cell line of choice. The standard service includes the optimization of transfection of DNA or RNA (at least 20 µg should be provided by the client) into the cell line of choice and final validation of construct expression by real-time qRT-PCR (mRNA expression) and/or Western blot (protein expression).

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Transient Transfection

Transient Transfection of siRNA, microRNA, mRNA, or plasmid DNA:

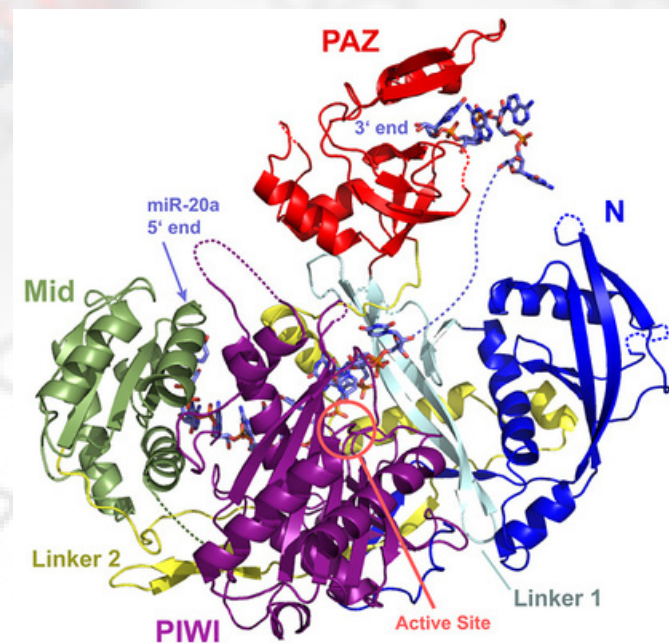
Transient transfection occurs when the transfected nucleic acids are introduced into the cell but are not permanently expressed or incorporated into the host cell genome. Subsequently, the nucleic acids are expressed for a short period of time but are eventually recognized as foreign genetic material and are degraded or diluted through mitosis and cell division. The main advantage of transient transfection is the rapid production of recombinant proteins that are of high quality, already activated and fully post-translationally modified.

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Standard Services

- *In vivo* siRNA delivery
- Quantitation of siRNA/shRNA functional knockdown effect by qRT-PCR and Western Blot
- siRNA transfection optimization
- Generation of stable RNAi cell lines (stably expressing shRNA targeting gene of interest)
- Transient (siRNA) and stable (shRNA) transfection services
- siRNA, shRNA, miRNA design and synthesis
- Altogen tetracycline-inducible RNAi systems
- RNAi Screens
- Genome-wide siRNA and microRNA high-throughput screens
- Gene targeting
- RNAi reporter cell lines generation
- *In vitro* functional testing of siRNA-induced mRNA knockdown
- RNAi screen pathway analysis and RNAi manufacturing
- siRNA and microRNA chemical modification to enhance stability *in vivo*
- PCR cloning: shRNA-encoding vector construction
- Reporter gene (RNAi) assays



Structure of human argonaute 2 complexed with a micro RNA

<http://www.hhmi.org>

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- PDX models are at the cutting edge of pre-clinical oncology research, and our team of scientists has a documented track record of establishing and performing these studies.
- Altogen Labs is compliant as a Good Laboratory Practices (GLP) environment.



PDX studies can present a clear picture of clinical trial success.

Photo credit: wisegeek.com

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

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