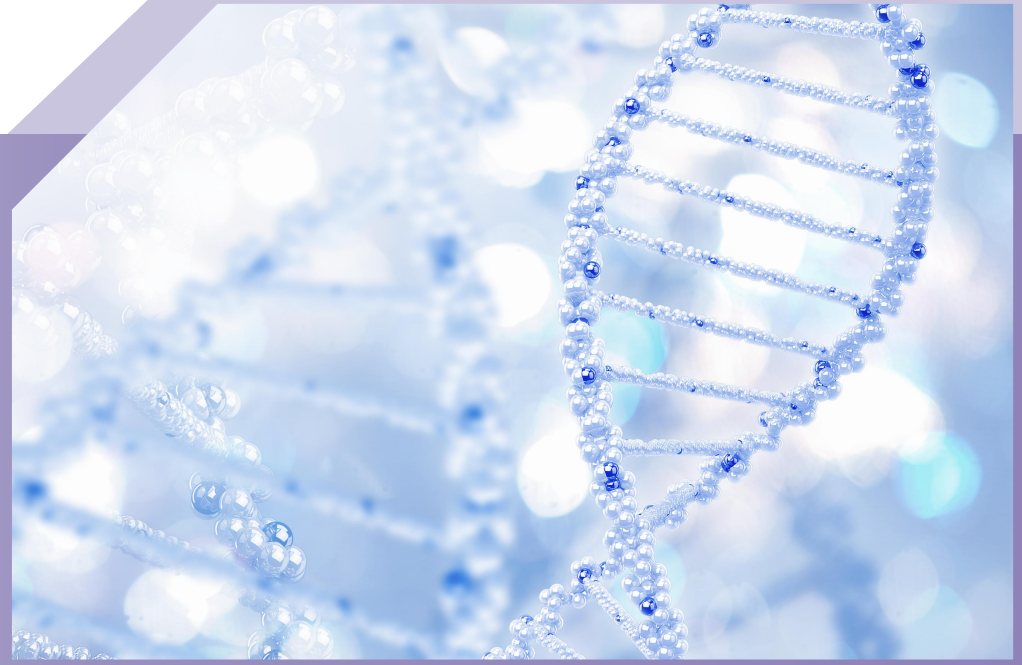
Protheragen
Rare Diseases

Spinal Muscular Atrophy (SMA)

Diagnostic and Therapeutic Development Solutions



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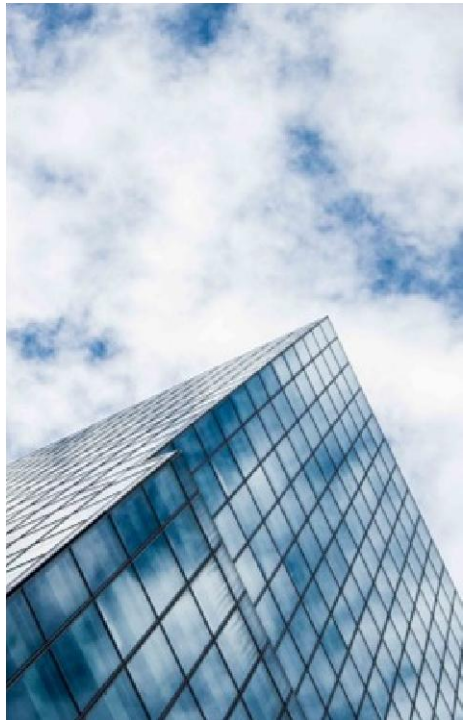

At **Protheragen**, we are dedicated to pioneering advances in *in vitro* diagnostic (IVD) and therapeutic development solutions for rare diseases like spinal muscular atrophy (SMA). With our expertise and commitment to innovation, we aim to address critical healthcare needs to facilitate early identification and timely intervention for SMA.

- Extensive Expertise in Rare Diseases
- Comprehensive and Customized Solutions
- Innovation and Collaborative Spirit

Overview of SMA

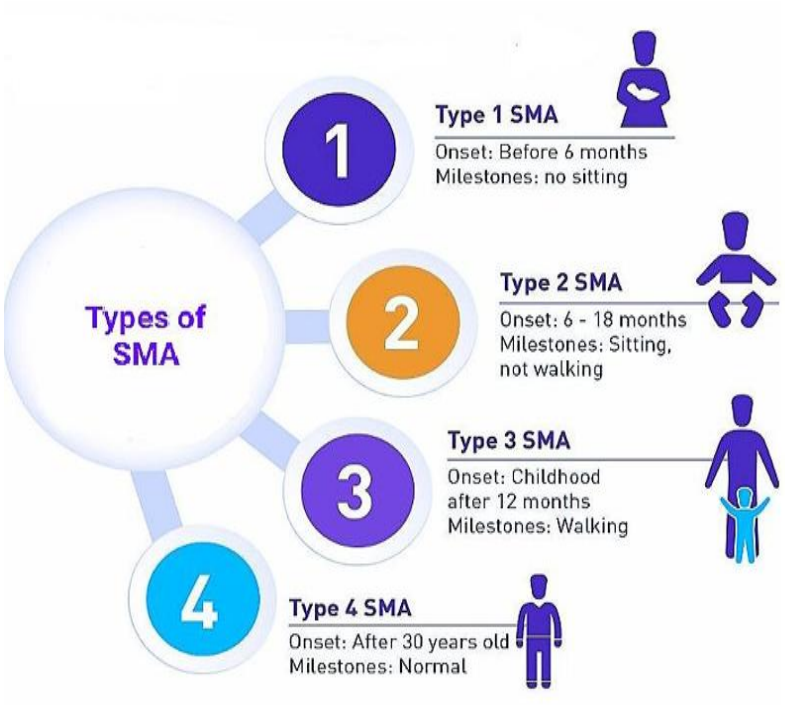

Fig.1 Types and milestones of SMA[1].

Introduction and Types of SMA

- Spinal muscular atrophy (SMA) is a rare genetic disorder characterized by the degeneration of motor neurons in the spinal cord, leading to muscle weakness and atrophy. The incidence of SMA is approximately 1 in 10,000 live births.
- SMA is classified into four types based on age of onset and highest motor milestones achieved, ranging from a severe infantile-onset form to a milder adult-onset form. Infantile-onset SMA, also known as Type I SMA, is the most severe form of the disease.

Pathogenesis of SMA

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SMN1 Gene Mutation: SMA is caused by homozygous deletions or mutations in the survival motor neuron 1 (SMN1) gene, located on chromosome 5q13. The SMN1 gene encodes the SMN protein, which is critical for the survival and function of motor neurons.

Role of SMN2 Gene: The SMN2 gene is similar to the SMN1 gene, but due to single nucleotide differences, it can only produce small amounts of functional SMN protein. The SMN2 gene influences the severity of SMA.

Pathogenesis of SMA: The loss of functional SMN1 and limited compensation by SMN2 results in a severe deficiency of SMN protein. Insufficient SMN protein levels lead to degeneration of spinal motor neurons, causing SMA.

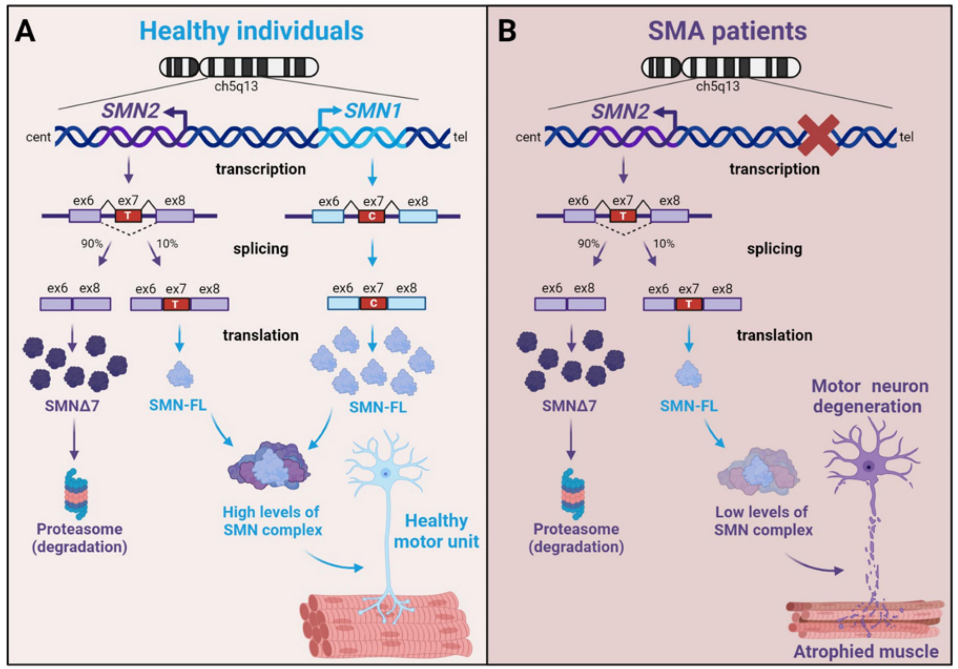

Fig.2 Mutation or deletion of the SMN1 gene results in decreased SMN protein levels[2].

Challenges in SMA Diagnostics and Therapeutics

Diagnostic Challenges: The various clinical manifestations of SMA may resemble other neuromuscular disorders, leading to diagnostic challenges and delays in accurate diagnosis, which is detrimental to timely therapeutics and intervention for SMA. Therefore, there is an urgent need for precise and effective IVD tools to enhance the comprehensive management of SMA.

Therapeutic Challenges: Developing therapeutics that can effectively increase the level of functional SMN protein is a key approach to addressing SMA. While existing gene replacement therapies and small molecule modulators have emerged, refining their efficiency, accessibility, and long-term outcomes remains a persistent hurdle. Urgent action is imperative to diversify therapeutic modalities, facilitating prompt intervention in SMA.

SMA Diagnostic Development Solutions

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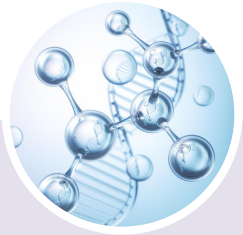
At Protheragen, we are committed to advancing the field of SMA diagnostics through innovative, reliable, and comprehensive IVD development services. Our expertise spans the entire diagnostic development pipeline, from reagent/kit development to the creation of cutting-edge devices and companion diagnostics. By leveraging state-of-the-art technologies and a deep understanding of the pathogenesis of SMA, we develop user-friendly IVD tools to facilitate early and accurate diagnosis of SMA.



IVD Reagent/Kit Development

Protheragen offers customizable reagent and kit development services to meet the unique needs of our clients, ensuring compatibility with various diagnostic platforms.

IVD Device Development

With our expertise in assay development and bioengineering, we work closely with you to design and manufacture IVD devices that adhere to the highest quality standards.

Point-of-Care Test Development

Our point-of-care test (POCT) development service focuses on developing rapid and user-friendly diagnostic solutions that can be performed at the point of patient care.

Companion Diagnostics Development

Leveraging our expertise in biomarker identification and regulatory compliance, we develop companion diagnostics to provide actionable insights for personalized therapies.

Case Study - SMA Genetic Testing Kit Development

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Protheragen is at the forefront of genetic testing innovation, providing customized services to meet the unique needs of our customers. The following delivery examples showcase the featured detection technologies used in our SMA genetic test kit development.

Fluorescent PCR Technology

We uses fluorescence PCR technology to bring the target gene and the internal reference gene to a plateau phase during competitive amplification. The concentration of the amplification product effectively reflects the relative relationship of the copy numbers of the two genes in the substrate template. Fluorescent dyes specifically bind to double-stranded DNA emitting fluorescence, while single-stranded DNA does not produce fluorescence.

Multiple Melting Curve Analysis

By real-time monitoring the change in fluorescence signal values during the melting process of double-stranded DNA and based on the differences in melt temperature and concentration of different amplification products, the target gene and the internal reference gene form distinct melt peaks. The melting peaks are normalized by software to distinguish different copy numbers of genes.

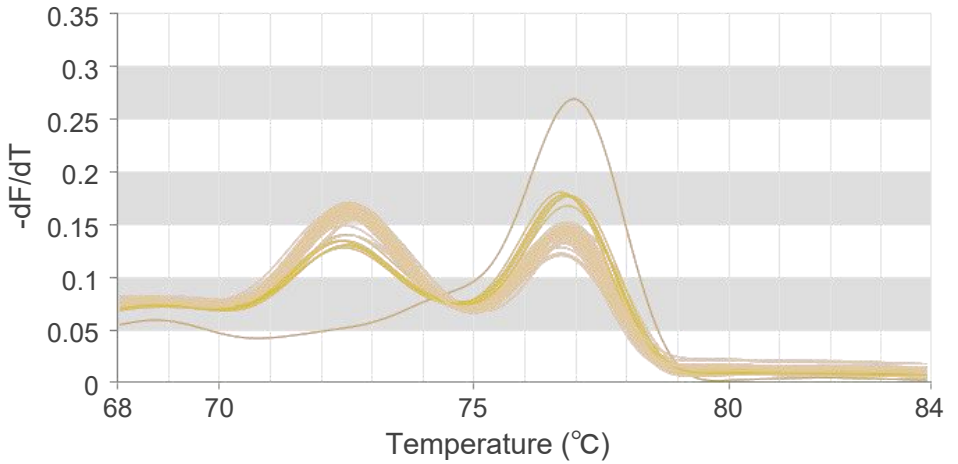

Fig.2 Melting curve.

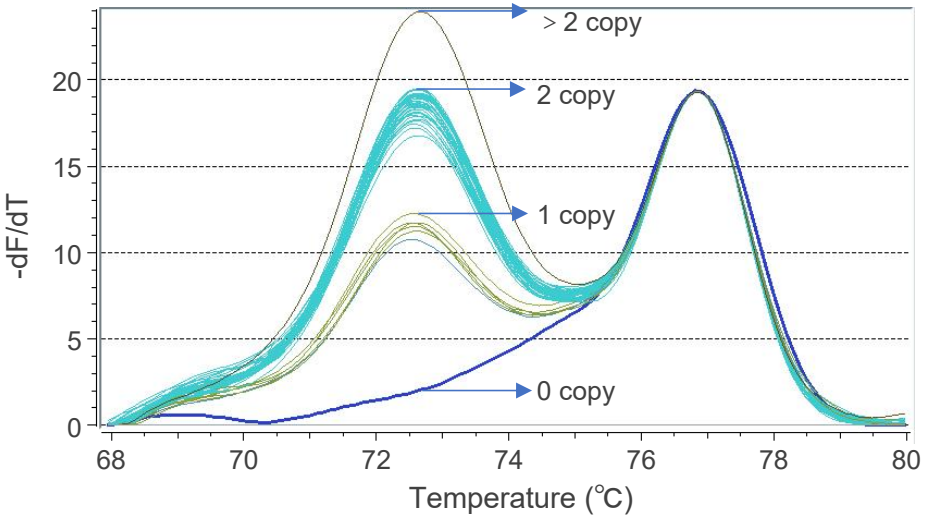

Fig.3 The copy number results after normalization processing.

Case Study - SMA Genetic Testing Kit Development

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Table.1 Test results of Protheragen's kit and MLPA reagent (SMN1 gene exon 7)

Detection Methods		MLPA Method			Total
		0 Copy	1 Copy	≥2 Copy	
Protheragen's SMN1 Gene Testing Kit	0 Copy	258	0	0	258
	1 Copy	0	37	0	37
	≥2 Copy	0	0	1283	1283
	Total	258	37	1283	1578

Table.2 Test results of Protheragen's kit and MLPA reagent (SMN1 gene exon 8)

Detection Methods		MLPA Method			Total
		0 Copy	1 Copy	≥2 Copy	
Protheragen's SMN1 Gene Testing Kit	0 Copy	223	0	0	223
	1 Copy	0	60	0	60
	≥2 Copy	0	0	1295	1295
	Total	223	60	1295	1578

Comparison of Detection Methods

- The SMN1 gene testing kit developed by **Protheragen** and the control MLPA assay (gold standard) were tested in 1578 human blood samples, with a 100% concordance rate in the test results.
- These test results highlight the outstanding accuracy and reliability of our SMN1 gene testing kit.

LEADER IN SMA SOLUTIONS

SMA Therapeutic Development Solutions

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At Protheragen, we are dedicated to offering a seamless and comprehensive solution for the therapeutic development targeting SMA. Our integrated services cover every crucial step of the drug development process, from initial target identification to toxicology evaluation, ensuring a holistic approach to addressing the complexities of SMA therapeutics.

Target Identification Services

Through advanced techniques and bioinformatics analysis, we pinpoint potential therapeutic targets, laying the foundation for the development of precise and effective therapeutic strategies.

Therapeutic Development Services

Leveraging our expertise in drug discovery and development, our therapeutic development services aim to translate promising targets into innovative therapeutic interventions.

Disease Model Development Services

By creating and validating SMA-specific *in vitro* or *in vivo* models, we facilitate the preclinical evaluation of drug candidates to accelerate drug development to commercialization.

Pharmacokinetics & Toxicology Evaluation

Protheragen offers comprehensive pharmacokinetic (PK) and toxicology evaluation services to ensure the safety and efficacy of potential SMA therapeutics.

SMA Target Identification Services

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Target Identification

- **Multi-Omics Profiling:** Whole-exome sequencing, single-cell RNA sequencing, TMT mass spectrometry, *etc.*
- **AI-Powered Target Prediction:** Integrate machine learning with SMA-specific datasets to prioritize targets.
- **CRISPR Screening:** Genome-wide CRISPR-Cas9 knockout/activation screen.

Target Validation

- **Functional Genomics:** CRISPR-Cas12a editing, phenotypic rescue assays, *etc.*
- **Advanced Disease Models:** iPSC-Derived Motor Neurons, 3D Neuromuscular Junction (NMJ) Chips, Zebrafish SMA Models, *etc.*
- **In Silico Modeling:** Molecular docking, systems biology modeling, *etc.*

Table.3 Identified therapeutic targets for SMA.

Type	Target	Mechanism
SMN-Dependent Targets	SMN2 exon 7 inclusion	Promote inclusion of exon 7 in SMN2 transcripts, increasing functional SMN protein production.
	SMN1 gene	Deliver functional SMN1 gene through gene therapy to restore SMN protein expression.
SMN-Independent Targets	Plastin 3 (PLS3)	Stabilize the actin cytoskeleton of motor neurons, protecting neurons.
	Zinc finger protein 1 (ZPR1)	Regulate the assembly of the SMN complex, enhancing SMN protein function.
	Olesoxime	Enhance motor neuron survival by modulating mitochondrial pores.
	Ubiquitin-like modifier activator 1 (UBA1)	Modulate the ubiquitin-proteasome pathway, reducing motor neuron degradation.
	Chondrolectin	Modulate neuromuscular junction (NMJ) integrity.

SMA Therapeutic Development Services

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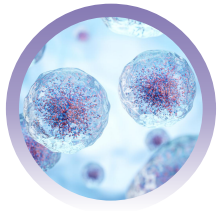
At Protheragen, we develop innovative therapies for SMA that address both SMN-dependent and SMN-independent pathways. Our approach integrates cutting-edge technologies and deep expertise in SMA biology to create therapeutics that can halt disease progression, restore motor function, and improve prognosis outcomes.

Small Molecule Drug Development

Develop orally bioavailable small molecules to modulate SMN2 splicing or target downstream pathways.

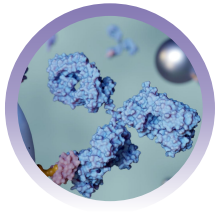


Cell Therapy Development

Engineer stem cells or immune cells to replace or repair damaged motor neurons in SMA.

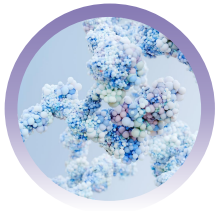
Gene Therapy Development

Deliver functional SMN1 or neuroprotective genes to motor neurons using viral vectors.



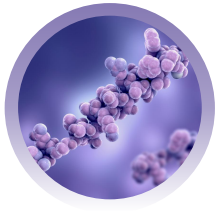
Therapeutic Antibody Development

Develop monoclonal antibodies (mAbs) to target neuroinflammation or extracellular modifiers in SMA.



Therapeutic Protein Development

Develop recombinant proteins to replace deficient SMN protein or enhance neuroprotection.



Therapeutic Peptide Development

Design peptides to modulate protein-protein interactions or enhance SMN2 splicing.

SMA Disease Model Development Services

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Protheragen specializes in developing advanced cell-based models and organoid models for SMA therapy research, providing researchers with powerful tools to study disease mechanisms and evaluate potential therapeutics.

- Patient-Derived Induced Pluripotent Stem Cells (iPSCs)
- Motor Neuron Cultures
- Astrocyte and Glial Co-cultures
- SMN1/SMN2-Edited Cell Lines
- Neuromuscular Junction (NMJ) Organoids
- Motor Neuron Organoids
- Spinal Cord Organoids
- Brain Organoids

SMA Disease Model Development Services

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Animal Models

- At **Protheragen**, we provide a wide range of animal models for SMA research, covering multiple species to support preclinical studies and therapeutic development.
- These models are designed to replicate the genetic, molecular, and phenotypic features of SMA, allowing researchers to study the **pharmacodynamics (PD)**, **pharmacokinetics (PK)**, and **toxicology** of potential therapeutics and advance translational research.

Chemical-induced Models		
Chemicals such as vincristine or β-neurotoxins are administered to animals to induce motor neuron degeneration and muscle atrophy, mimicking SMA-like symptoms.		
Optional Models	• Vincristine-induced model • β-neurotoxin-induced model	
Genetically Engineered Models		
Genetically engineered models are usually obtained using gene editing or transgenic techniques, such as CRISPR/Cas9-mediated SMN1 gene knockout.		
Optional Models	• $SMN1^{-/-}$ model • $SMN2^{tg/tg}$ model • $SMN\Delta7^{tg/tg}$ model • $SMN1^{Hung-/-}$ model	• SMN1A2G model • $SMN2^{Hungtg/-}$ model • SMN2 transgenic model • More
Optional Species	Mice, Rats, Zebrafish, Pigs, Drosophila, Caenorhabditis elegans, Non-Human Primates, Others	

Pharmacokinetics & Toxicology Evaluation

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Pharmacokinetics (PK) Evaluation:

- Drug Absorption Assessment
- Drug Distribution Assessment
- Drug Metabolism Assessment
- Drug Excretion Assessment
- Half-life and Clearance Assessment

Toxicology Evaluation:

- Acute Toxicity Studies
- Subchronic and Chronic Toxicity Studies
- Genotoxicity Studies
- Carcinogenicity Studies
- Reproductive and Developmental Toxicity Studies
- Immunotoxicity Studies
- Safety Pharmacology

Service Advantages

Customized Solutions

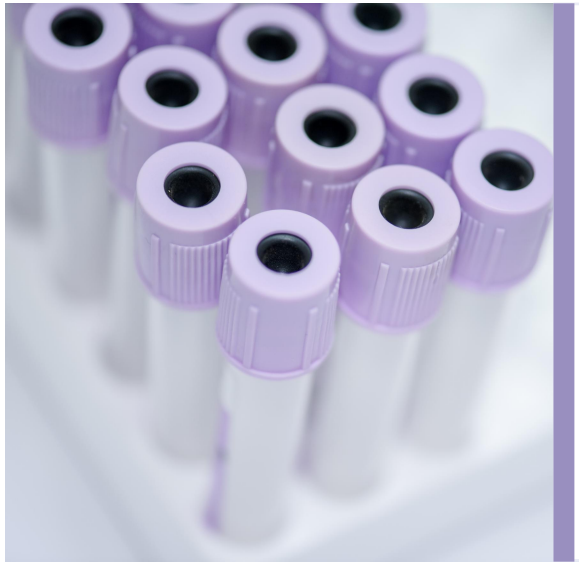
Rich Expertise and Experience

Cutting-edge Technology Platform

Comprehensive Research Support

Reliable and Trustworthy Partner

Collaboration Opportunities



Accelerating the Commercialization of SMA Diagnostics and Therapeutics

Protheragen offers comprehensive solutions designed to accelerate the commercialization of SMA diagnostics and therapeutics. From pioneering advancements in SMA diagnostic tools to spearheading breakthroughs in therapeutic modalities, we are committed to shepherding the complete lifecycle management of SMA interventions. Collaborate with us to drive innovation for SMA diagnosis and therapy.

Reference

[1] Mahendra Dwivedi, *et al.* "A review on spinal muscular atrophy: An inherited neuromuscular disease." *IP International Journal of Comprehensive and Advanced Pharmacology 8* (2023): 22-26.

[2] Bolado-Carrancio, Alfonso, Olga Tapia, and José C. Rodríguez-Rey. "Ubiquitination Insight from Spinal Muscular Atrophy-From Pathogenesis to Therapy: A Muscle Perspective." *International Journal of Molecular Sciences* 25.16 (2024): 8800.

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