Unlabeled/Investigational Uses

I will not be discussing unlabeled/investigational uses of medical devices or pharmaceuticals during this presentation.

Pranesh Rao, MS
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Asuragen, a Bio-Techne Brand

Presenter Financial Disclosure

I have the following financial relationships to report within the past 24 months with ACCME defined ineligible companies:

I am an employee of Asuragen, a Bio-Techne Brand

Pranesh Rao, MS

Scientist, R&D

Asuragen, a Bio-Techne Brand

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A whole exon targeted PCR/Nanopore sequencing assay that reveals SNVs, indels and CNVs across *SMN1* and *SMN2* with implications for SMA carriers and disease severity

Pranesh Rao, MS
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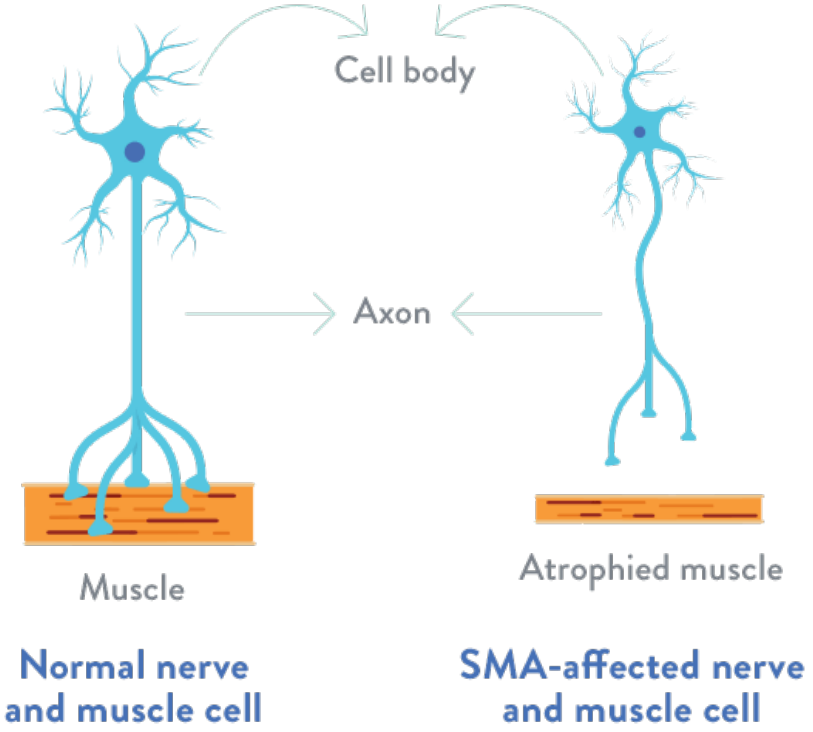
What is Spinal Muscular Atrophy (SMA)?

A potentially fatal disease with a high carrier rate affecting diverse populations

Loss of the *SMN1* gene function results progressive weakness of neuromuscular functions



https://www.togetherinsma.com/
https://www.mda.org/disease/spinal-muscular-atrophy

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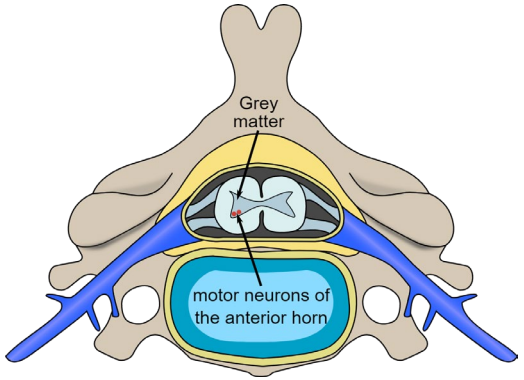
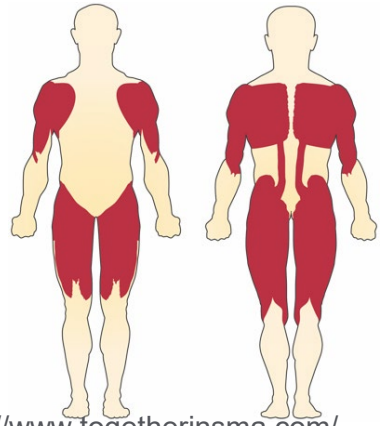


Loss of the *SMN1* gene function results progressive weakness of neuromuscular functions

Loss of anterior horn cells of the spinal cord and brain stem nuclei causing progressive weakness in skeletal muscles, and a wide range of phenotypes. Over 50% of cases can lead to death.

Historically, a leading genetic cause of infant death



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What is Spinal Muscular Atrophy (SMA)?

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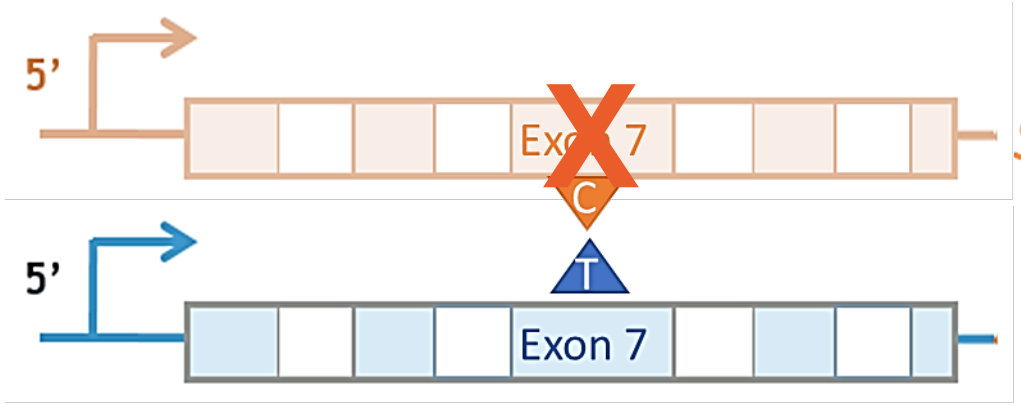
Historically, a leading genetic cause of infant death

Recent breakthrough therapies can ameliorate disease and are driving increased testing & newborn screening

www.curesma.org

SMN1, SMN2 and SMA

SMA carriers and patients are informed by *SMN1* and/or *SMN2* copy numbers



SMN1

No SMN protein

SMN2

Mostly non-functional SMN protein

SMN1 copy number	Diagnosis/ Carrier Status	Abilities	Prevalence
0	SMA	Depends on disease severity	1 in ~10,000
1	Carrier	Normal function	1 in ~50

Type	Highest Function	*SMN2* Copy#
1	Never Sit	2
2	Never Stand	3
3	Stand Alone	3-4
4	Stand Alone	>4

General population carrier screening recommended by ACMG[1,2] and ACOG[3]

1. Prior TW. *Genet in Med*. 2008.
2. Gregg, A. R. et al. *Genet Med*. 2021.
3. Committee Opinion No. 691. Obstetrics Gynecol 2017

SMN1/2 sequencing assay

Assay should resolve complex variations and high homology to inform:

- **Diagnostic and Carrier Risk**
- **Disease severity**

SMN1 copy number

SMN1 variants *c.*3+80T>G* & *c.*211_*212del*

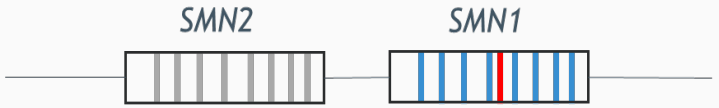
SMN1/2 hybrids

SMN2 copy number

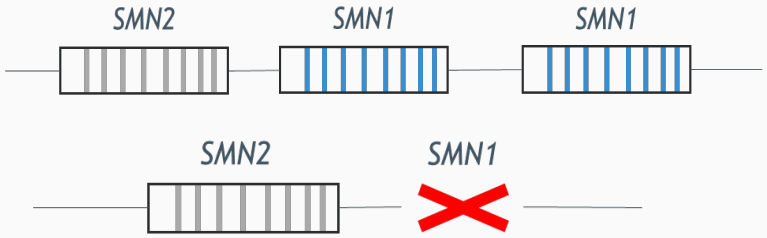
SMN2 variant c.859G>C

- ~95% gene deletions

- <5% other SNVs

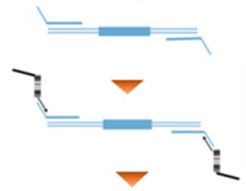


- ~5-30% [2+0] silent carriers



Variable copy number for both paralogs

Streamlined PCR/Nanopore workflow‡ addresses current assay challenges



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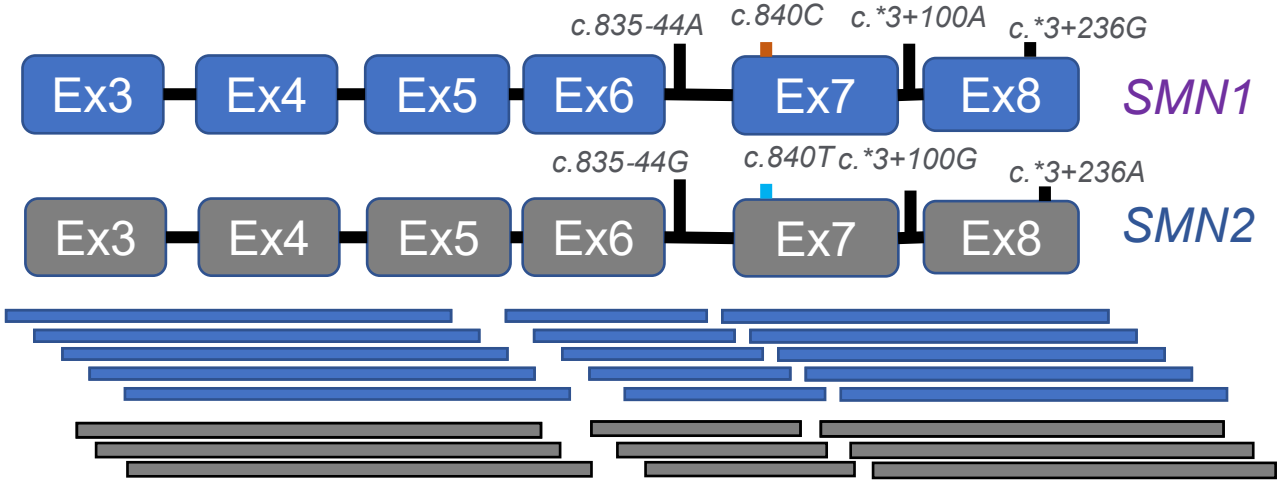
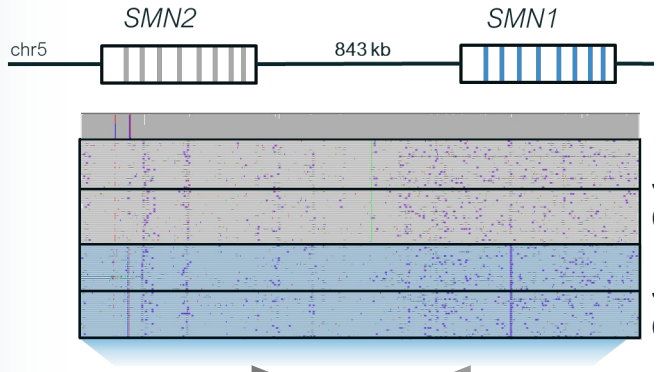
Nanopore

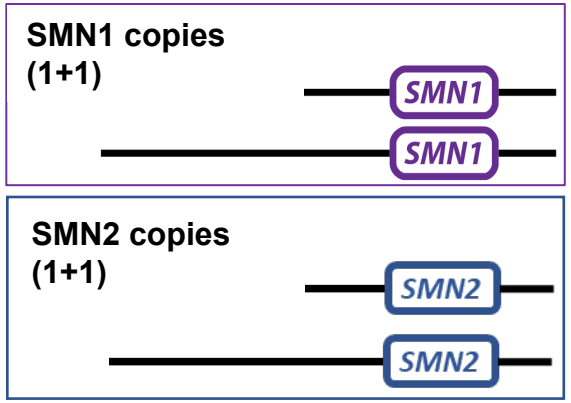
Long-Range PCR

Long-Read Sequencing



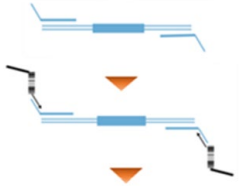


Data Analysis



‡ - Prototype assay

Streamlined PCR/Nanopore workflow‡ addresses current assay challenges



Asuragen

Nanopore

Long-Range PCR

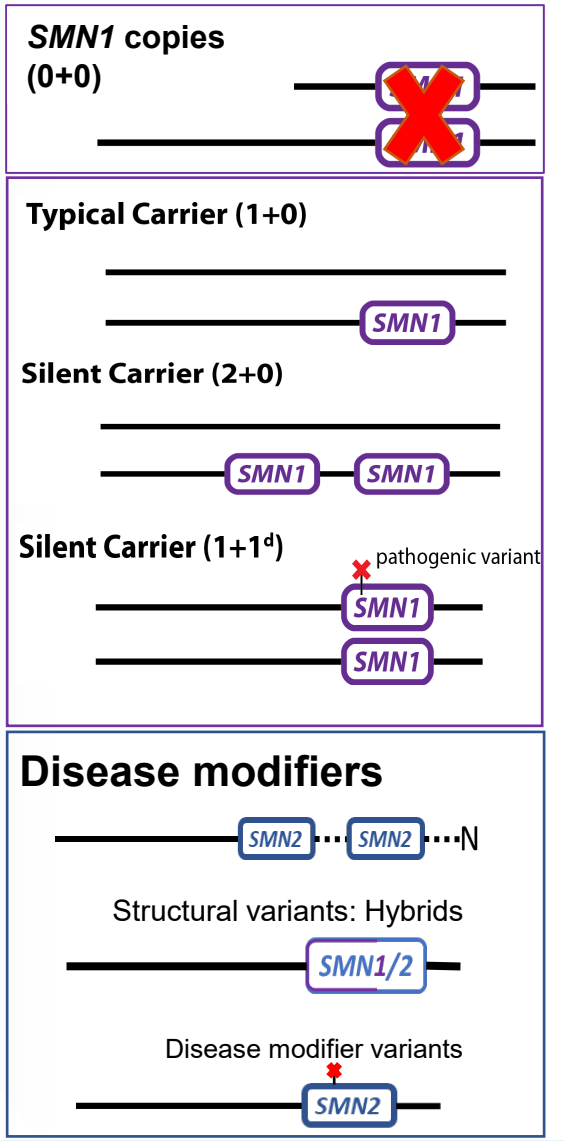
Long-Read Sequencing

CNV prediction ML model trained on an independent cohort of:

- 102 cell line samples
- 227 whole blood samples derived from single automated gDNA isolation method

Data presented on analysis of:

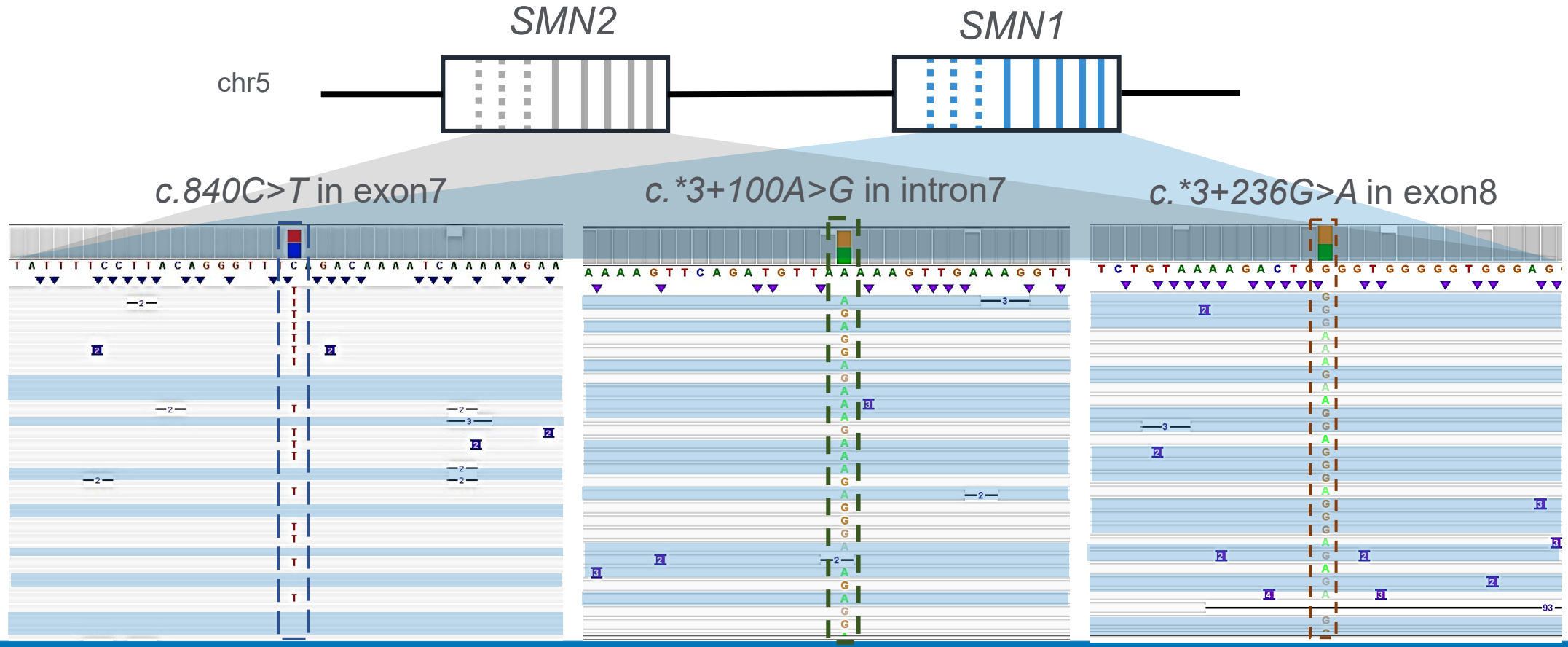
- 61 *SMN1/2* cell-lines representative of CNVs and intragenic variants
- 287 presumed normal whole blood samples with diverse methods of gDNA isolation



‡ - Prototype assay

Nanopore reads span *SMN1/2* exons 3 through 8

Reads aligned to their respective reference genes using paralog-specific variants (PSVs)



PSVs used in decision tree machine learning model to predict *SMN1/2* copy numbers‡

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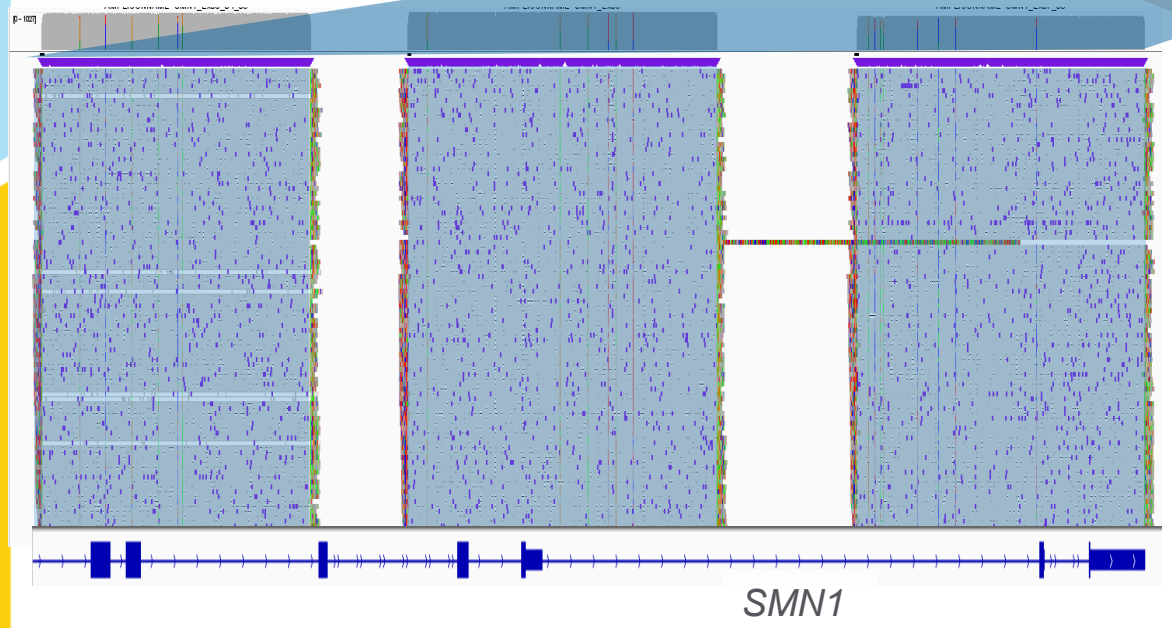
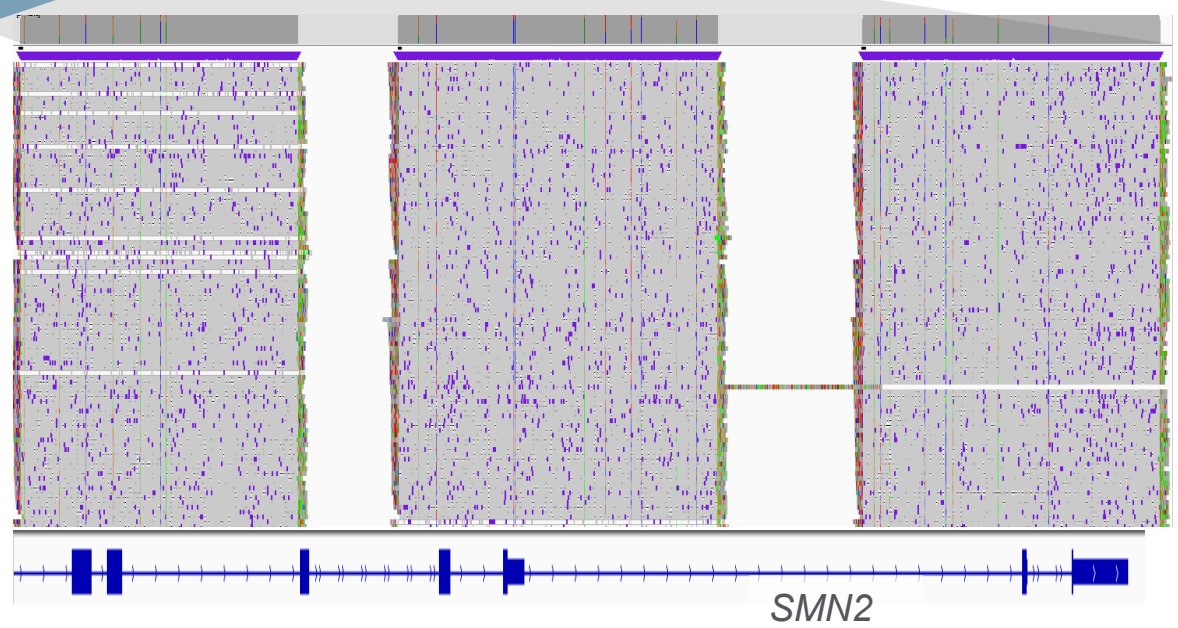
Sequence deconvolution by paralog-specific variants (PSVs)



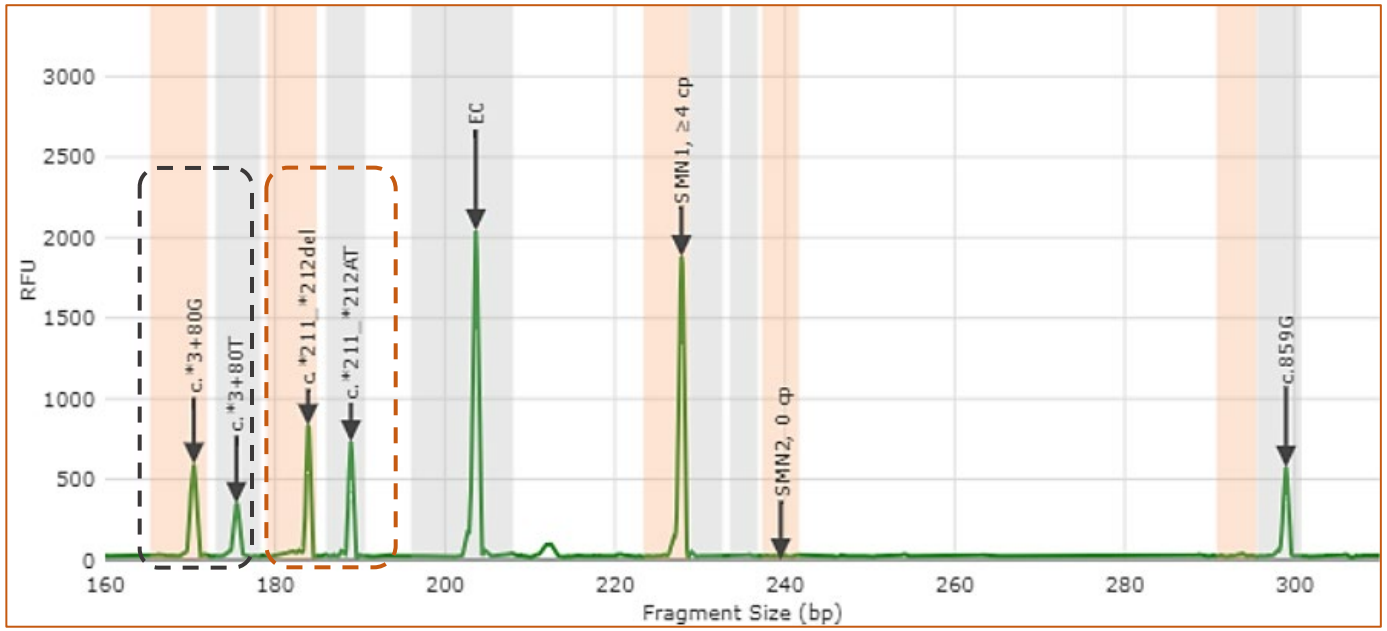


Results confirmed with comparator assay: AmplideX® PCR/CE *SMN1/2* Plus Kit*

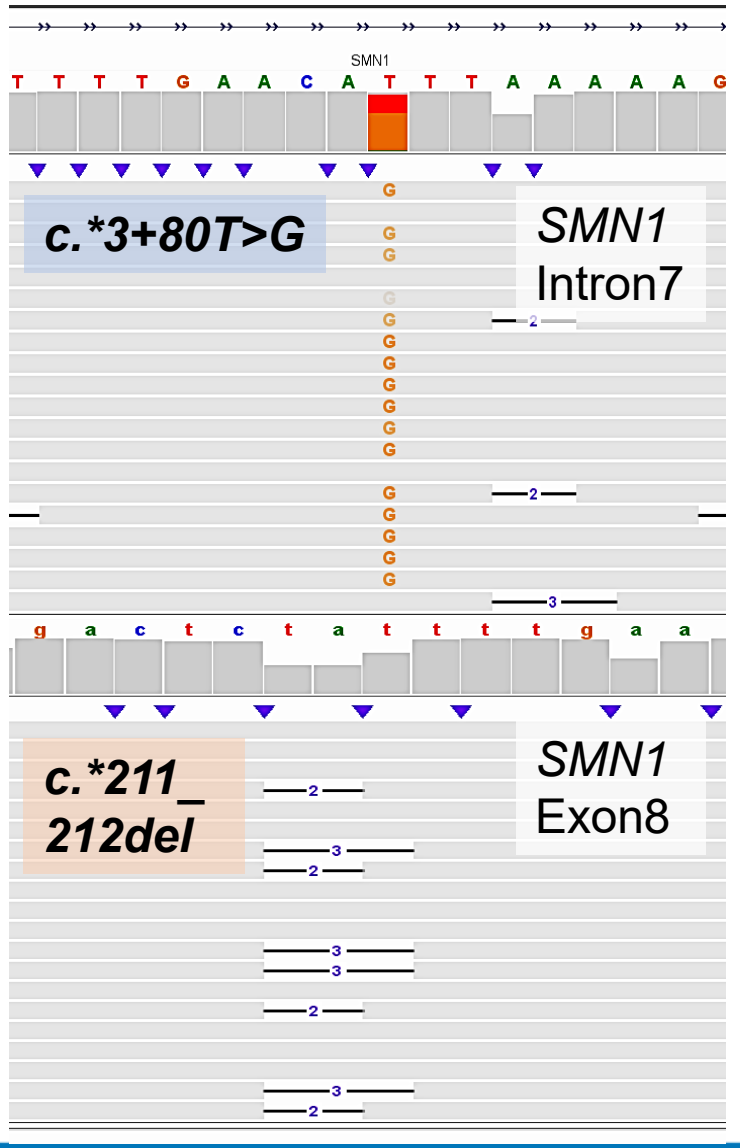
‡ - Prototype assay

* - Research Use Only. Not for use in diagnostic procedures

SMN1 silent carrier analysis from *c.*3+80T>G* & *c.*211_212del* variant allele frequency‡



AmplideX® PCR/CE *SMN1/2* Plus kit* used as the comparator method



‡ - Prototype assay
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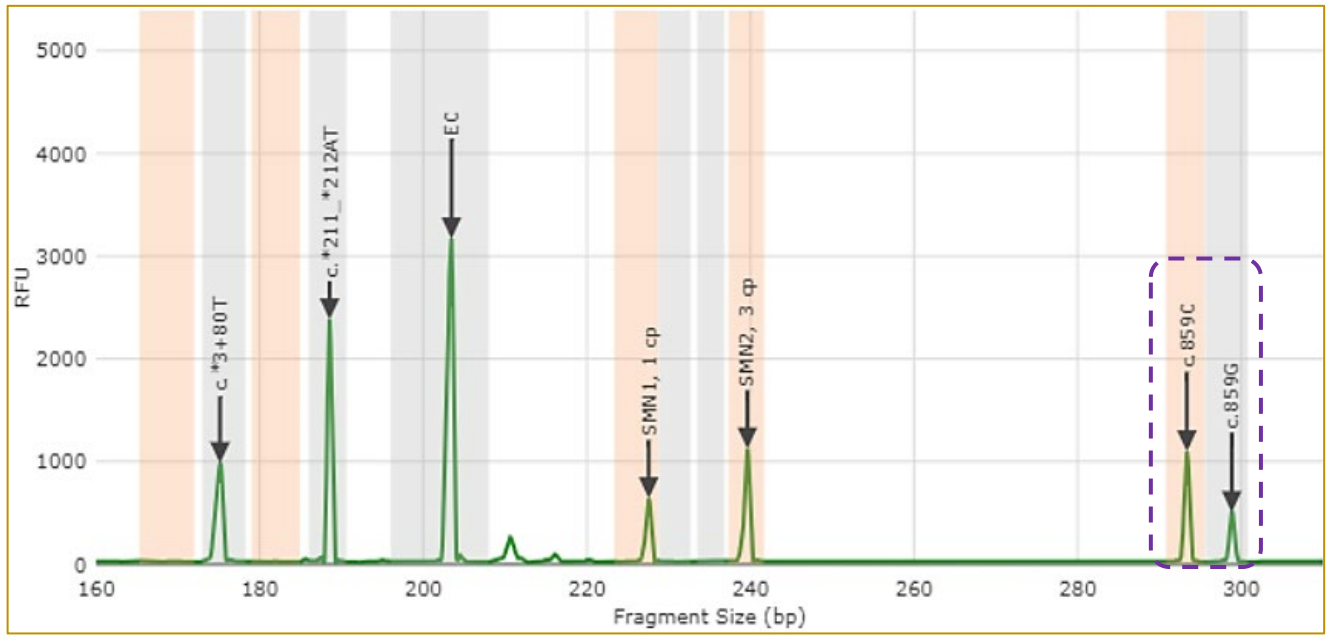
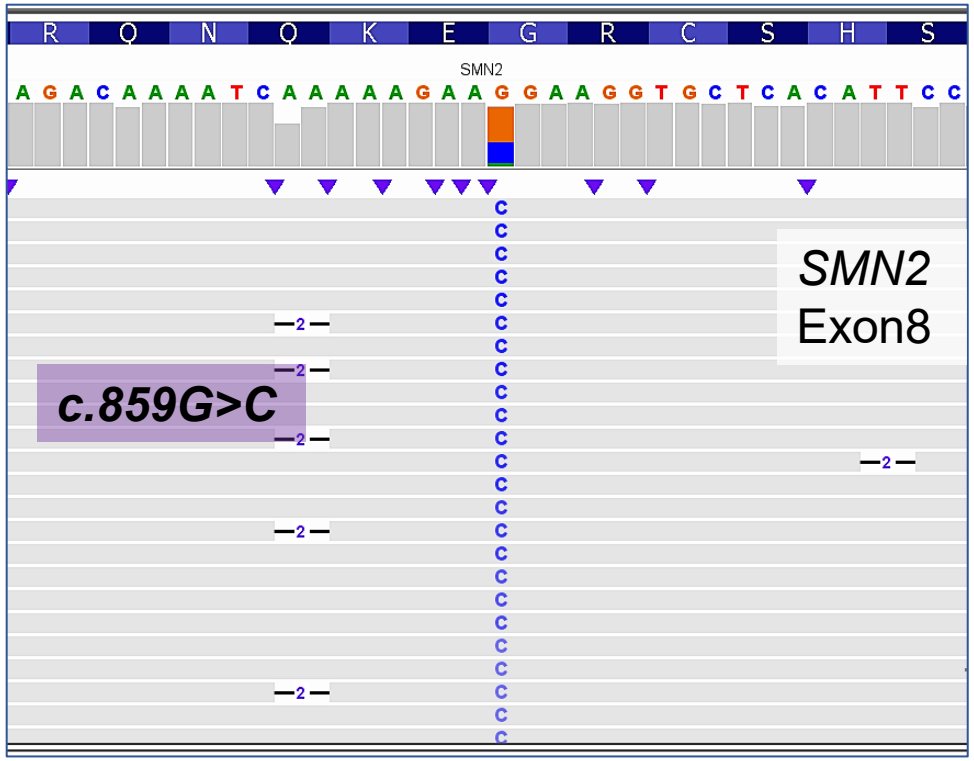
SMN2 disease modifier analysis using *c.859G>C* variant allele frequency‡





AmplideX® PCR/CE *SMN1/2* Plus kit* used as the comparator method

‡ - Prototype assay
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SMN1/2 copy numbers up to 3 or more copies called with >95% accuracy in whole blood samples (n=287)

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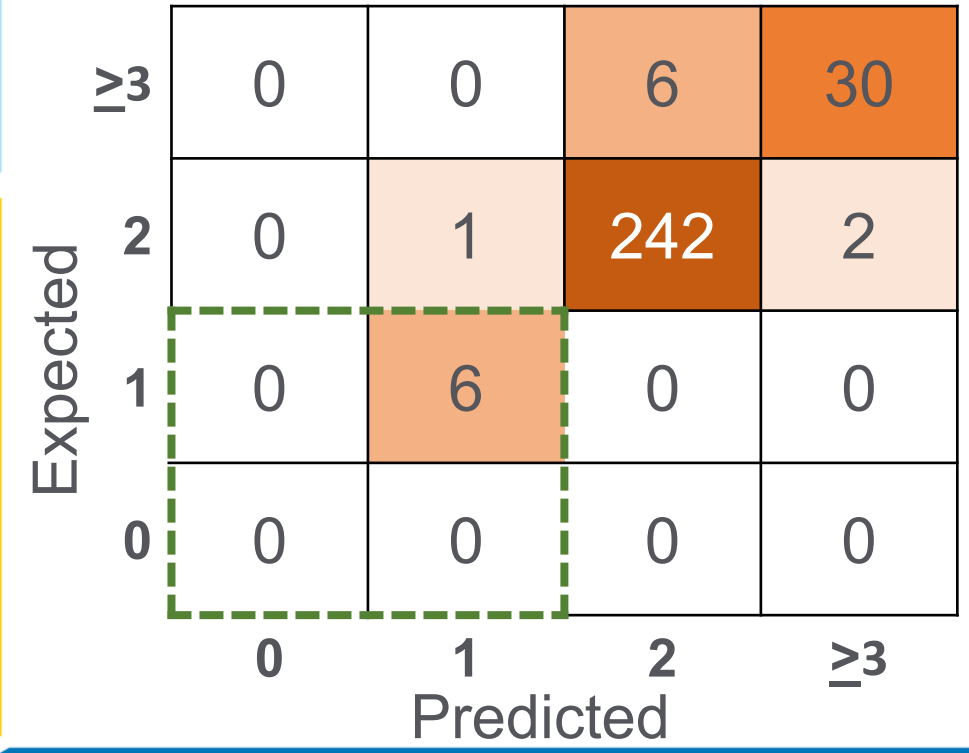
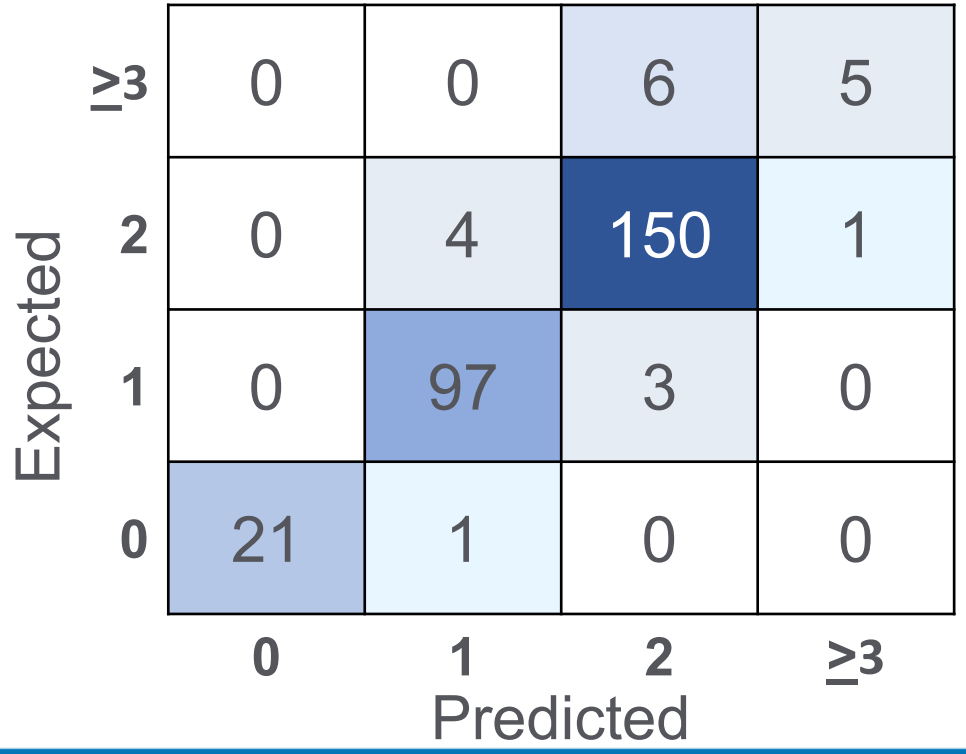
Independently trained with 102 cell-lines & 227 whole blood samples, single gDNA isolation method

SMN1 copy number accuracy 96.8%



SMN2 copy number accuracy 95.1%



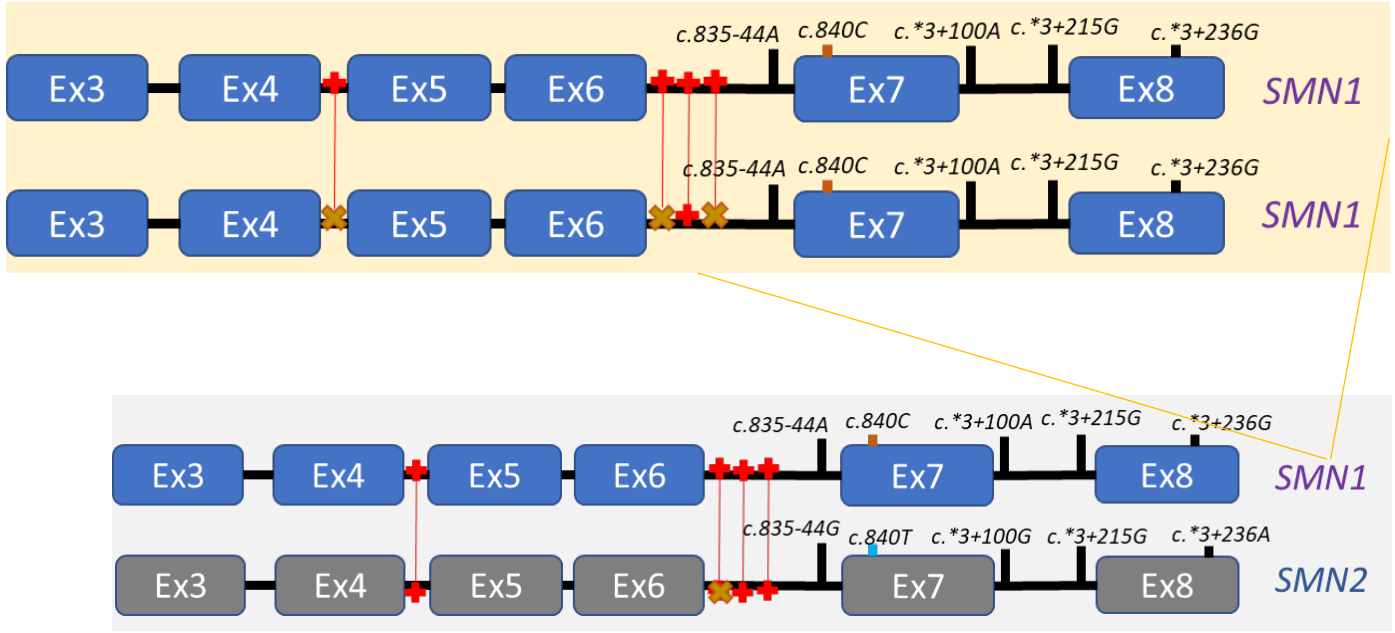
Reconciliation of outlier samples with longer-range PCR‡

SMN1 copy number prediction (*SMN1:SMN2*=2:1) discordant with comparator method=**3**:1

Longer amplicons resolve sequence population variation between and within genes

Clustering of read PSVs/SNPs reveals 4 distinct *SMN1* and *SMN2* clusters. *SMN1:SMN2*=**3**:1

Outlier sample: RS4939



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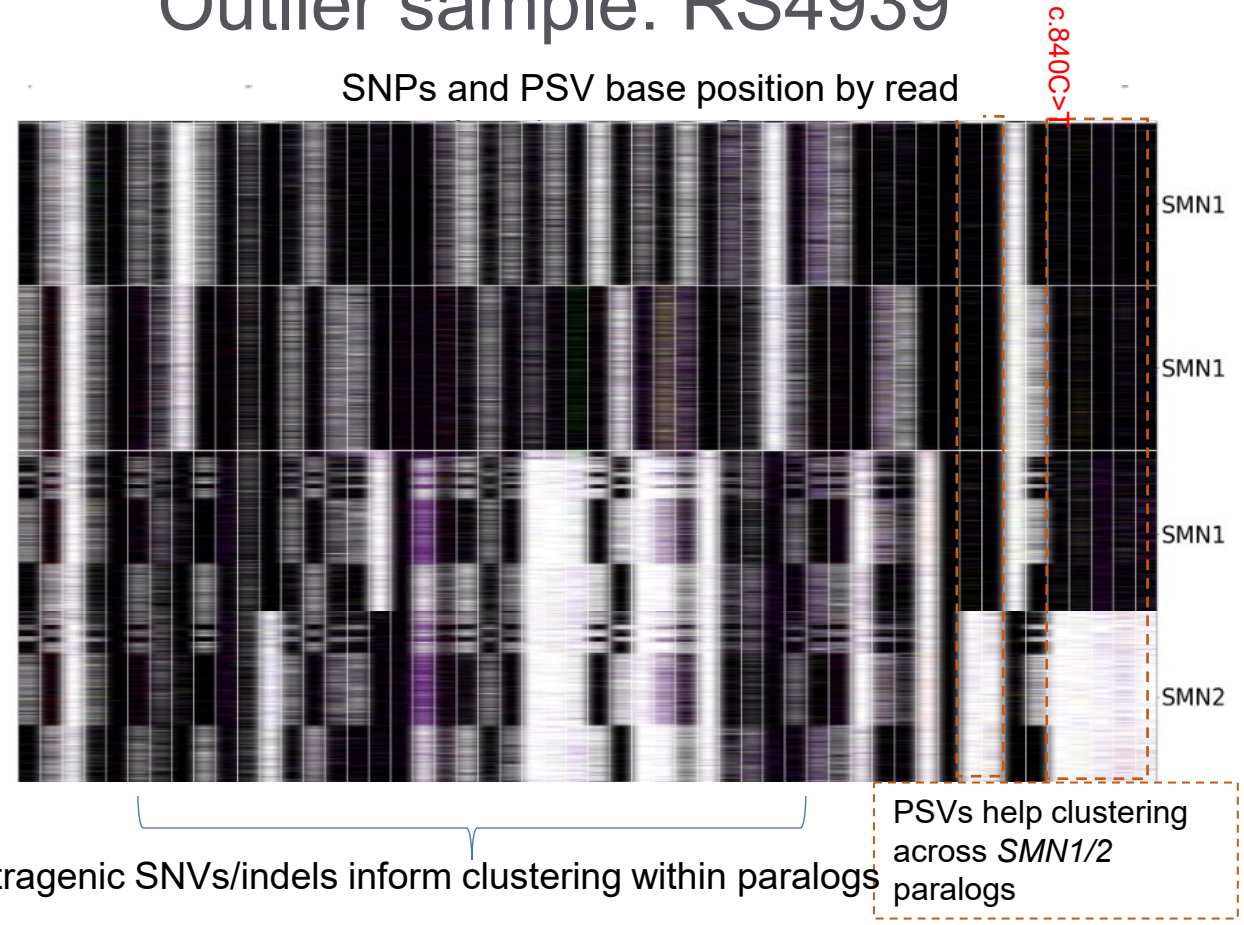

‡ - Prototype assay

SMN1 silent carrier variant & *SMN2* DM variant calls showed 99.7% agreement with reference assay

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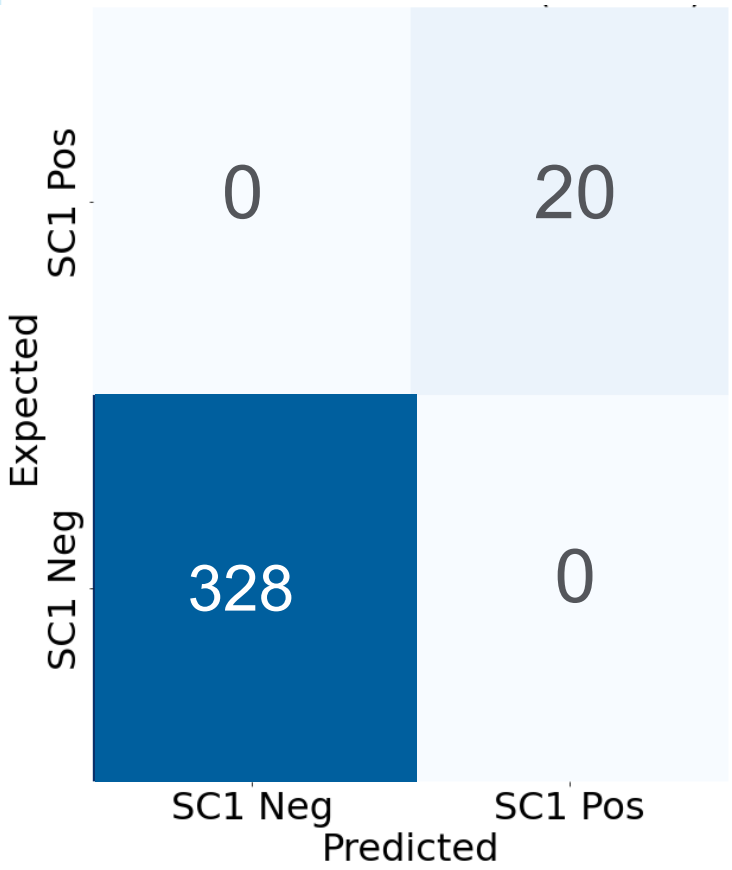
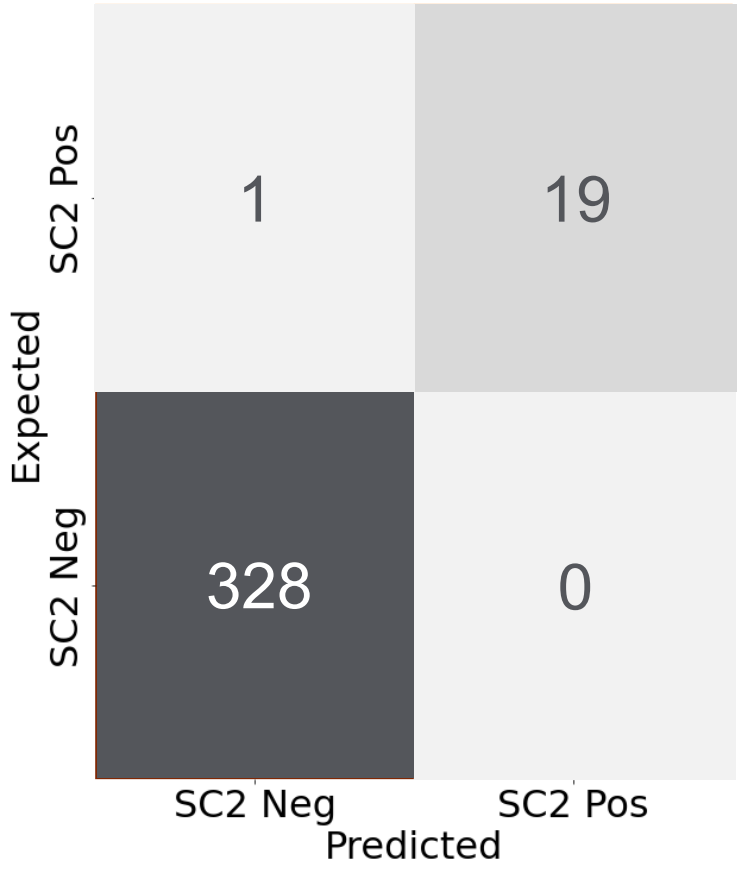
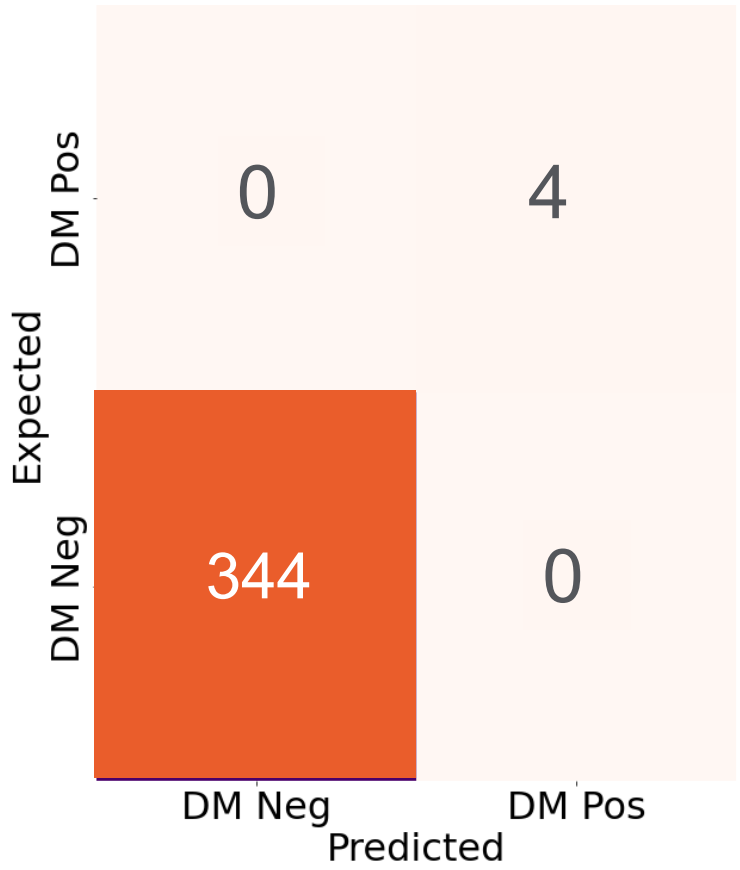
SC1: c.*3+80T>G



SC2: c.*211_*212del



DM: c.859G>C



Conclusions

- PCR/Nanopore sequencing enables comprehensive and accurate genotyping of *SMN1* and *SMN2*‡
- CNVs, large exon deletions, SNVs and indels were analyzed using PSVs with a machine learning approach to achieve reliable gene-specific assignments
- Using 348 samples, genotype accuracy was 95-100% across all variant types compared to reference methods

Assay has potential to help:

Standardize SMA screening and molecular diagnostics

+

Expand to both conventional and challenging genes

=

Address gaps in equity with many current testing regimes, consistent with recent ACMG recommendations

‡ - Prototype assay

Acknowledgements

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www.curesma.org

Ryan Routsong

Dr. Bradley Martin

Dr. Bryan Killinger

Christopher J Fraher

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