A Rapid, High-throughput PCR Fragment-sizing Assay for Quantifying Spinal Muscular Atrophy (*SMN1*, *SMN2*) Copy Numbers

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Summary

- Spinal muscular atrophy is a lethal autosomal recessive disease resulting from *SMN1* (5q13.2) disruptions, with deletions comprising most cases.
- Multiplex Ligation-dependent Probe Amplification (MLPA) is the most common current methodology for *SMN1* assessment. Unfortunately, this approach requires technical rigor, a 2-day workflow, and relatively high costs.
- Herein we describe prototype assays (AmplideX® PCR/CE *SMN1* and *SMN2**) that quantify >3 gene copy numbers in under three hours and can be combined with *FMR1* repeat genotyping.

Introduction

Spinal muscular atrophy (SMA) is a progressive neuromuscular disease and the primary genetic cause of infant death. The illness's etiology is characterized by loss-of-function mutations to Survival Motor Neuron gene 1 (*SMN1*), commonly manifesting as deletions that minimally encompass exon 7. A virtually identical gene, *SMN2*, can partially compensate for this event but requires an otherwise rare alternative splice variant to produce a functional protein.

The recent FDA approval of the antisense oligonucleotide nusinersen, marketed as SPINRAZA®, provides the first effective means of promoting *SMN2* alternative splicing and subsequent SMA treatment. Consequently, there is increased interest in both newborn and carrier screening for SMA (~1:50 carrier incidence). Herein we report the performance of prototype AmplideX® PCR/CE *SMN1* and *SMN2* reagents, quantitative assays for the two critical *SMN* genes that overcome many of the workflow and operational limitations associated with commonly used MLPA-based approaches.

Materials and Methods

For each reaction, 2 µL of gDNA samples (cell line or whole blood, 10-40 ng/µL) were combined with 7.5 µL of a PCR mastermix (containing buffer, dNTPs, and polymerase) and 5.5 µL labeled primer mastermix within 96-well PCR plates. Amplification was performed on a Veriti Thermal Cycler (Thermo Fisher) and resolved using capillary electrophoresis (CE).

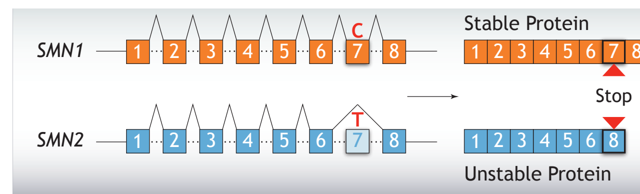


Figure 1. *SMN1* and *SMN2* Maturation. The sequences differ within their exons by a single base (red). While transcriptionally silent, this alteration leads to *SMN2* exon 7 skipping/loss, utilization of an alternative downstream stop codon, and resultant protein degradation.

Samples were injected for 20 sec/2.5 kV and run for 20 minutes/19.5kV on a 3500xL Genetic Analyzer (Thermo Fisher). Output data were visualized with GeneMapper™ Software 5 (Thermo Fisher) and calculations for the copy number performed in Excel.

FMR1 triplet repeat amplification was performed using an AmplideX® PCR/CE *FMR1* Kit† (Asuragen) following the manufacturer's protocol. 2 µL ea of *FMR1* and *SMN1* PCR amplicons were then combined with HiDi/Rox (15 µL total) and injected as noted above.

Results

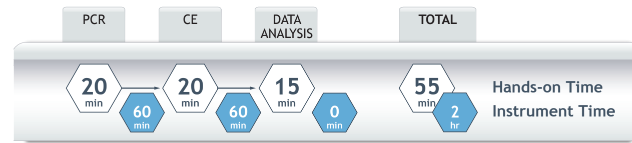
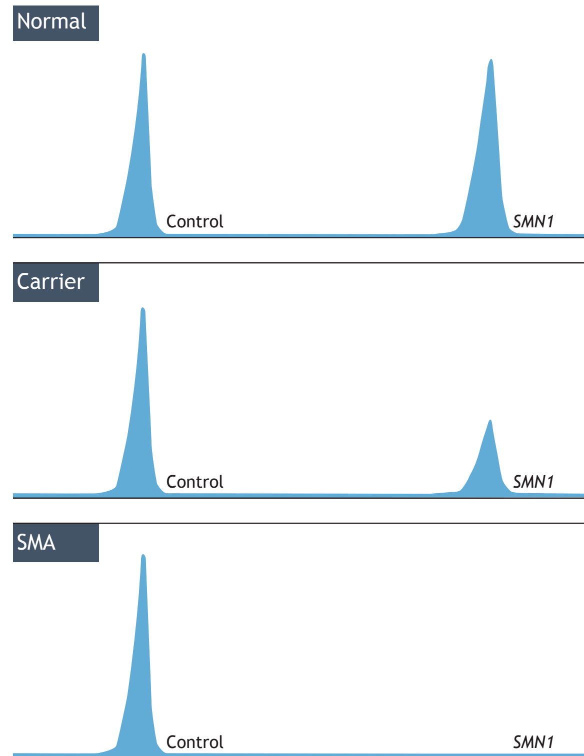


Figure 2. Assay Workflow. The process takes less than 3 hours per 24 well injection, and less than 6 hours for an entire 96 well plate. No unconventional steps, reagents, or equipment are required.

3A



3B

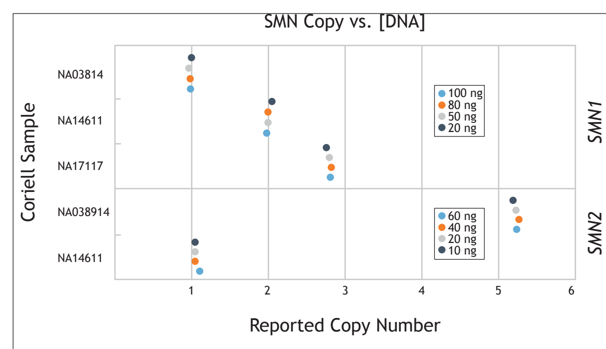
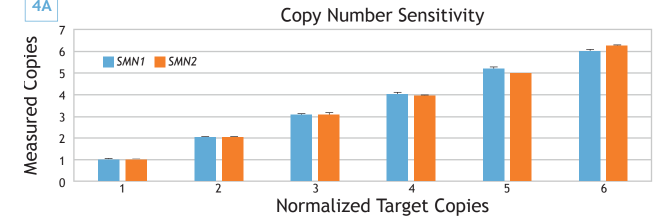


Figure 3. Quantification of *SMN1* in Cell-line and Blood-Derived DNA. A) Example raw trace outputs from Normal (NA14611), Carrier (NA23687), and SMA (NA23689) annotated Coriell samples. **B)** Calculated copy number; response is linear across a range of DNA input levels.

4A



4B

Cell-line gDNA Sample ID	*SMN1* Copy	*SMN2* Copy
NA00232	0	2
NA03813	0	indeterminate
NA03814	1	5
NA03815	1	1
NA05756	2	1
NA09677	0	3
NA10684	0	2
NA11254	2	0
NA14611	2	1
NA14820	2	1
NA17117	3	0
NA22592	0	indeterminate
NA23687	1	2
NA23688	1	2
NA23689	0	3
RU001	2	2
RU002	2	2
RU003	2	3
RU004	2	2
RU005	2	2
RU006	2	2
RU007	2	1
RU008	2	1
RU009	2	1
RU010	2	3
RU011	2	3

Whole Blood gDNA Sample ID	*SMN1* Copy	*SMN2* Copy
F16	3	1
F17	2	1
F18	2	0
F19	2	3
F20	3	2
F21	2	1
F22	2	2
F23	2	1
F24	2	2
M16	2	2
M17	2	2
M18	2	2
M19	2	2
M20	2	2
M21	2	1
M22	2	2
M23	2	2
M24	2	2
M25	2	2
M26	2	1
M27	2	2
M28	2	1
M29	2	2

Figure 4. Copy Number Quantification in Synthetic, Cell-Line and Whole Blood Samples. A) *SMN1* and *SMN2* gBlocks (IDT) titrated into a negative human DNA background (NA20232 or NA11254) are quantifiable up to 6 copies. **B)** Calculated copy number of additional cell-line and blood samples. All outputs matched previously reported copy numbers (where available).

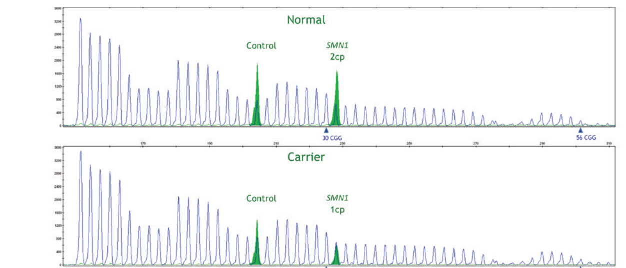


Figure 5. *SMN1* and *FMR1* PCR Products are Easily Resolved and Quantified Following Co-Injection. *SMN1* peaks (Normal: NA14820 and Carrier: NA03815, 20 ng/PCR) are highlighted in the *FMR1* (30, 56 CGG, 40 ng/PCR) overlay.

Conclusions

- Prototype AmplideX® PCR/CE *SMN1* and *SMN2* reagents can correctly quantify gene copy numbers in Coriell and blood-derived samples.
- Resolution achieves at least 3-5 *SMN1/2* copies, with an optimal range of 20-80 ng of input material.
- *FMR1* PCR products can be co-injected, allowing simultaneous assessment of two of the most relevant genes used in carrier screening.
- Turn-around-time is significantly faster than comparable PCR/CE technologies using a simple workflow.

*This product is under development. Future availability and performance cannot be ensured.
†Research Use Only. Not for use in diagnostic procedures.

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