Streamlined, Single-tube PCR Assay that Quantifies *SMN1* and *SMN2* Copy Numbers using Capillary Electrophoresis

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Summary

- Spinal Muscular Atrophy (SMA) is a genetic disease caused by deletion of or mutation in the *SMN1* gene, where disease severity is modulated by the *SMN2* copy number.
- We developed a prototype AmplideX® PCR/CE *SMN1*/2 kit† in a single-tube PCR assay, providing accurate quantification of *SMN1* and *SMN2* using capillary electrophoresis in less than 3 hours.
- Here we describe feasibility data demonstrating high specificity, broad input range, and reliable genotyping to more than 3 copies of both *SMN1* and *SMN2*, including detection of rare hybrid genes (gene conversions).

Introduction

Spinal Muscular Atrophy (SMA), an autosomal recessive neuromuscular disease caused by a loss of *SMN1* gene function, is the primary genetic cause of infant death. The copy number of the highly similar *SMN2* gene is an important predictor of the severity of SMA, as the *SMN2* gene can produce some functional SMN protein that partially restores biological function. The antisense oligonucleotide nusinersen (marketed as SPINRAZA) promotes *SMN2* alternative splicing to enhance the effectiveness of *SMN2* functional replacement of *SMN1*. Early diagnosis of SMA, along with knowledge of *SMN2* copy number, is critical for effective medical management. Herein, we report the performance of a prototype AmplideX PCR/CE *SMN1*/2 kit†, a single-tube PCR assay that quantifies *SMN1* and *SMN2* copy number including *SMN1*/2 hybrids (consistent with gene conversion) using capillary electrophoresis (CE).

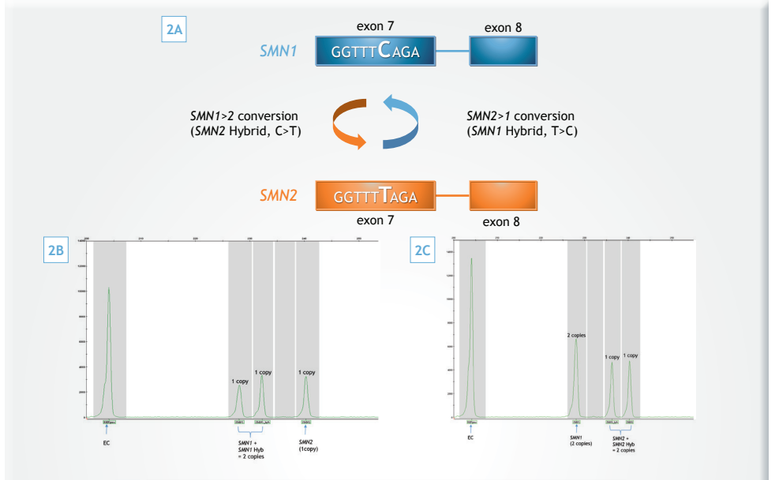
Materials and Methods

We developed a multiplexed PCR that simultaneously amplifies *SMN1*, *SMN2*, and an endogenous control (EC) in a single well. This PCR can also identify *SMN1*/2 gene conversion events. The PCR products were separated and quantified via Applied Biosystems™ 3500xl Genetic Analyzer with POP7 polymer with a 2.5kV, 20sec injection and 40min run time. The copy number of *SMN1*, *SMN2*, or *SMN1*/2 hybrid was calculated as the peak area ratio of target gene and EC normalized to a calibrator. If gene conversion was detected, *SMN1* and *SMN2* copy number was determined from the sum of *SMN1* and an *SMN1* hybrid and the sum of *SMN2* and an *SMN2* hybrid, respectively, indicating number of copies of exon 7 for each gene.

Figure 1. Assay Workflow. The workflow is streamlined with total assay time less than 3 hours. Total hands-on time is 45 minutes. CE instrument time is for a single injection, or 24 samples using an Applied Biosystems™ 3500xl Genetic Analyzer.

†This product is under development. Future availability and performance cannot be ensured.
Presented at AMP 2018

Results



2D

Sample	Sample Type	*SMN1* Copy	*SMN1* Hyb Copy	*SMN2* Copy	*SMN2* Hyb Copy
NA23255	Cell line	0	0	3	0
NA23686	Cell line	0	0	2	0
NA00232	Cell line	0	0	2	0
NA03184	Cell line	1	0	5	0
NA20294	Cell line	3	0	1	0
NA19235	Cell line	4	0	0	0
NA03813	Cell line	0	0	3	0
NA22592	Cell line	0	0	3	0
1	Blood	2	1	1	0
2	Blood	1	1	1	0
3	Blood	1	0	2	0
4	Blood	2	0	0	0
5	Blood	3	0	1	0
AJC3666	Buccal	1	0	1	0
AJC3764	Buccal	3	0	1	0

Figure 2. Assay Outputs Quantify *SMN1* and *SMN2* Copy Numbers Along with Hybrid Genes. A) Diagram illustrating gene conversion of exon 7 between *SMN1* and *SMN2*. **B)** CE trace of sample with *SMN1* Hybrid. **C)** CE trace of sample with *SMN2* Hybrid. **D)** Examples of *SMN1* and *SMN2* copy numbers determined by the prototype assay using cell-line, blood and buccal DNA. *SMN2>1* conversion was detected in blood #1 and #2 samples.

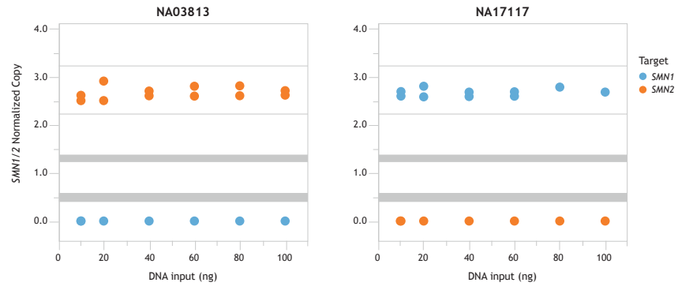


Figure 3. Specificity Study. Two DNA samples derived from Coriell cell lines with the absence of either *SMN1* or *SMN2* were tested with DNA inputs up to 100 ng. Expected *SMN1* and *SMN2* copy numbers for NA03813 are 0 and 3, respectively, and for NA17117 are 3 and 0. *SMN1* and *SMN2* copy number for both DNA samples determined by the assay agreed with expected copy numbers up to 100 ng DNA input. The assay is specific for *SMN1* and *SMN2* detection.

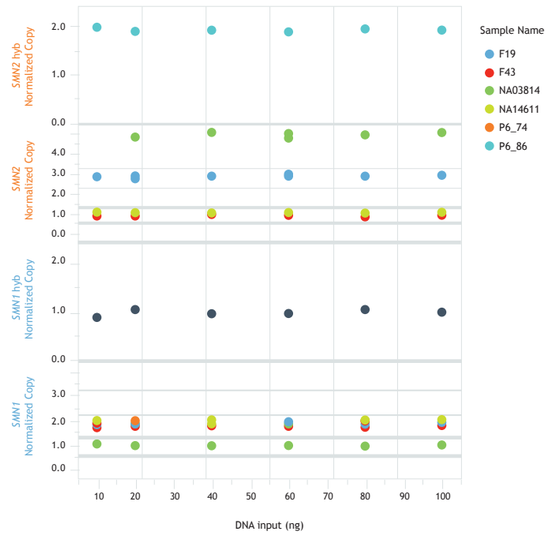


Figure 4. DNA Input Range. DNA input ranging from 10 to 100 ng was evaluated using two cell line samples (Coriell) and four blood samples. The expected *SMN1* and *SMN2* copy number was determined using independent methods.† White bars designate bins for calling *SMN1* and *SMN2* copy number. All samples with DNA input from 10 to 100 ng produced expected copy numbers (ranging from 1 to ≥ 4).

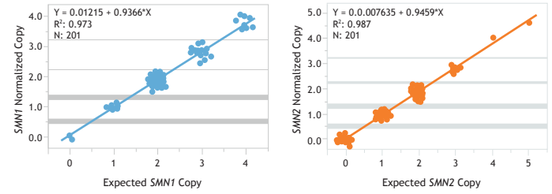


Figure 5. Accuracy Study with 201 Samples. Samples derived from cell line, blood and buccal were tested and compared to an independent assay. White bars designate bins for calling *SMN1* and *SMN2* copy number. Expected copies were determined using an orthogonal method. The percent agreement between the two methods was 100% for both *SMN1* and *SMN2*.

Conclusions

- The prototype assay described here detects and accurately quantifies *SMN1* and *SMN2* copy numbers, including hybrid genes, within 3 hours including 45 minutes hands-on time.
- Copy numbers were accurately quantified using 10 to 100 ng DNA, and were reliably determined with DNA derived from >200 cell line, blood and buccal cell sources.
- The AmplideX PCR/CE *SMN1*/2 assay offers a rapid and robust single-tube PCR with reduced complexity compared to existing methods.

Reference

1. Stabley DL, Harris AW, Holbrook J, et al. *SMN1* and *SMN2* copy numbers in cell lines derived from patients with spinal muscular atrophy as measured by array digital PCR. Molecular Genetics & Genomic Medicine. 2015;3(4):248-257. doi:10.1002/mgg3.141.

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