CarrierMax™ SMN1/SMN2 Reagent Kit- A PCR/CE based SMA assay that shows excellent sensitivity and specificity in determining SMN1 copy number for carrier status

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ABSTRACT

Here we present an assay that uses a method of QF-PCR amplification combining CE to detect homozygous and heterozygous deletions of exon 7 and/or exon 8 of SMN1/2 and SNPs related to haplotype as well gene conversion event for detection of carrier status.

INTRODUCTION

Spinal Muscular Atrophy (SMA), an autosomal recessive neuromuscular disorder, is the primary genetic cause of infant death with an incidence of ~1/10,000 and a carrier rate of ~1/50. SMN1 is the disease-causing gene of SMA. Approximately 95% of SMA patients have a homozygous deletion of SMN1, and the remaining 5% of patients have a heterozygous deletion of SMN1 with a pathogenic SMN1 variant. The identification of SMA carrier status in large population is necessary for congenital defects prevention. That demands the ability to distinguish the copy numbers of SMN1 gene between one and two, which means to distinguish carriers and normal persons. The high homology between SMN1 and SMN2 creates a research challenges for the copy number determination.

MATERIALS AND METHODS

To ensure the quantitative performance of the assay, we describe a method that uses the same pair of primers to amplify SMN1 and endogenous control gene in the same reaction with the same amplification efficiency. The assay is made up of two separate PCR/CE reactions.

The first reaction is to detect copy number of SMN genes. SMNP, a retropseudogene located in 9p21 with no known function, were employed as the endogenous control. As shown in Figure 1, SMN1 and SMNP, but not SMN2 can be amplified by one pair of primers. SMN2 and SMNP, but no SMN1 can be amplified by another pair of primers. These primers are incorporated in a single multiplex reaction. All those PCR amplicons are all different length and can be separated and quantified via Capillary electrophoresis (CE). The copy number of SMN1 (or SMN2) was calculated as the peak area ratio of target gene and control (SMNP).

Since the copy number primers in the first PCR reaction did not directly detect the copy number of the functional c.840C>T site that distinguish the functional differences of SMN1 and SMN2 gene. The C to T change from SMN1 to SMN2 cause the splicing loss of 90% of transcripts in SMN2 that leads to SMN2 locus only about 10% functional compare to SMN1. It would be important to rule out gene conversion event at this important nucleotide as gene conversion is more likely between SMN1 and SMN2 given the similarity of the two gene sequences. Thus a second PCR reaction is necessary to detect gene conversion event in order to correctly determine SMN1 copies. The primer design is illustrated in Figure 2.

In addition the second PCR also have additional primer sets for detecting two SNPs (g.27134T>G and g.27706-27707 del AT) associated with haplotype silent carriers where both copies of SMN1 resides on the same chromosome.

One of the pairs of primers for either SMN1 or SMN2 incorporates a FAM (6-carboxyfluoroscein) fluorescent label for the

Furthermore, we incorporated a set of amelogenin primers for sex determination, this is for the purpose of PCR positive control as well as matching the two separate PCR reaction from the same sample. These primers are labeled with HEX (hexachloro-fluorescein) fluorescent dye for detection.

Lastly, we use a ROX (6-carboxy-X-rhodamine) labeled size standards ranging from 70bps to 500pbs for precise sizing of the amplicons.

This assay has been validated on the Applied Boisystems 3500 series and SeqStudio™ Genetic Analyzers. On the 3500 series we use 50cm capillary array and POP-7™ polymer; on the SeqStudio the V2 catrtidge incorporated a 28cm capillary array and POP-1™ polymer. Both instrument utilize the standard fragment analysis run module.

The resulting fragment data is then analyzed in GeneMapper 6.0 with specifically designed panel and bin. The resulting genotype is then exported in csv format and imported into the CarrierMax™ software for final report output.

Figure 1. SMN1/SMN2 copy number detection primers

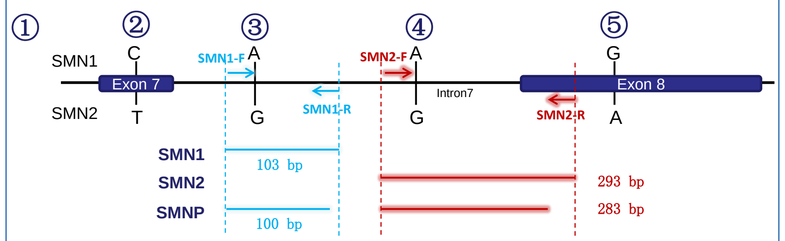


Figure 2. SMN1/SMN2 Gene conversion primers

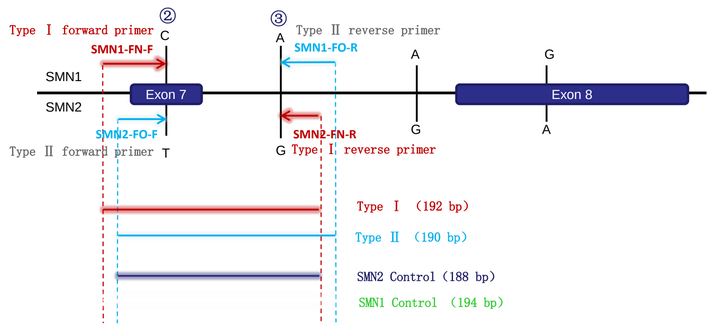


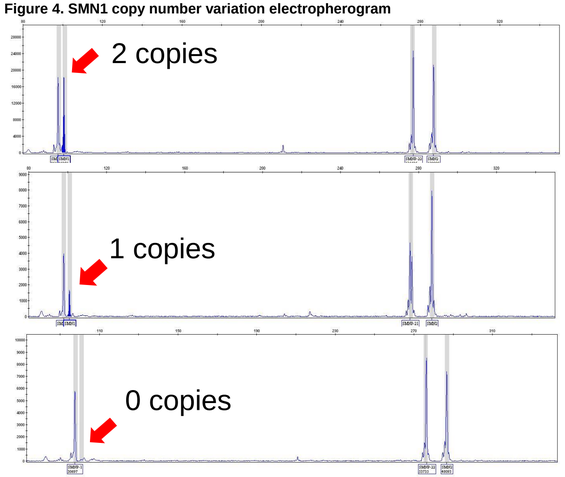
Figure 3. Instruments, reagent kit and softwares used in this study

Table 1. Example CarrierMax™ Software result report

Sample Name	SMN1 Copy Number	SMN2 Copy Number	2+0 SNPs	Classification
Control2	2	2		Normal
Control1	0	2		Homozygote affected
Type1	1	2		Carrier
Type2	2	2		Normal
g.27134T-G	2	2	g.27134T-G	Risk Factor
g.27706-27707delAT	2	2	g.27706-27707delAT	Risk Factor

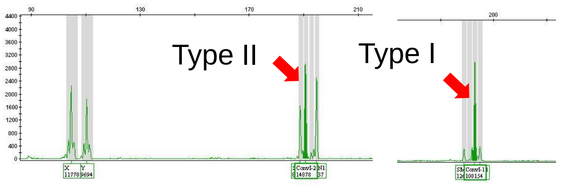
RESULTS

Figure 4. SMN1 copy number variation electropherogram



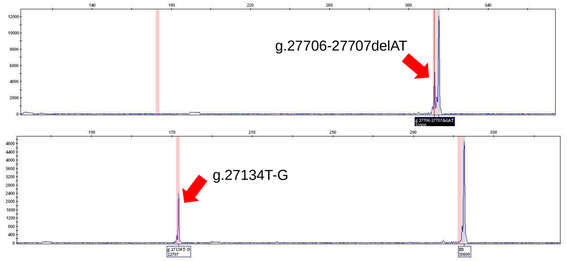
Electropherogram showing SMN1 copy number variation reflected in the SMN1 specific peak as pointed by arrow.

Figure 5. SMN1 SMN2 gene conversion detection



Type II conversion result in over call of SMN1 copy. When Type II gene conversion is detected the software automatically change the normal call to a carrier call. Type I conversion undercall of SMN1 copy number. If Type I gene conversion is detected the software will change the carrier call to normal.

Figure 6. SMN1 SMN2 haplotype carrier detection



Electropherogram showing detection of SNP genotypes associated with haplotype that both copies of SMN1 on the same chromosome (2+0 carrier status, which also called silent carrier).

Table 2. Results of 20 clinical research samples fully concordant with MLPA

Sample Name	SMN1 Copy Number	SMN2 Copy Number	Classification	Concordant with MLPA Result
CNV-0-1	0	2	Homozygote affected	YES
CNV-0-2	0	2	Homozygote affected	YES
CNV-0-3	0	2	Homozygote affected	YES
CNV-1-1	1	2	Carrier	YES
CNV-1-2	1	2	Carrier	YES
CNV-1-3	1	2	Carrier	YES
CNV-1-4	1	3	Carrier	YES
CNV-2-1	2	0	Normal	YES
CNV-2-2	2	0	Normal	YES
CNV-2-3	2	1	Normal	YES
CNV-2-4	2	1	Normal	YES
CNV-2-5	2	2	Normal	YES
CNV-2-6	2	2	Normal	YES
CNV-2-7	2	2	Normal	YES
CNV-2-8	2	2	Normal	YES
CNV-2-9	2	2	Normal	YES
CNV-2-10	2	2	Normal	YES
CNV-2-11	2	2	Normal	YES
CNV-2-12	2	2	Normal	YES
CNV-2-13	2	2	Normal	YES

Results are highly concordant with MLPA (multiplex ligation-dependent probe amplification) which is a well-known industry standard using an orthogonal method to determine SMN copy numbers.

CONCLUSIONS

We shown the CarrierMax™ SMN1/SMN2 Reagent Kit can accurately determine SMN1 and SMN2 copy numbers as well as detect gene conversion and haplotyping that can affect the copy number outcome. The reagent kit is packaged with a reporter software that is able to generate a concise result report that classifies the sample SMN genotype status.

This kit can be used to verify SMN genetic result from high throughput expanded carrier screening research product such as the Thermo Fisher CarrierSeq™ ECS Kit on the Ion Torrent NGS platform or the Applied Biosystem CarrierScan Assay on the microarray platform.

REFERENCES

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