# Gene Conversions and Hybrid Peak Detection in AmplideX® PCR/CE *SMN1/2* Kit*

TECHNICAL NOTE

## Overview of the Kit

The AmplideX® PCR/CE *SMN1/2* Kit is an *in vitro* nucleic acid amplification kit for the determination of *SMN1* and *SMN2* exon 7 copy number[1]. It is a single-tube PCR assay that amplifies distinctive *SMN1* and *SMN2* gene regions and an endogenous control (EC) gene from purified genomic DNA.

In this method, fluorescently-labeled *SMN1*- and *SMN2*-specific amplicons are resolved by capillary electrophoresis (CE) and referenced to co-amplified EC gene products to determine copy numbers. Discrete amplicon peaks are generated from the EC, *SMN1*, *SMN2*, and/or *SMN1* or *SMN2* hybrid, or chimeric, genes. Example electropherograms are shown in Figure 1.

## *SMN1/2* Gene Conversions and Hybrid Peaks

Survival motor neuron genes (telomeric *SMN1* and centromeric *SMN2*) are located within a complex region of chromosome 5q13, which is prone to duplications and deletions. These two genes have equivalent promoters and differ only by five nucleotides within their 3' ends (in intron 6, exon 7, intron 7, and noncoding exon 8)[2]. Despite their high homology, only the *SMN1* gene is necessary for survival of motor neurons. A single nucleotide difference (c. 840C>T) between *SMN1* and *SMN2* in exon 7 disrupts an exonic splicing enhancer and reduces full-length *SMN2* transcripts[3]. Due to the functional impact of this critical difference when *SMN1* is deleted, methods for dosage analysis of the *SMN1* and *SMN2* genes target copy number quantification of exon 7.

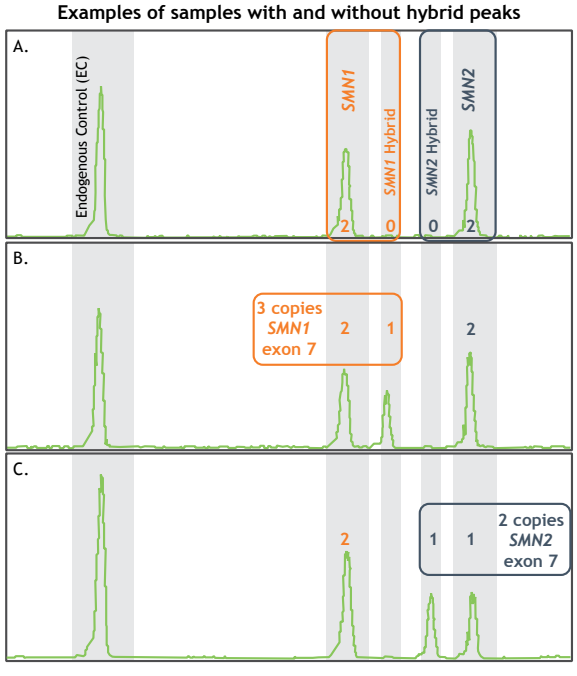


**Figure 1. A)** Commonly observed sample genotype with 2 copies of *SMN1* and 2 copies of *SMN2* gene (no hybrid peaks). **B)** Sample with *SMN1* hybrid peak (*SMN1* sequence in exon 7 with *SMN2* in intron 7). **C)** Sample with *SMN2* hybrid peak (*SMN2* sequence in exon 7 and *SMN1* in intron 7).

In addition to *SMN1* and *SMN2* gene dosage, the AmplideX PCR/CE *SMN1/2* Kit is also designed to detect and differentiate *SMN1*-to-*SMN2* and *SMN2*-to-*SMN1* gene conversions[4,5,6,7,12] within exon 7 and intron 7 regions. When present, these chimeras are detected as unique peaks in the CE trace, referred to as hybrid peaks.

An *SMN1* hybrid peak indicates a chimeric gene with *SMN1* sequence in exon 7 and *SMN2* sequence in intron 7. Similarly, an *SMN2* hybrid peak stems from *SMN1* gene conversion resulting in a *SMN2* sequence in exon 7 and an *SMN1* sequence in intron 7 (Figure 2). Importantly, only exon 7 status is used in the final copy number calculation. The presence of exon 7/intron 7 sequence mismatches due to gene conversion (as indicated by hybrid peaks) is currently provided for informational use only.

AmplideX®
PCR/CE *SMN1/2* Kit

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The AmplideX PCR/CE *SMN1/2* Macro enables streamlined analysis of *SMN1* and *SMN2* copy numbers, and reports genotypes as 0, 1, 2, 3, or ≥ 4 copies of exon 7 for both *SMN1* and *SMN2*. The exon 7 copy number represents the sum of gene-specific and hybrid gene integer copy numbers for both *SMN1* and *SMN2* (Figure 1). In addition to *SMN1* and *SMN2* genotypes, the macro output indicates a gene conversion event within the exon 7/intron 7 region in a separate hybrid peak column (labeled as "*SMN1*," "*SMN2*," or "both" when gene conversion is present)[8].

## Frequency of *SMN1/2* Gene Conversion Events

Conventional qPCR-based *SMN1/2* assays query c.840C>T in exon 7 and cannot flag *SMN* hybrid genes. These chimeras may be detected if a method is used to interrogate multiple loci that distinguish the two genes, as is the case for the AmplideX PCR/CE *SMN1/2* Kit. We note, however, that the junctions for *SMN1/2* chimeras are heterogeneous[7], and thus the identification of *SMN* hybrids across different assay designs that probe multiple gene-specific sequences may not agree with one another, even though each may produce an analytically accurate result.

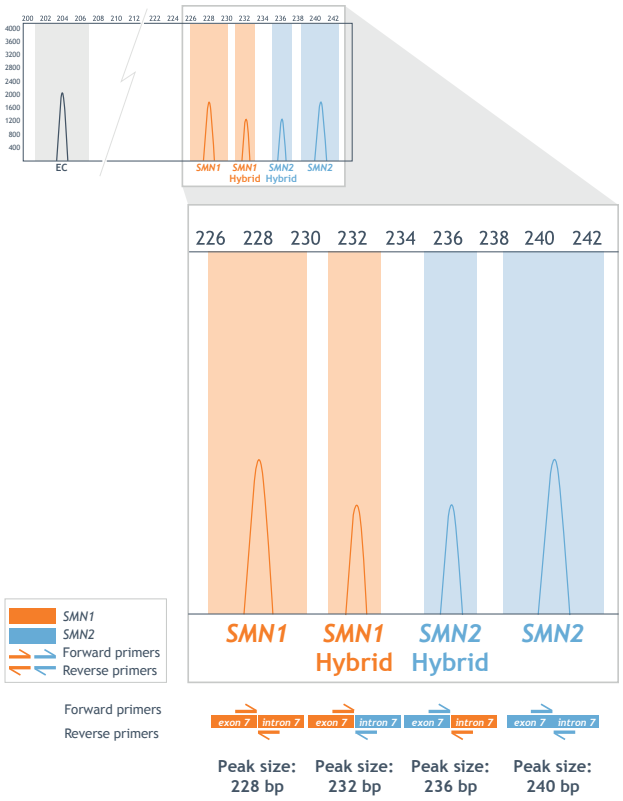


**Figure 2. Schematic of all SMN peak sizes in base pairs, and their corresponding exon 7/intron 7 status.**

Several studies have reported that *SMN1/2* gene conversions are present in approximately 1% of the population[9,10,11]. A similar rate of gene conversion was detected using the AmplideX PCR/CE *SMN1/2* Kit; a screen of 1426 human non-SMA samples uncovered 14 samples with *SMN* gene conversions (0.98%). Ten of the conversions (0.70%) had *SMN1* exon 7 along with *SMN2* intron 7 (*SMN1* hybrid peak) and four samples (0.28%) had *SMN2* exon 7 with *SMN1* intron 7 (*SMN2* hybrid peak). Dosage analysis of hybrid peaks from this sample set showed that the majority of samples had only 1 copy of a hybrid peak but cases of 2 copies have also been detected (two out of the fourteen samples with gene conversions had 2 copies of a hybrid peak). The aggregate exon 7 *SMN1* and *SMN2* copy number results for all gene conversion samples were confirmed with a qPCR-based orthogonal method.

## References


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