Next-generation sequencing assay accurately determines carrier status for Spinal Muscular Atrophy

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We developed an NGS-based assay for the most common cause of SMA that offers higher accuracy and throughput, lower cost, and higher dynamic range than the current state-of-the-art standalone qPCR assays.

Abstract

Spinal Muscular Atrophy (SMA) is a severe neuromuscular disease that is the second most common fatal autosomal recessive disorder. Nearly 95% of SMA-affected patients have *SMN1* genes that are rendered largely dysfunctional due to a splicing mutation that causes exon 7 to be preferentially omitted from the SMN protein. However, traditional methods such as allele-specific qPCR and MLPA are relatively expensive and low throughput in large scale carrier screening, and incapable of detecting novel variants. We describe a high-throughput next-generation sequencing (NGS) method that naturally fits in the context of an NGS-based expanded carrier screen and accurately determines the *SMN1* and *SMN2* copy numbers in a sample.

Map reads to *SMN1* and *SMN2*

We designed hybrid-capture probes common to *SMN1* and *SMN2*. NGS reads were assigned to each gene according to the single base that distinguishes them in exon 7.

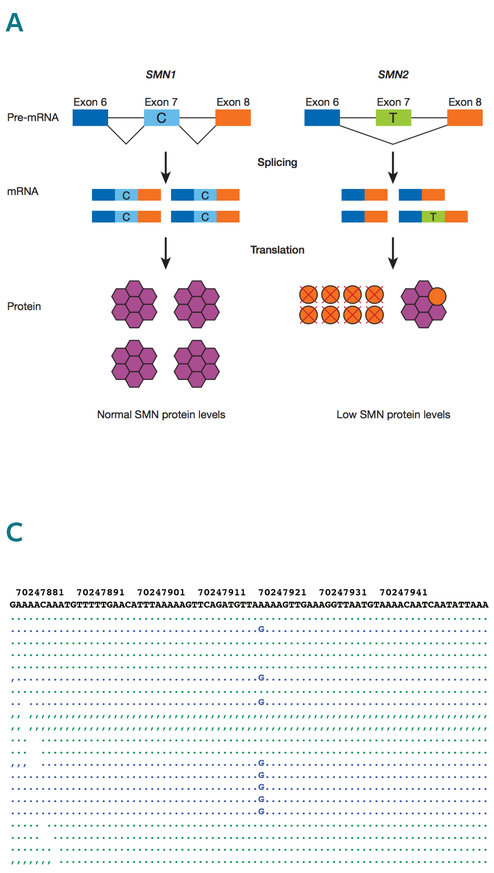

Figure 1. Schematic of SMA mechanism, and workflow mapping reads to SMN1 and SMN2. (A) Mechanism of the most common cause of SMA, a splicing variant in exon 7 (C→T at position 840) distinguishes SMN1 and SMN2 (image from Life Technologies). (B) Hybrid capture probes were designed for targeted sequencing of SMN1/2 regions. (C) Reads are assigned to SMN1 (blue) or SMN2 (green) based on the splicing variant shown in the pileup.

Call copy number

Relative read depths were calculated by normalizing to the depth in regions elsewhere in the genome of comparable GC content, where median copy number is expected to be two. Copy-number calls and associated confidence scores were calculated from a distance test that found the most likely integer copy number for each gene.

Figure 2. Normalized read depths are used to determine the copy number of each sample. (A) Unnormalized read depths for SMN1; (B) Normalized read depths for SMN1.

NGS assay determines *SMN1* and *SMN2* copy numbers with high accuracy

1. The NGS assay allows higher accuracy across a broader dynamic range than qPCR, enabling confident copy-number calls in excess of two copies for *SMN1* and as many as six copies for *SMN2*.
2. Multiple tiled hybrid capture probes prevent false positive results arising from genomic variants within particular probe regions.
3. NGS assay enables novel variant detection.

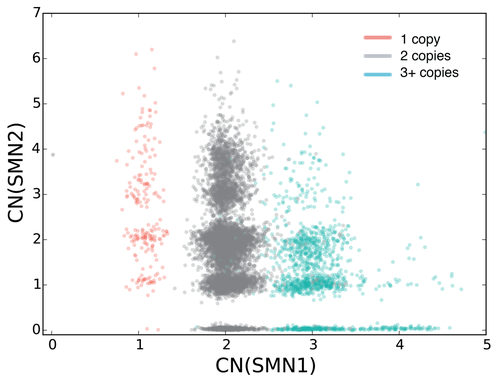

Figure 3. Confident call of copy number in excess of two copies for both SMN1 and SMN2. Colors indicate copy number calls from qPCR assay.

Our NGS-based method correctly determines copy number of *SMN1* and *SMN2*, and resolves SMA carrier status with > 95% detection rate on a set of more than 87,000 clinical samples.

qPCR \ NGS	carrier	non-carrier
carrier	1.82e-2	1.17e-4
non-carrier	4.69e-4	0.981

Table 1. Confusion matrix: performance of NGS assay (with cutoff 1.45 for calling carrier) vs. qPCR.

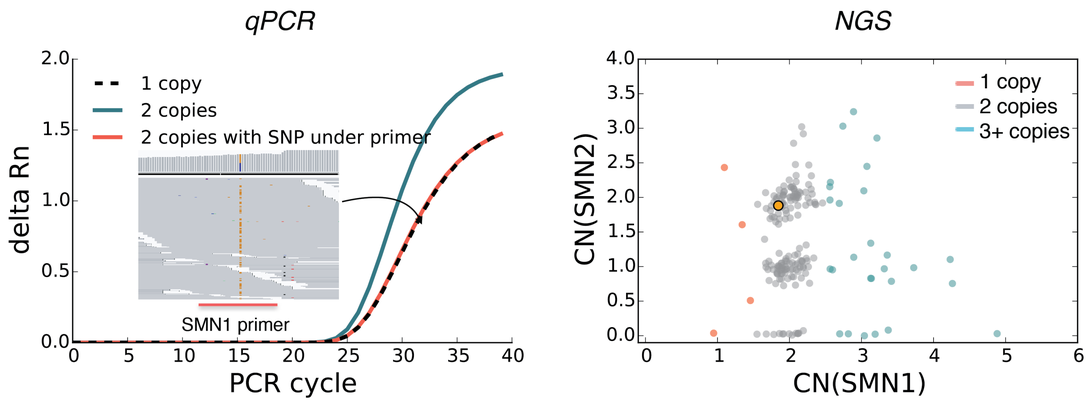

Figure 4. NGS assay for SMA is more robust to false positives and false negatives relative to qPCR methods. Figure shows an example of a qPCR false positive due to SNP in the primer binding site, that is correctly called in NGS. (ACMG guidelines: Technical standards and guidelines for spinal muscular atrophy testing, Genetics In Medicine | Volume 13, Number 7, July 2011)