A novel digitalMLPA assay for comprehensive copy number variant detection in genes associated with cardiac disorders

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Introduction

Cardiac disorders are a major cause of mortality worldwide and are often driven by genetic alterations, including germline copy number variants (CNVs). Molecular genetic testing has evolved from single-gene assays to Next-Generation Sequencing (NGS)-based approaches. However, accurate CNV detection using these methods remains challenging and often requires complex bioinformatics analysis.

To address this limitation, we are developing **NXtec D011 Cardiac Panel**. This digitalMLPA assay detects CNVs across more than 60 genes and genomic regions associated with cardiac disorders, including cardiomyopathies, arrhythmia syndromes, familial hypercholesterolemia, and syndromic congenital heart disease.

digitalMLPA is a multiplex PCR-based technology that enables comprehensive CNV analysis through the simultaneous hybridisation of hundreds of probes, coupled with the high coverage of NGS. NXtec D011 Cardiac Panel contains over 900 target probes covering the genes and genomic regions outlined in ***Table 1***.

Table 1. **Genes and regions targeted by NXtec D011 Cardiac Panel.**

Genes	*ACTC1*	*ACTN2*	*ALPK3*	*APOB*	*BAG3*	*CACNA1C*	*CALM1*
CALM2	*CALM3*	*CASQ2*	*CHD7*	*CSRP3*	*DES*	*DSC2*	*DSG2*
DSP	*ELN*	*ETS1*	*FHL1*	*FHOD3*	*FLNC*	*GLA*	*JAG1*
JPH2	*JUP*	*KCNE1*	*KCNE2*	*KCNH2*	*KCNJ2*	*KCNJ5*	*KCNQ1*
LAMP2	*LDLR*	*LMNA*	*MYBPC3*	*MYH7*	*MYL2*	*MYL3*	*NEXN*
PCSK9	*PKP2*	*PLN*	*PRKAG2*	*PTPN11*	*RAF1*	*RBM20*	*RIT1*
RYR2	*SCN5A*	*TBX5*	*TECRL*	*TMEM43*	*TNNC1*	*TNNI3*	*TNNT2*
TPM1	*TRDN*	*TTN*	*TTR*	*VCL*	**Regions**	1p36	22q11

Materials and Methods

The performance of NXtec D011 Cardiac Panel was evaluated by testing 39 DNA samples harbouring a diverse range of previously characterised CNVs that together cover 47 genes/regions targeted by this assay. The sample set consisted of cell line-derived DNA samples obtained from the Coriell Institute and externally provided DNA samples, for which independently determined genotype information was available.

Reactions were performed according to the digitalMLPA NXtec Protocol (MRC Holland), using ~40 ng of purified genomic DNA (***Figure 1***). digitalMLPA-derived PCR amplicons were pooled and sequenced on a NextSeq 1000 Sequencing System (Illumina). The resulting FASTQ files were analysed using Coffalyser digitalMLPA for CNV calling. All test samples were normalised against dedicated reference samples of the same sex to obtain the final probe ratios.

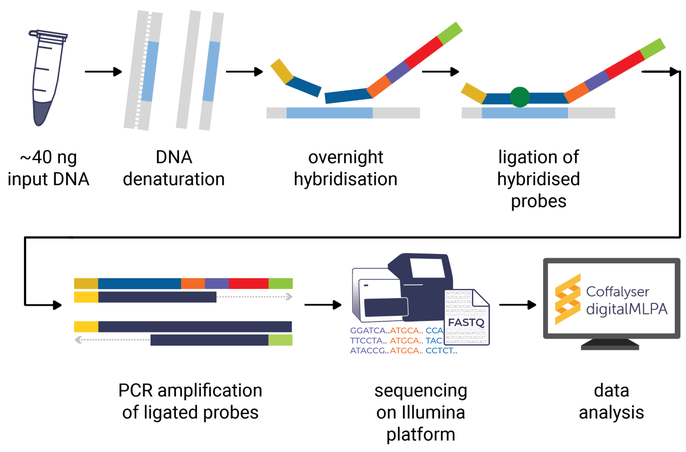

Figure 1. **Schematic overview of the digitalMLPA workflow.** DNA is denatured and the left and right probe oligonucleotides hybridise to their adjacent target sequences. The hybridised oligonucleotides are then ligated and exponentially amplified by PCR. The resulting products are sequenced on an Illumina platform and analysed using Coffalyser digitalMLPA software.

Conclusions

digitalMLPA assay NXtec D011 Cardiac Panel is a promising tool for reliable identification of CNVs in a wide range of genes and chromosomal regions associated with cardiac disorders:

- Simultaneous analysis of 62 target genes/regions in one single reaction, reducing the number of individual tests per sample.
- Accurate detection of CNVs from whole gene to single-exon level.
- Simple protocol and user-friendly analysis software.

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Results

The digitalMLPA assay accurately detected all known CNVs across the sample set tested, except for one sample (***Figure 3***). The observed CNVs included both intragenic and whole gene deletions and duplications affecting 45 genes, as well as partial deletions and duplications of chromosomal regions 1p36 and 22q11 (***Figure 2***).

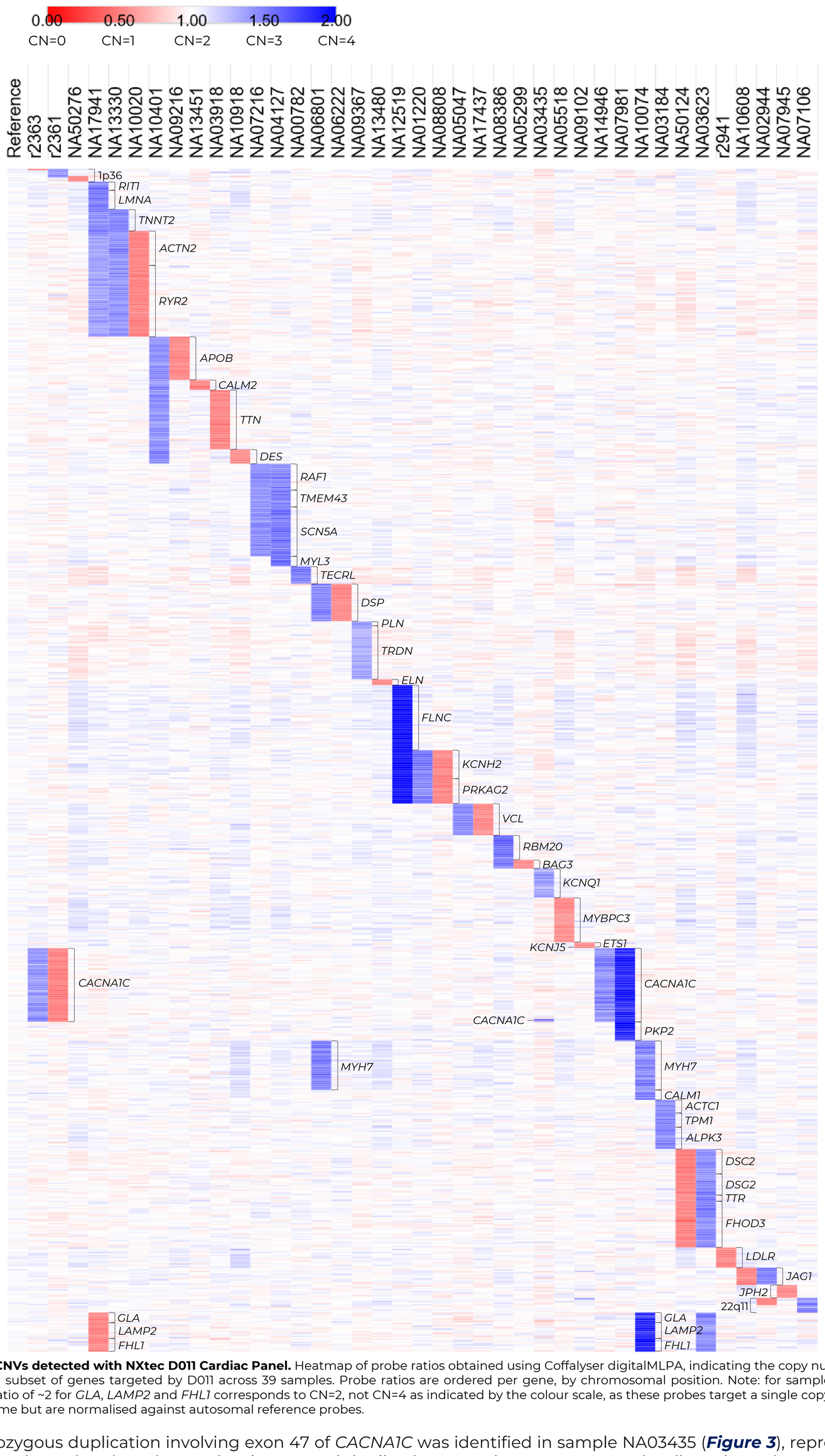

Figure 2. **CNVs detected with NXtec D011 Cardiac Panel.** Heatmap of probe ratios obtained using Coffalyser digitalMLPA, indicating the copy number (CN) status of a subset of genes targeted by D011 across 39 samples. Probe ratios are ordered per gene, by chromosomal position. Note: for sample NA10074 (male), a ratio of ~2 for *GLA*, *LAMP2* and *FHL1* corresponds to CN=2, not CN=4 as indicated by the colour scale, as these probes target a single copy on the X-chromosome but are normalised against autosomal reference probes.

A heterozygous duplication involving exon 47 of *CACNA1C* was identified in sample NA03435 (***Figure 3***), representing a smaller aberration than the previously reported duplication spanning exons 38-47. The discordance between these findings requires additional investigation.

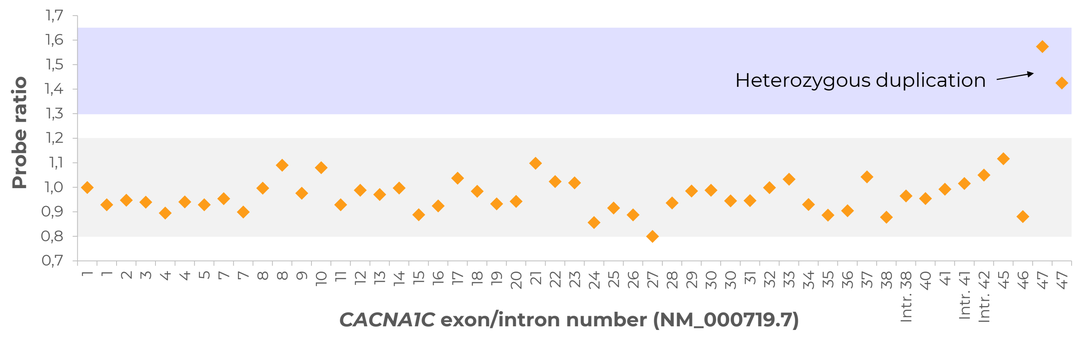

Figure 3. **Probe ratios for sample NA03435 carrying a *CACNA1C* exon 47 duplication.** Each datapoint represents one probe. The y-axis indicates the probe ratio, which is normalised to the reference samples. The x-axis indicates the *CACNA1C* exon/intron targeted by one probe of D011. A ratio of 0.80-1.20 corresponds to CN=2, while a ratio of 1.30-1.65 corresponds to CN=3.

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