Genome-wide detection of gross copy number alterations associated with genetic conditions using digitalMLPA

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INTRODUCTION

Fast and reliable characterisation of gross copy number (CN) changes is essential for cytogenetic analysis. Classical cytogenetic testing using karyotyping and FISH, as well as next-generation sequencing (NGS)-based approaches and optical genome mapping (OGM), remain relatively costly, labour-intensive, and require more elaborate bioinformatics analysis, resulting in longer turnaround times. qPCR-based methods typically only target a limited number of regions in a single assay.

Thus, there is an urgent need for genome-wide CN characterisation of DNA samples for cytogenetic applications in a cost-effective and high-throughput manner.

Here, we showcase the SALSA® digitalMLPA™ Probemix D024 Genome-wide CNV Characterisation (test version), a SALSA® digitalMLPA™ assay consisting of ~1200 probes, for genome-wide CN characterisation at a ~2-4 Mb resolution. In this study, this assay was tested using a panel of cell lines harbouring clinically significant copy number variations (CNVs).

MATERIALS AND METHODS

Experiments were performed using SALSA® digitalMLPA™ Probemix D024 Genome-wide CNV Characterisation (see Figure 1 and 2). digitalMLPA-derived PCR amplicons were sequenced on a NextSeq 1000 Sequencing System (Illumina). FASTQ files were analysed using data analysis software Coffalyser digitalMLPA™ (MRC Holland). The CNVPANEL01, comprising 43 cell lines harbouring clinically significant cytogenetic aberrations, was purchased from the Coriell Institute.

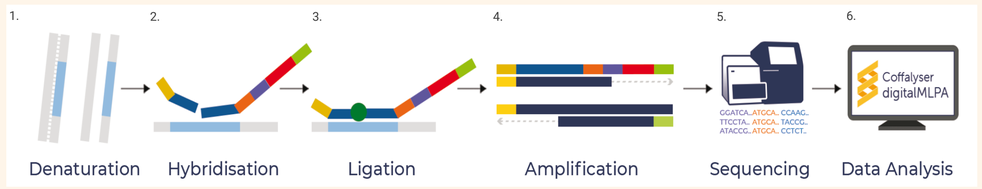

Figure 1. digitalMLPA workflow
digitalMLPA steps. 1) DNA denaturation. 2) Hybridisation: the left probe oligo (LPO) and right probe oligo (RPO) bind to their target DNA. 3) Ligation: hybridised probes are ligated. 4) Amplification: ligated probes are amplified by PCR. 5) Sequencing: PCR products are quantified on an Illumina platform. 6) Data analysis is performed using Coffalyser digitalMLPA™.

SALSA® digitalMLPA™ Probemix D024 Genome-wide CNV Characterisation content:

- ~1200 probes covering all chromosomes at a resolution of ~2-4 Mb.
- 78 SNV probes that allow sample differentiation and identification.
- >160 control probes and fragments to aid in data normalisation, detection of experimental deviations when performing this assay, and to check for impurities in and fragmentation of the DNA samples that could influence the digitalMLPA reaction.

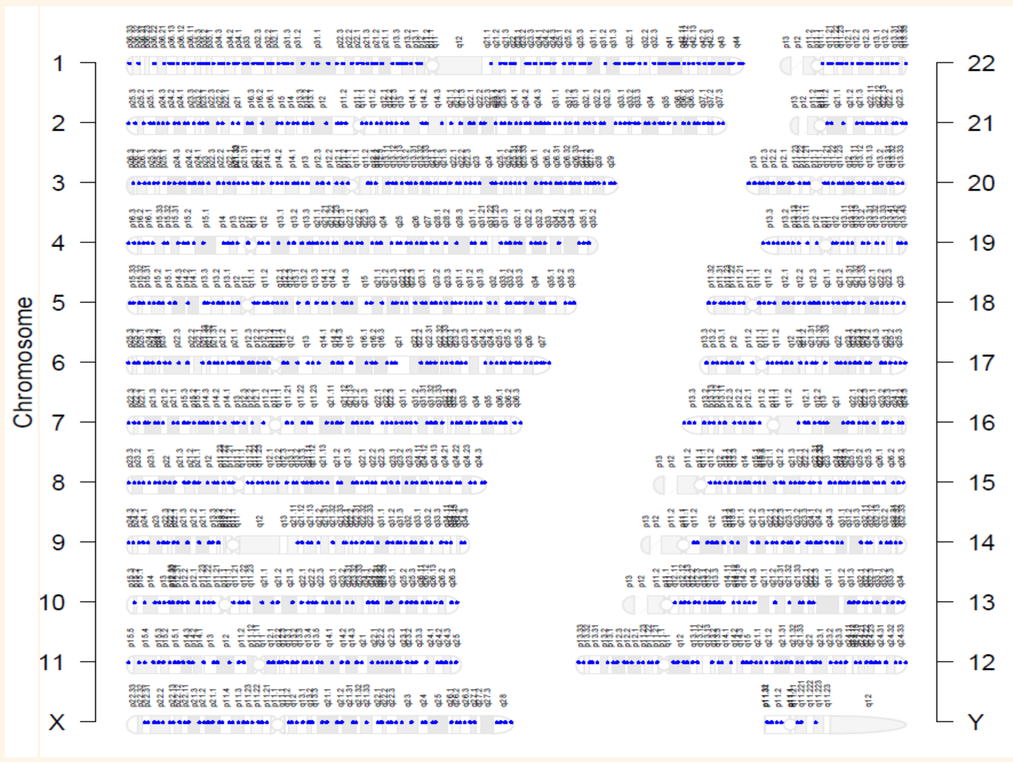

Figure 2. D024 Genome-wide CNV Characterisation content
Chromosome plot showing the genome-wide coverage of the D024 Genome-wide CNV Characterisation probemix. Each blue dot represents one digitalMLPA probe target site.

RESULTS

Genome-wide detection of copy number variations using D024 Genome-wide CNV Characterisation

The copy number status of the CNVPANEL01 was analysed using D024 Genome-wide CNV Characterisation (test version). A heatmap showing the copy number results for each sample obtained using Coffalyser digitalMLPA™ is shown in Figure 3. The results were compared to the existing data from the Coriell Institute, revealing a high concordance between the two datasets (98.5% for n = 51084 datapoints).

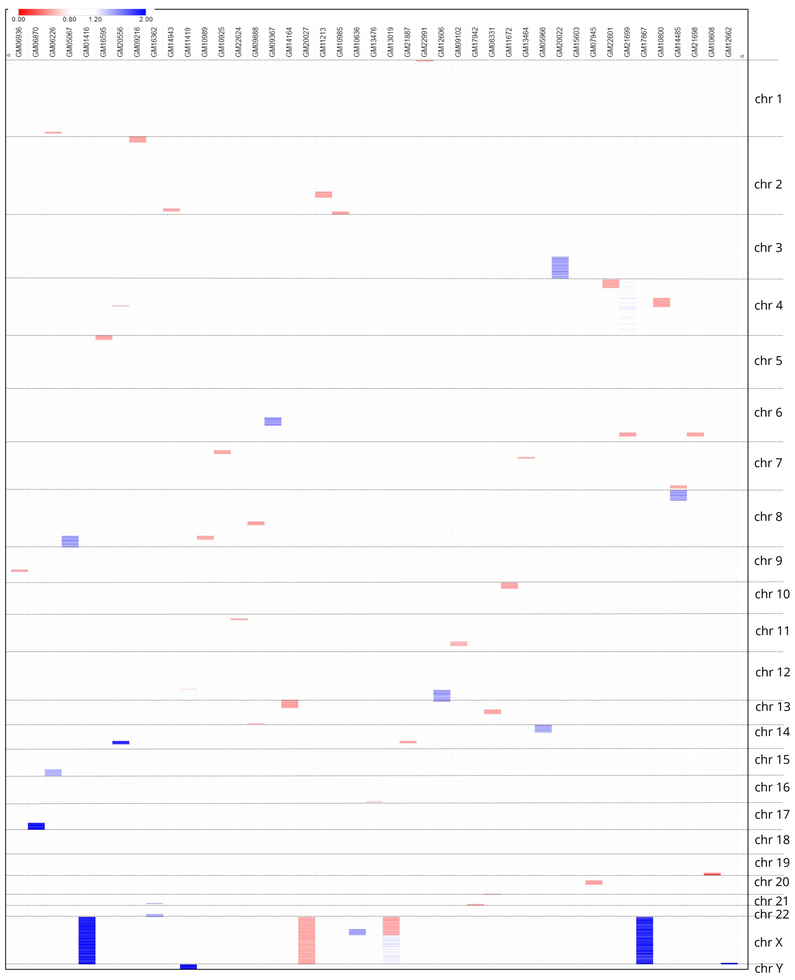

Figure 3. Detection of a wide range of copy number variations in the Coriell CNVPANEL01 using D024 Genome-wide CNV Characterisation
Heatmap showing the copy number variations for the 43 samples of the Coriell CNVPANEL01. Colour coding: normal copy number (probe ratio ~1.0) is white, deletions (probe ratio <0.8) are shown in red and gains (probe ratio >1.20) are shown in blue.

digitalMLPA as a fast, reliable and cost-effective method for genome-wide CN characterisation

Using the digitalMLPA assay D024 Genome-wide CNV Characterisation, it is possible to reliably detect gross copy number changes, as well as smaller subchromosomal CNVs at a ~2-4 Mb resolution in a single reaction (see Figure 4).

In Table 1, various methods that are used for genome-wide CN determination are compared. Altogether, digitalMLPA is a simple and user-friendly technique with short hands-on time, and a turnaround time of only 48-72 hours. Importantly, this technique requires as little as 20 ng DNA input material per sample, and is compatible with Illumina sequencing platforms. With free of charge and easy-to-use data analysis software (Coffalyser digitalMLPA™), it is a cost-effective approach for reliable genome-wide CN characterisation.

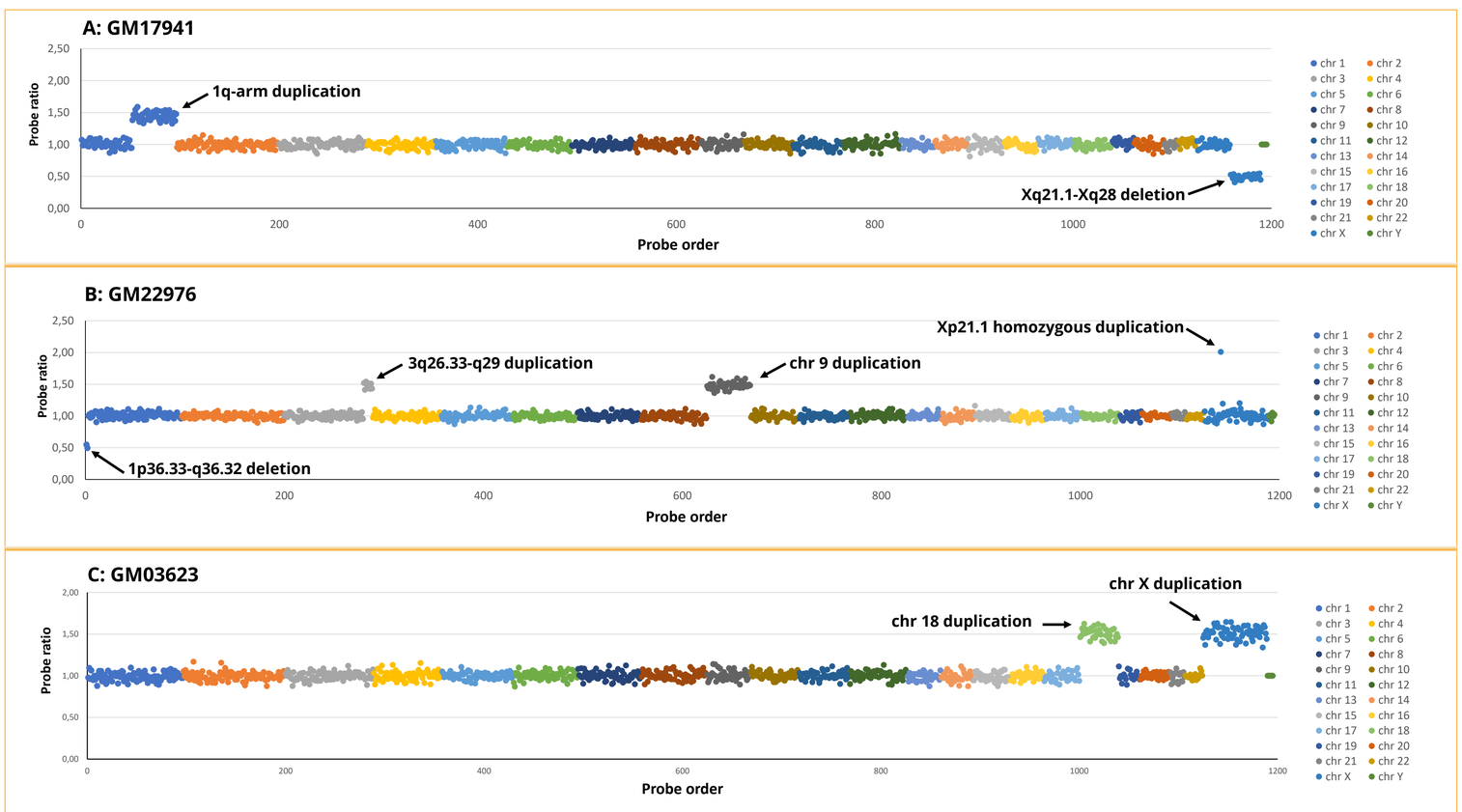

Figure 4. Detection of a diverse range of copy number variations using D024 Genome-wide CNV Characterisation
A-C: Three examples of copy number profiles from Coriell samples determined using D024 Genome-wide CNV Characterisation. The digitalMLPA probes are organised on the x-axis, based on chromosomomal location. The y-axis indicates the probe ratio normalised to the reference samples. Copy number varations detected with digitalMLPA are consistent with those described by Coriell for these samples.

Table 1. Comparison of different techniques used for genome-wide CN determination

Feature	Karyotyping	Array CGH	SNP Array	Whole Genome Sequencing (WGS)	Low coverage WGS	Optical Genome Mapping	digitalMLPA (D024)
Turnaround time	3- ≥7 days	2–3 days	2–3 days	5–7 days	3–5 days	5-7 days	2 days
Hands-on time	Moderate	Moderate	Moderate	High	Moderate	Low-Moderate	Low
DNA input required	Dividing cells required	~500 ng	200–500 ng	~500–1000 ng	~50–100 ng	> 100 ng of ultra high molecular weight DNA	~20-40 ng
Estimated cost per sample	€€	€€	€€€	€€€€	€€€	€€€€	€
Bioinformatics needs	None - Manual scoring needed	Low–Moderate	Moderate	High (significant pipeline)	Moderate to High	Moderate (custom pipeline)	None
Resolution for CNV detection	5-10 Mb	5–100 kb	10–50 kb	Base-pair level	100kb–1 Mb (depth-dependent)	>0.5-50 kb	~2-4 Mb
Advantages	Detects large balanced rearrangements	Well-established; Cost-effective	Also detects LOH, UPD	Very high resolution; Detects all variant types	Cheaper than WGS; Scalable	Can detect all classes of SVs and CNVs	Simple; Fast; Cost-effective
Disadvantages	Low resolution; Dividing cells required	Misses balanced events; lower resolution	May miss complex rearrangements	High cost; complex data analysis	Lower resolution than WGS	High cost; Requires UHMW DNA	Limited to designed probes

CONCLUSIONS

- CN data obtained with D024 Genome-wide CNV Characterisation showed a high concordance with the Coriell CNVPANEL01 dataset.
- Using this digitalMLPA assay, a wide range of gross CN variations can be reliably determined genome-wide in one reaction in a cost-effective manner, with a short (48-72h) turnaround time as compared to other techniques.

For further information please contact Sander Palit at s.palit@mrcholland.com

digitalMLPA is for research use only. Not for use in diagnostic procedures.
The product described concerns a trial version that is not available for general purchase.

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