MRC Holland

digitalMLPA™

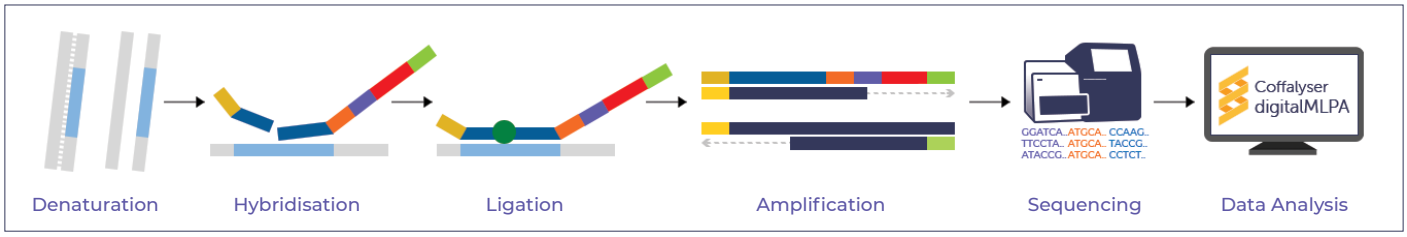
Confidence in Copy Number Determination

digitalMLPA™

Where MLPA meets NGS

- ✓ **Specific:** unparalleled copy number certainty for complex genetic regions
- ✓ **Cost-effective:** up to 1600 DNA targets in one reaction
- ✓ **Simple:** easy hands-on steps and no library quantification needed
- ✓ **Robust:** only 20 ng of sample DNA needed; uniform coverage generated
- ✓ **Straightforward:** free software, simple analysis, clear-cut results

digitalMLPA is a multiplex PCR followed by Illumina sequencing-based amplicon quantification, for the detection of CNVs and selected SNVs. digitalMLPA amplifies ligated probes with a universal PCR primer pair, enabling unbiased amplification. With digitalMLPA, up to 1600 unique sequences can be detected and quantified in a single reaction.



The digitalMLPA technique is similar to SALSA® MLPA®, the gold standard for CNV detection, but with the ability to examine many more targets in a single reaction. Analysis is done using free, easy-to-use software – so no bioinformatic skills are needed.

NXtec™ probemixes	
Predisposition to Cancer	**NXtec D001 Hereditary Cancer Panel 1** Focused, cost-effective panel targeting key genes associated with a hereditary predisposition to breast, ovarian, colorectal, gastric, prostate, pancreatic and endometrial cancer, and melanoma.
	NXtec D002 Hereditary Cancer Panel 2 Broad panel targeting genes associated with a wide range of cancer types, including breast, ovarian, colorectal, gastric, prostate, pancreatic and endometrial cancer, melanoma, neurofibromatoses, retinoblastoma, Wilms' tumour, and more.
Multiple Myeloma	**NXtec D006 Multiple Myeloma** Extensive panel covering all copy number alterations (CNAs) included in current multiple myeloma staging protocols. This assay enables multiplex detection of routinely analysed regions such as 1p, 1q, 13q, 17p and hyperdiploidy, while also supporting research into emerging molecular markers, including CAR T cell targets like *BCMA*, *GPRC5D* and *FcRH5*.
Acute Lymphoblastic Leukemia	**NXtec D007 Acute Lymphoblastic Leukemia** Targeted panel for highly specific genome-wide CNA detection across 73 ALL-related genes and regions. Captures a broad spectrum of key alterations, including (partial) chromosome gains and losses, high-level amplifications, intrachromosomal gene fusions, and intragenic CNAs. Enables comprehensive genomic profiling to support advanced research into the molecular stratification of ALL, including *IKZF1*^{del} and *IKZF1*^{plus} profiles.
Neuromuscular	**NXtec D008 DMD** High-resolution coverage of the *DMD* gene with two probes per exon for the detection of CNVs associated with Duchenne & Becker muscular dystrophies (DMD & BMD).
Chromosomal Profiling	**NXtec D024 KaryoProfiler** Genome-wide panel for assessing large-scale chromosomal copy number changes at 2–4 Mb resolution. Enables wide range of possibilities for cytogenetic and karyotype analysis across diverse applications, from products of conception and constitutional CNV characterisation to stem cell and cell line stability studies, with detection of mosaicism, subclonality and sample cross-contamination.*
Carrier Status	**NXtec D028 Carrier Panel 1** Comprehensive panel for the detection of CNVs and selected SNVs associated with spinal muscular atrophy (SMA), Duchenne & Becker muscular dystrophy (DMD & BMD), alpha- and beta-thalassemia, cystic fibrosis (CF), congenital adrenal hyperplasia (CAH), DFNB1 hearing loss, juvenile neuronal ceroid lipofuscinosis and cystinosis.

D008 expected Q3

*Not suitable for balanced structural variants or cancer cell karyotyping.

Features	Advantages
Wide assay coverage	Up to 1600 DNA targets per reaction
Quick turnaround	From DNA to sequencer in <24 hours
Low DNA input	Requires only 20 ng of sample DNA
Highly specific	Can discriminate 1 nt differences, allowing for: o reliable gene-pseudogene distinction (e.g. *PMS2*/*PMS2CL*) o analysis of complex regions (e.g. *PMS2*, *PTEN, SMN, HBA, HBB, CYP21A2*) o detection of selected SNVs (e.g. *BRAF* p.V600E, *MITF* c.952G>A)
Wide range of CNV detection	CNV detection ranging from whole chromosomes to single exons
Simple library prep	No library quantification needed
	No DNA enrichment needed, thus removing associated bias
Uniform coverage for accurate results	Universal PCR primer pair eliminates amplification bias
	Robust even with varying read depths (recommended read depth: 600x)
	Efficient amplification of probes in AT and GC-rich regions (e.g. *CEBPA*, *STK11, DMD*)
Highly-targeted	Sequencing of probes, *not* sample DNA, meaning: o reduced chance of incidental findings o no allelic dropout caused by SNVs interfering with primer binding o simplified data analysis; no dependence on alignment to a reference genome
Extensive quality control	Robust data normalisation due to extensive number of reference probes
	Built-in quality control for enzymatic activity, sample fragmentation, depurination, denaturation, read depth and other reaction conditions
	Free software for quality control and result calculation

Protocol

1. DNA denaturation
- Sample DNA is mixed with a unique barcode solution and denatured.

2. Probe hybridisation to sample DNA
- A probemix consisting of up to 1600 probes is added to the denatured DNA/barcode sample mix.

3. Ligation of hybridised probes
- Hybridised digitalMLPA probes are ligated to form a fully amplifiable probe.

4. PCR amplification
- Ligated digitalMLPA probes are all amplified using a single PCR primer pair.

5. Illumina sequencing
- Equal volumes of digitalMLPA PCR reactions are mixed and diluted.
- Diluted PCR products are loaded directly on an Illumina sequencer.

6. Data analysis
- Data analysis software is used for reaction quality control, probe quantification and ratio determination to identify aberrations.

Throughput

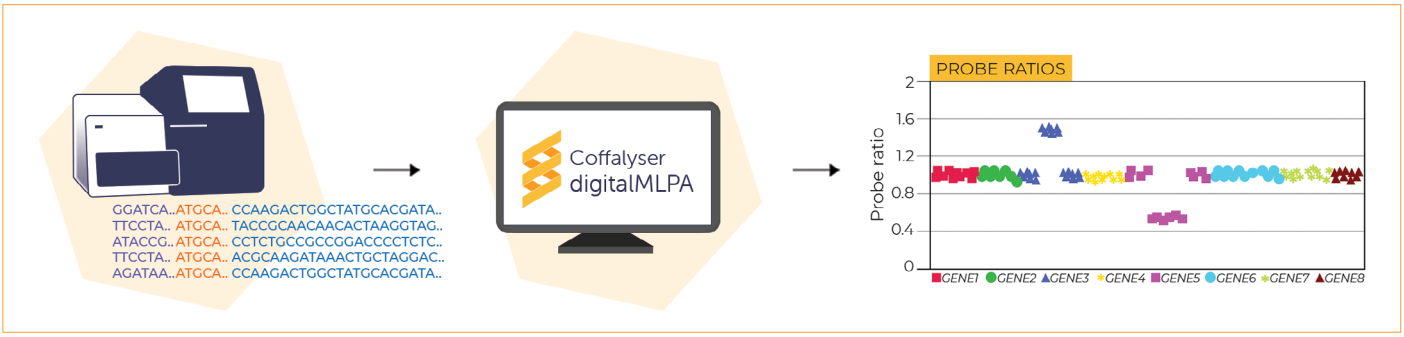
Illumina instrument[1]	Samples per run[2]
iSeq 100	Up to 11
MiSeq System (v3 chemistry), MiniSeq System	Up to 69
All NextSeq, HiSeq and NovaSeq Systems	Up to 384 [3]

[1] Besides the instrument, the number of samples also depends on the sequencing kit size used.
[2] Sample numbers based on 600-probe digitalMLPA assay at an average read depth of 600x.
[3] Four barcode plates are available to label a maximum of 384 samples; check barcode plate-compatibility in the product documentation.

Please read Instructions for use for more detailed information.

Coffalyser digitalMLPA™
Data analysis software for clear CNV calling

- ✓ **Simple:** FASTQ files are directly loaded into the software
- ✓ **Smart:** automatic digitalMLPA sequence read and probemix recognition
- ✓ **Reliable:** extensively tested and validated
- ✓ **Safe:** thorough built-in quality control



Coffalyser digitalMLPA is free and easy-to-use software developed by MRC Holland and built specifically for the analysis of digitalMLPA data. The software automatically recognises and extracts digitalMLPA sequence reads from FASTQ files. This is followed by advanced data quality checks, and the return of a clear report displaying all detected aberrant regions.

Interested in digitalMLPA?
For ordering and more information, visit
mrcholland.com or email info@mrcholland.com.

MRC Holland

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