MRC Holland

SALSA® digitalMLPA

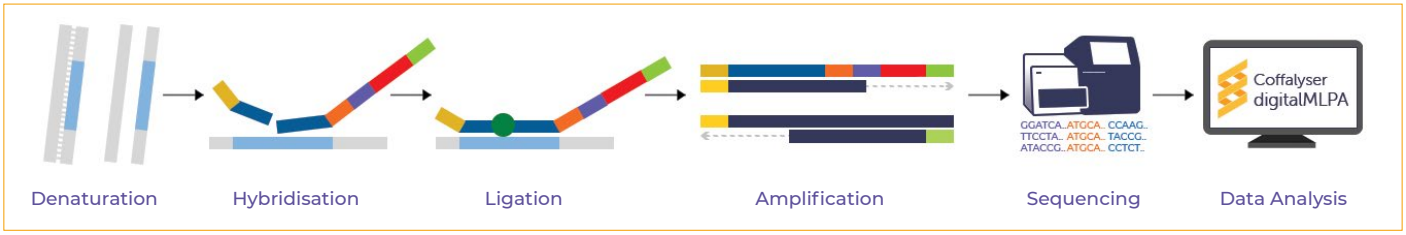
Confidence in Copy Number Determination

SALSA® digitalMLPA – where MLPA meets NGS

The fastest way to broad copy number certainty

- ✓ **Reliable:** proven copy number detection technology[+]
- ✓ **Robust:** only 20 ng of sample DNA needed; uniform coverage
- ✓ **Quality control:** control probes included in each reaction
- ✓ **Simple:** easy hands-on steps and no library quantification needed
- ✓ **Cost-effective:** up to 1000 probes in one reaction
- ✓ **Sample certainty:** SNV probes present to distinguish samples
- ✓ **Easy analysis:** user-friendly analysis with free Coffalyser digitalMLPA software

SALSA® digital Multiplex Ligation-dependent Probe Amplification (digitalMLPA) is a multiplex PCR followed by Illumina sequencing-based amplicon quantification, for the detection of copy number variations (CNV) and specific point mutations. digitalMLPA amplifies ligated probes with a universal primer pair enabling unbiased amplification. With digitalMLPA, up to 1000 unique sequences can be detected and quantified in a single reaction.



The digitalMLPA technique is similar to conventional MLPA, the gold standard for CNV detection of multiple DNA sequences, but with the ability to examine many more targets in a single reaction. digitalMLPA samples can be combined with other NGS sequencing libraries in a single run to give simultaneous results for reliable CNV quantification and NGS sequence analysis. This saves time and money, ensuring sample result turnaround times are met.

digitalMLPA data analysis is count-based, uses free software (Coffalyser digitalMLPA), and can be done on any Windows 10-based personal computer. Coffalyser digitalMLPA returns two reports for easy reaction quality determination and result interpretation. Best of all, no bioinformatician is needed for result interpretation.

SALSA® digitalMLPA is for Research Use Only. Not for use in diagnostic procedures.

Call CNVs with confidence, reduce your turnaround time, and lower your sample costs with SALSA® digitalMLPA.

[+] Benard-Slagter *et. al. J Mol Diagn.* 2017 Sep;19(5):659-672.

Features	Advantages
Low DNA input	Optimal results with 20-200 ng of sample DNA
Highly specific	Probes easily distinguish differences of 1 nt allowing for: o easy distinction between genes and pseudogenes o detection of select SNVs with dedicated probes o analysis of complex regions (e.g. *PMS2*, *HBA*)
Wide range of CNV detection	CNV detection ranging from whole chromosomes to single exons
Uniform coverage for accurate results	Universal primer pair eliminates amplification bias
	Reliable CNV calling with low sequence read depth due to low probe read depth variability
	Efficient amplification of probes in both AT and GC-rich regions
No direct DNA sequencing	Sequencing of optimized probes - not test DNA - meaning: o reduced chance of incidental findings & privacy problems o less allelic dropout due to SNVs in primer binding sequences o simplified data analysis; no dependence on alignment to a reference genome
No capture panel use	No sequence bias introduced by capture and capture fragmentation
Extensive quality control	Robust data normalization due to having a large number of reference probes
	Built-in quality control for enzymatic activity, sample fragmentation, depurination, denaturation, read numbers, and reaction conditions
	Free software for quality control and result calculation
	SNV probes included for sample identification and detection of sample contamination

digitalMLPA protocol

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

2. Probe hybridisation to sample DNA
- A digitalMLPA probemix consisting of up to 1000 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
- Coffalyser digitalMLPA is used for reaction quality control, probe quantification and ratio determination to identify sample aberrations.

Extensive multiplexing

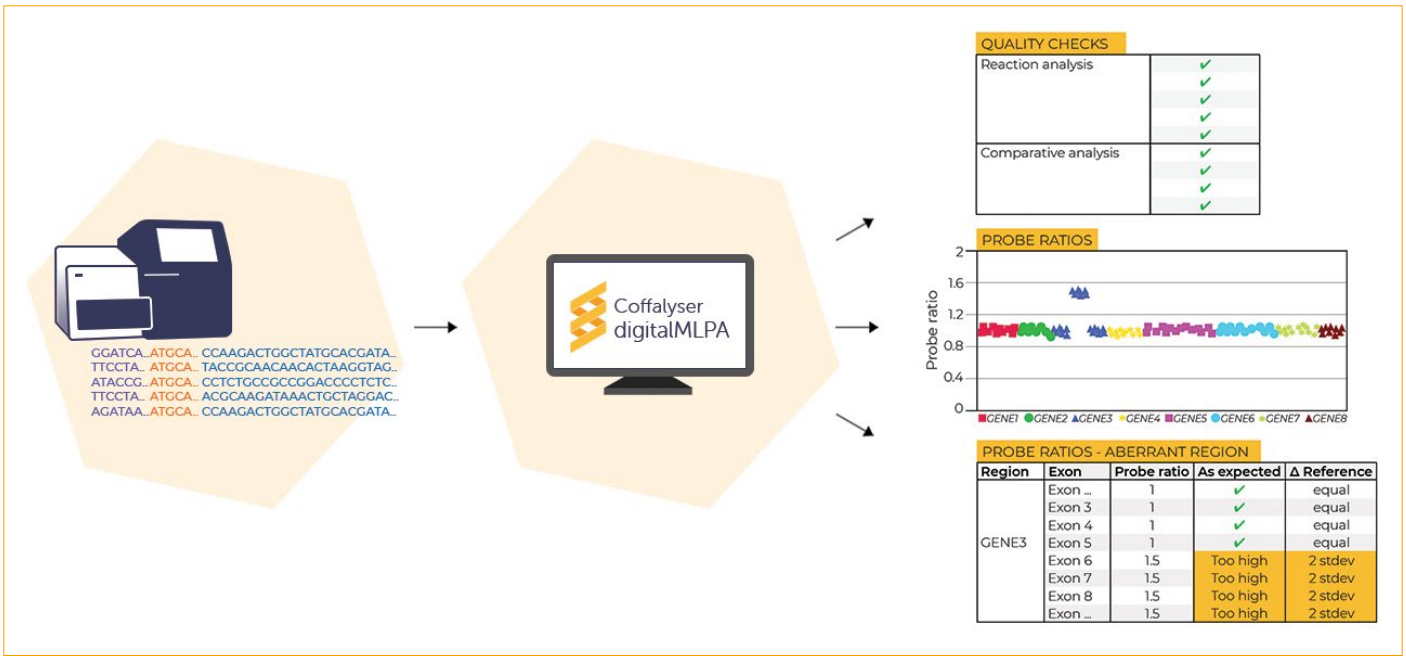
Instrument*	Samples per run** **600 probes** (1000 probes)
iSeq 100	**10** (6)
MiSeq System (v3 chemistry)	**64** (38)
MiniSeq System	**64** (38)
NextSeq™ 500/550 System	**192**#
HiSeq 2000/2500 System	**192**#

* 110 nt single end reads (longer reads, PE reads and NextSeq™ 75 nt SE reads are also suitable)
** Samples per run at an intended average read depth of 400x
# Sample number restricted by barcode availability, number does not differ for 600 or 1000 probes, will increase to 384 samples by year 2021

Coffalyser digitalMLPA

Data analysis software for clear CNV calling

- ✓ **Complimentary:** no additional costs for data analysis software
- ✓ **Simple:** FASTQ files are loaded directly into the software
- ✓ **Smart:** automatic digitalMLPA read and probemix recognition
- ✓ **Easy:** can be run by any user on a personal Windows 10-based computer
- ✓ **Reliable:** extensively tested and validated
- ✓ **Safe:** extensive built-in quality control by analysis of > 100 control probes



Coffalyser digitalMLPA is free software developed and supported by MRC Holland for the analysis of digitalMLPA data. Coffalyser digitalMLPA automatically recognises and extracts digitalMLPA sequence reads from FASTQ files for direct use by the software. The software performs advanced data quality checks and returns a clear report with all identified aberrant regions.

Interested in SALSA® digitalMLPA?
For ordering and more information, visit
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