MRC Holland

# SALSA® digitalMLPA™

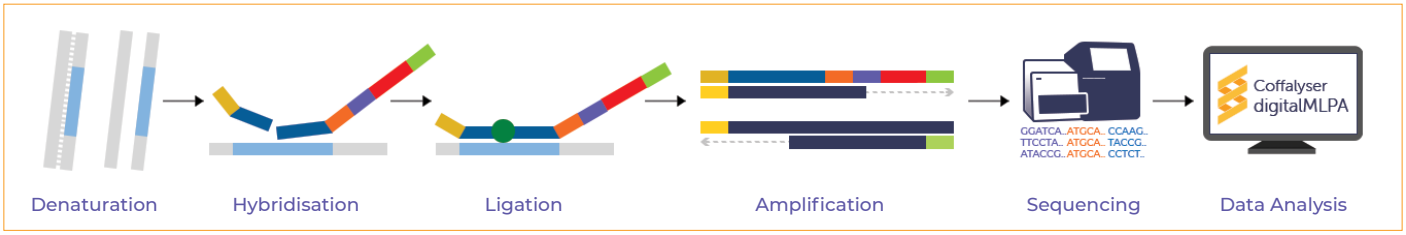
## Confidence in Copy Number Determination

# SALSA® digitalMLPA™

*Where MLPA meets NGS*

- ✓ **Specific:** unparalleled copy number certainty for complex genetic regions
- ✓ **Cost-effective:** up to 1000 probes 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

SALSA® 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 SALSA® MLPA®, the gold standard for CNV detection, 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 turnaround times are met.

Analysis is done using free, easy-to-use software – so no bioinformatic skills are needed.

| Applications | |
|---|---|
| **Predisposition to Cancer** | **SALSA® digitalMLPA™ Probemix D001 Hereditary Cancer Panel 1**<br>Genes associated with a hereditary predisposition to breast, ovarian, colorectal, gastric, prostate, pancreatic and endometrial cancer and melanoma. |
| **Multiple Myeloma** | **SALSA® digitalMLPA™ Probemix D006 Multiple Myeloma**<br>1p, 1q, 13q, 17p and trisomies 3, 5, 7, 9, 11, 15, 19, 21. |
| **Acute Lymphoblastic Leukemia** | **SALSA® digitalMLPA™ Probemix D007 Acute Lymphoblastic Leukemia**<br>55 ALL-related genetic regions; hyperdiploidy and hypodiploidy. |

*Call CNVs with confidence and reduce your turnaround time with minimal DNA input.*

| Features | Advantages |
|---|---|
| Wide assay coverage | 600-1000 DNA targets per reaction |
| Quick turnaround | Results in 48-72h |
| Low DNA input | Requires only 20 ng of sample DNA |
| Highly specific | Can discriminate 1 nt differences, allowing for:<br>o reliable gene-pseudogene distinction (e.g. *PMS2*/*PMS2CL*)<br>o analysis of complex regions (e.g. *PMS2*, *PTEN*)<br>o detection of select SNVs (e.g. *BRAF* V600E, *MITF* p.E318K) |
| 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 primer pair eliminates amplification bias |
| | Robust even with varying read depths (min. read depth: 400x) |
| | Efficient amplification of probes in both AT and GC-rich regions (e.g. *STK11*, *PTEN*, *IKZF1*) |
| Highly-targeted | Sequencing of probes, *not* sample DNA, meaning:<br>o reduced chance of incidental findings<br>o no allelic dropout caused by SNVs interfering with primer binding<br>o simplified data analysis; no dependence on alignment to a reference genome |
| Extensive quality control | Robust data normalization 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 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™ software is used for reaction quality control, probe quantification and ratio determination to identify sample aberrations.

# Throughput

| Illumina instrument[1] | Samples per run[2] |
|---|---|
| iSeq 100 | Up to 13 |
| MiSeq System (v3 chemistry), MiniSeq System | Up to 83 |
| All NextSeq, HiSeq and NovaSeq Systems | Up to 384 [3] |

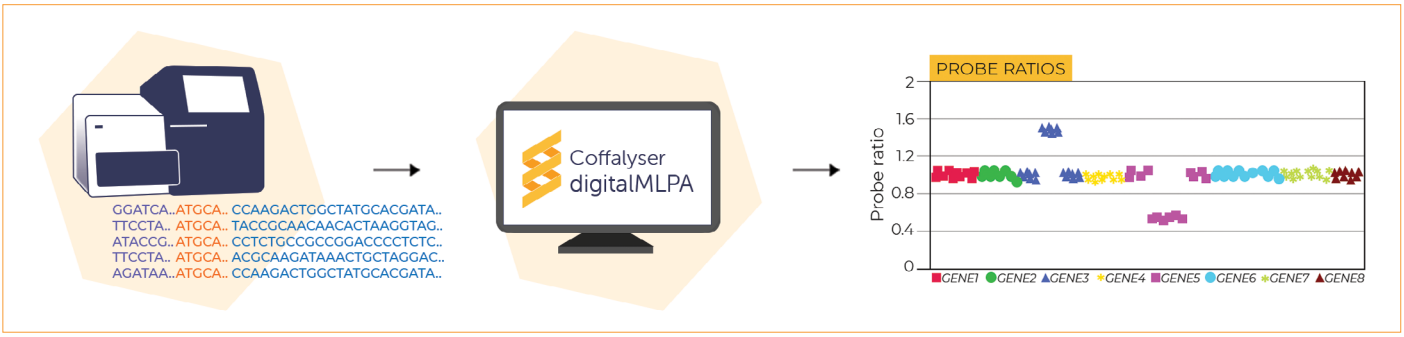
[1] Besides the instrument, the number of samples also depends on the sequencing kit size used.
[2] Sample numbers based on 600-probe digitalMLPA assay at an average read depth of 500x.
[3] Four barcode plates are available to uniquely label 384 (96x4) samples: BP01-IL, BP02-IL (available on the website) and BP03-IL and BP04-IL (available for D001 Hereditary Cancer Panel 1 upon request).

**Please read Instructions for use for more detailed instructions.**

# Coffalyser digitalMLPA™
## Data analysis software for clear CNV calling

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

Coffalyser digitalMLPA




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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