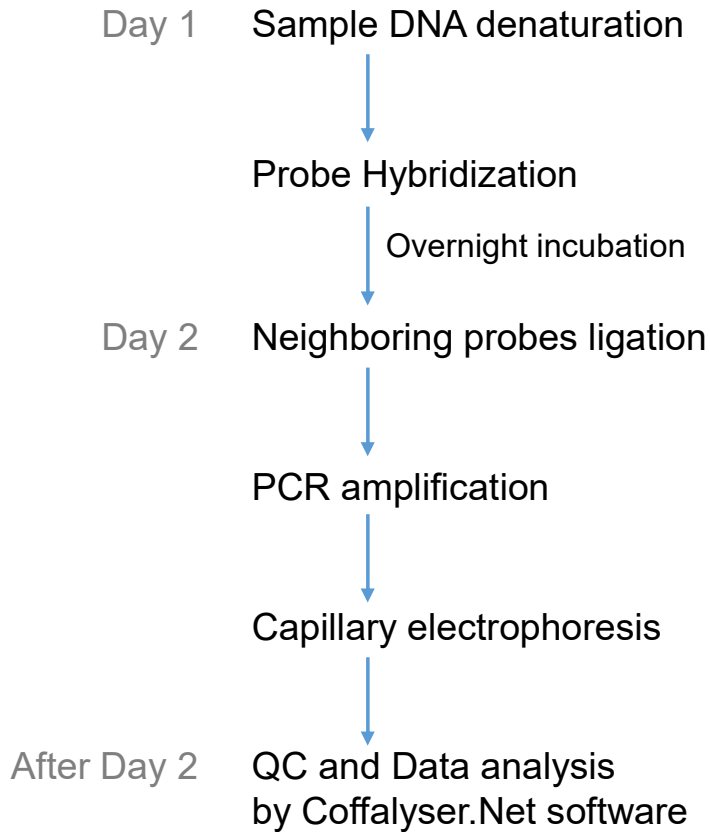


An overview of the MLPA workflow

A



B BRCA1 and BRCA2 relevant probe sets

Discovery sets:

P002-D1-0918
BRCA1-v01

P090-C1-0319
BRCA2-v01

P239-C1-0319
BRCA1 Region-v01
(Extended to BRCA1 up- and
downstream genes)

C Control samples

Control samples	Purposes
3 normal DNA samples with intact BRCA1 and BRCA2	To evaluate the overall assay performance and as normalization control for calculating the final ratio (FR)
SALSA Artificial duplication DNA: SD024-S01	As positive duplication control for P002:BRCA1 and P090:BRCA2 MLPA assays
BRCA009 Blood DNA (BRCA009-B)	As positive duplication and deletion control for P239:BRCA1 up- and downstream MLPA assay

MLPA assay QC checklist

QC1.Electrophoresis data	QC2.QC Fragments check	QC3.Control samples	QC4.Data quality check in Coffalyzer.Net
Strength of capillary electrophoresis (CE) signal: PASSED	Benchmark fragment at 92nt: DETECTED	A. Positive control (SD024) Artificial duplication in P002:BRCA1 and BRCA2:P090: DETECTED (BRCA009-B) In-house positive control for P239: DETECTED Two chromosome X-specific fragment: DETECTED One chromosome Y-specific fragment: DETECTED	Maximum FRSS, FMRS and CAS quality scores: PASSED
Size standard and CE quality: PASSED	Four DNA quantity fragments: DETECTED and ABOVE THRESHOLD		No missing probe: PASSED
Regularity, distribution and shape of peaks: PASSED	Two DNA denaturation fragments: DETECTED and ABOVE THRESHOLD		Bin size correct and no peak overlapping: PASSED
MLPA peaks fall into the optimal range of our CE platform: PASSED	Chromosome X-specific fragment (For sex checking): MATCHED	B. No DNA control Only five major peaks <121nt: DETECTED	Lot number of the probes was accurately shown: PASSED
	Chromosome Y-specific fragment (For sex checking): MATCHED	C. Reference samples Three normal references: INCLUDED Variation of individual probe among three references (SD<=0.10): PASSED Normal BRCA1/2 copy number: DETECTED	
		D. Non-BRCA specific reference probes Variation of reference probes (SD<=0.10): PASSED Final ratio of each reference probe fall within normal copy number range: PASSED	

P002:BRCA1 (QC PASSED)
P090:BRCA2 (QC PASSED)
P239:BRCA1 up- and downstreams (QC PASSED)

Derived from the MLPA checklists generated by Daisy