

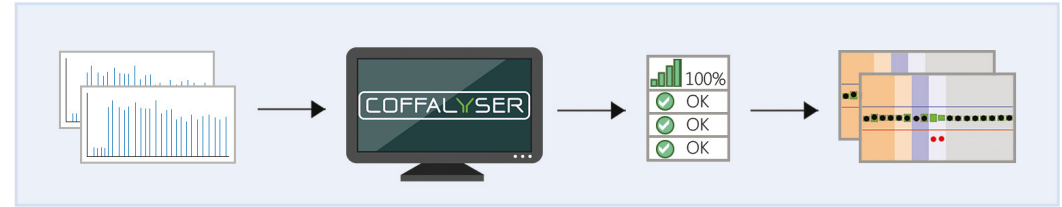
MLPA[®]

Cilia Disorders

MRC-Holland
MLPA[®]

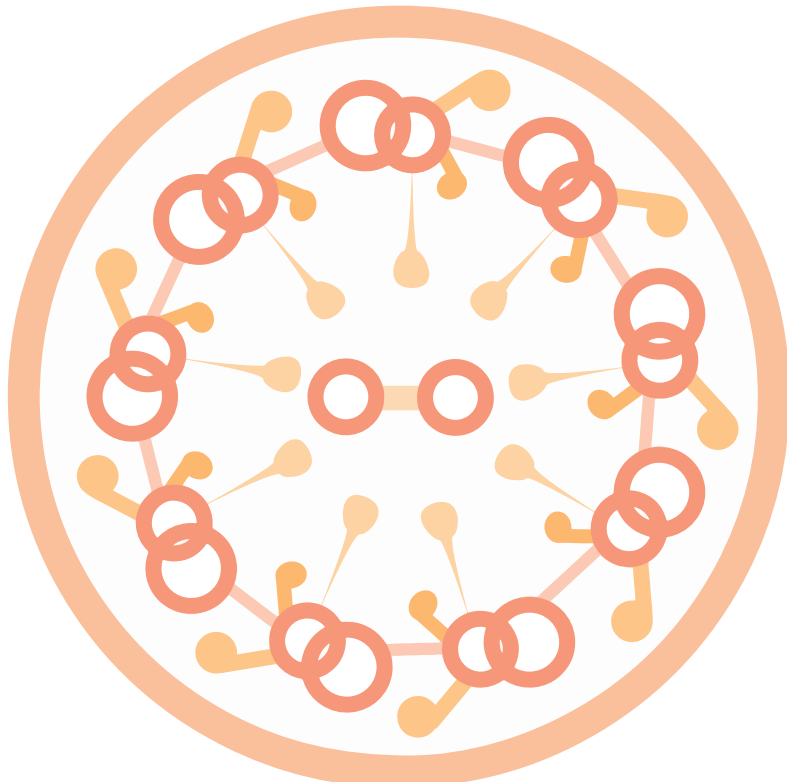
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Coffalyser.Net[™]



Free MLPA data analysis software designed and supported by MRC-Holland.

- User-friendly software and reliable MLPA data analysis
- Extensive quality control developed specifically for MLPA
- Always free access to the latest analysis panels (Coffalyser sheets)
- Server-client model that allows data sharing
- Available free of charge!



Collaborations with scientists

Most novel MLPA applications are developed in close collaboration with scientists around the world. Results obtained with MLPA probemixes have been described in thousands of scientific publications. Researchers are encouraged to contact us with requests for new MLPA applications or feedback on current panels on info@mlpa.com.



DNA Copy Number Quantification for Cilia Disorders

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MLPA[®]

MLPA[®] & Cilia Disorders

Multiplex Ligation-dependent Probe Amplification (MLPA) is a multiplex PCR-based method that can detect the copy number of up to 60 DNA sequences in a single reaction. 96 DNA samples can be handled simultaneously, with results being available within 24 hours.

Besides copy number changes, MLPA allows the detection of selected point mutations. Furthermore, MLPA is able to detect methylation patterns in DNA when used in combination with a methylation-sensitive restriction enzyme (MS-MLPA). MLPA is used worldwide for diagnostics and research of human genetic disorders and tumours.

				
<p>Simultaneous detection</p> <p>of copy number, methylation and targeted point mutations.</p>	<p>Low input</p> <p>Requires only 50 ng of DNA. Suitable for FFPE tissue DNA.</p>	<p>Time-efficient</p> <p>Results available within 24 hours.</p>	<p>Short hands-on time</p> <p>MLPA is performed in 5 simple steps.</p>	<p>Cost-effective</p> <p>One MLPA reaction costs EUR 12/USD 15.</p>

MLPA[®] protocol

1. DNA denaturation

- Heat a 5 µl DNA sample for 5 minutes at 98°C

2. Hybridisation of probes to sample DNA

- Cool down to room temperature, open tubes
- Add 3 µl Hybridisation master mix
- Incubate 1 minute at 95°C + 16 hours hybridisation at 60°C

3. Ligation of hybridised probes

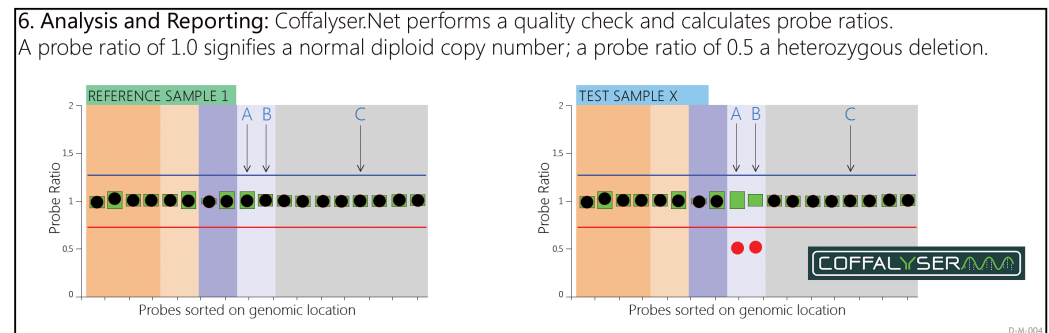
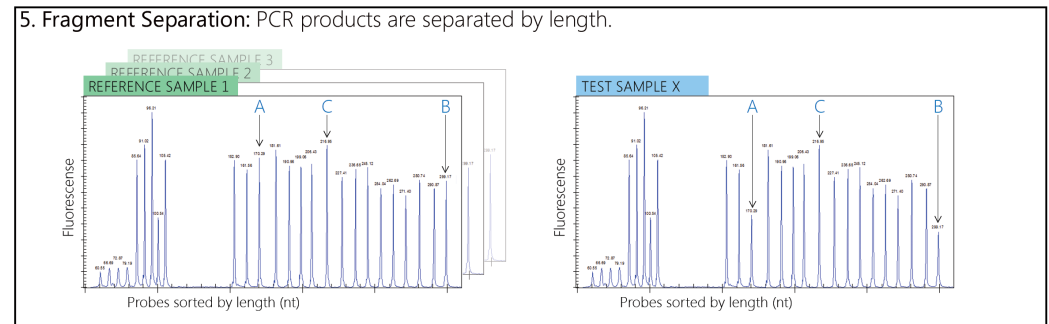
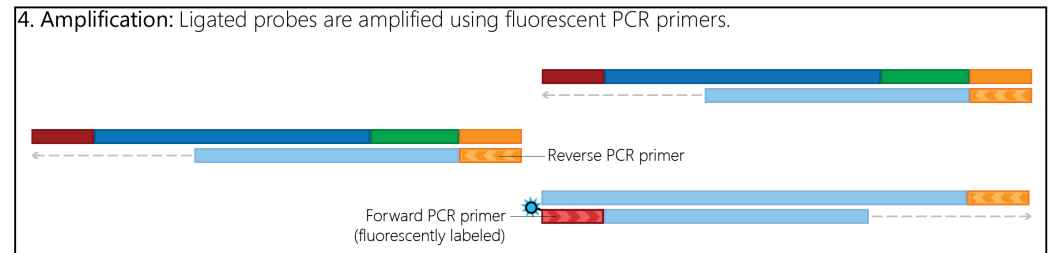
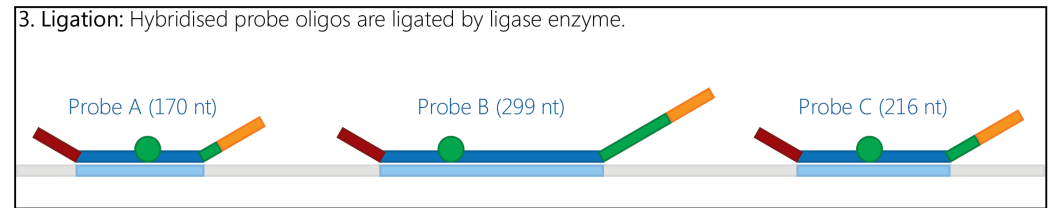
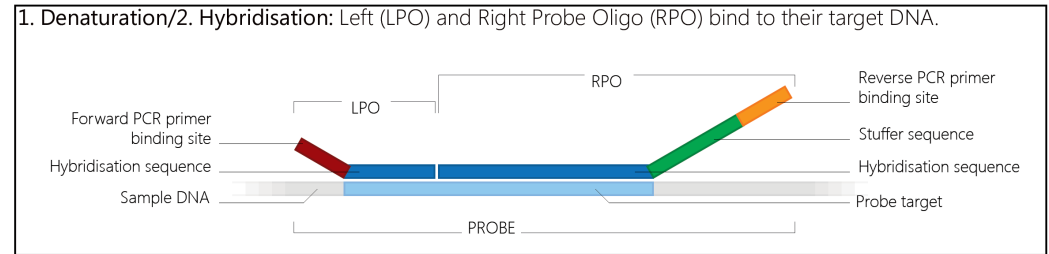
- Lower thermocycler temperature to 54°C, open tubes
- Add 32 µl Ligase-65 master mix, incubate 15 minutes at 54°C
- Heat to inactivate the ligase enzyme: 5 minutes at 98°C

4. PCR amplification of ligated probes

- Cool down to room temperature, open tubes
- Add 10 µl Polymerase master mix at room temperature
- Start PCR

5. Fragment separation by capillary electrophoresis

How MLPA[®] works



Probemixes: Cilia Disorders

The following lists give an overview of current MLPA probemixes for cilia-related genes or disorders. Over 400 MLPA probemixes are available and new assays are constantly developed in close collaboration with scientists around the world. See www.mlpa.com for a complete overview.

Application	Probemix	Genes/region
Autosomal Dominant Polycystic Kidney Disease (ADPKD)	P351	PKD1
	P352	PKD1, PKD2
Autosomal Recessive Polycystic Kidney Disease (ARPKD)	P341	PKHD1
	P342	PKHD1
Birt-Hogg-Dube Syndrome	P256	FLCN
Heterotaxy Syndrome	P434	ACVR2B, CFC1, NODAL, ZIC3
Leber Congenital Amaurosis (LCA)	P222	CEP290, GUCY2D, RDH12, RPGRIP1
Nephronophthisis 1	P387	NPHP1
Primary Ciliary Dyskinesia (PCD) (Kartagener Syndrome, Immotile Ciliary Syndrome)	P237	DNAI1
	P238	DNAH5
Retinitis Pigmentosa (RP)	P235	IMPDH1, PRPF31, RHO, RP1
	P366	CHM, RP2, RPGR
Usher Syndrome	P292	PCDH15
	P361	USH2A
	P362	USH2A
Von Hippel-Lindau Syndrome	P016	VHL

MLPA probemixes are for Research Use Only. Not for Use in Diagnostic Procedures unless explicitly stated otherwise.

* For this gene/application, both copy number and DNA methylation can be determined.