

## SALSA MLPA KIT P054 FOXL2-TWIST1

Lot 0905. As compared to the previous lot 0704, three probes have been added telomeric of the TWIST gene.

This probemix includes probes for the TWIST1, FOXL2, FOXC1, FOXC2, ATR, PITX2 and GPR143 (former OA1) genes. Mutations in most of these genes result mainly in ophthalmogenetic anomalies. Please note that the GPR143 gene is on the X-chromosome and that we may replace the GPR143 probes in a separate probemix in the future.

The protein coded by the TWIST1 gene may affect the transcription of fibroblast growth factor receptors (FGFRs), a gene family implicated in craniosynostosis. TWIST proteins may also regulate cytokine signalling. The TWIST gene is located on 7p21, contains two exons and spans only 1.9 Kb. Mutations in the TWIST gene are the major cause of Saethre-Chotzen syndrome (SCS, MIM 101400). It has been estimated that 11% of SCS patients have a deletion of one copy of the TWIST gene. The majority of patients with a TWIST deletion are also developmentally delayed, presumably due to haploinsufficiency of nearby genes. We included two probes for the TWISTNB gene, located at a distance of 590 Kb from TWIST in this gene poor region. There is no proof however that deletion of TWISTNB is the cause of the developmental delay.

The FOXL2 gene product is a forkhead transcription factor. The blepharophimosis syndrome (BPES; MIM 110100), an autosomal dominant syndrome in which an eyelid malformation is associated (type I) or not (type II) with premature ovarian failure (POF), can be caused by mutations in FOXL2. The FOXL2 gene is located on 3q23 and consists of a single 2.7 kb exon. Several FOXL2 deletions have been identified. At least one of these deletions spans several Mb of chromosomal DNA and may extend to the ATR gene located 3.6 Mb telomeric of FOXL2.

Other genes in the probemix are ATR (3q22; involved in cell cycle progression), PITX2 (4q25-q26; Rieger syndrome MIM 180500), GPR143 (former OA1; Xp22.3; ocular albinism type I) and forehead transcription factors FOXC1 (6p25) and FOXC2 (16q24.3)

This SALSA MLPA kit is designed to detect deletions/duplications of one or more exons of the aforementioned genes. In female heterozygotes, a 35-50% reduced relative peak area of the amplification product of that probe is expected. However, mutations and/or polymorphisms very close to the probe ligation site may also result in a reduced relative peak area. Therefore, apparent deletions detected by a single probe always require confirmation by other methods. We have no information on what percentage of defects in these genes is caused by deletions/duplications of complete exons. Please note that most defects in these genes are expected to be small (point) mutations, most of which will not be detected by this MLPA test.

**SALSA® MLPA® kits are sold by MRC-Holland for research purposes and to demonstrate the possibilities of the MLPA technique. This kit is not CE/FDA certified for use in diagnostic procedures. SALSA MLPA kits are supplied with all necessary buffers and enzymes. Purchase of the SALSA MLPA test kits includes a limited license to use these products for research purposes.**

The use of this SALSA MLPA kit requires a thermocycler with heated lid and sequence type electrophoresis equipment. Different fluorescent PCR primers are available. The MLPA technique has been first described in Nucleic Acid Research 30, e57 (2002).

### More information

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## Data analysis

The P054 FOXL2-TWIST probemix contains 34 MLPA probes with amplification products between 139 and 436 nt. In addition, it contains 5 control fragments generating an amplification product smaller than 120 nt: four DNA Quantity fragments (Q-fragments) at 64-70-76-82 nt and one synthetic control fragment of 92 nt. More information on how to interpret observations on these control fragments can be found in the MLPA protocol.

Data generated by this probemix should be normalized with a more robust method. The signals of target-specific probes should be normalized against every single reference probe separately, thereby creating as many ratios as there are reference probes. The median of all produced ratios gives an estimate of the final probe ratio, or ploidy status, of the sample's target-specific probes sequences in an MLPA mix. This way, the signal of each selected reference probe will be used as a normalization constant. With the normalization constant, the ratio between reference and patient sample is determined for each target-specific probe.

When only small numbers of samples are tested, visual comparison of peak profiles should be sufficient to easily identify exon deletions. Comparison of results should preferably be performed within one experiment. Only samples purified by the same method should be compared. Confirmation of most exons deletions and amplifications can be done by e.g. Southern blots or long range PCR.

Note that Coffalyser, the MLPA analysis tool developed at MRC-Holland, can be downloaded free of charge from our website [www.mlpa.com](http://www.mlpa.com).

This probemix was developed by J. Coffa & J.P. Schouten at MRC-Holland. In case the results obtained with this probemix lead to a scientific publication, it would be very much appreciated if the first probemix designer could be made a coauthor.

Info/remarks/suggestions for improvement: [info@mlpa.com](mailto:info@mlpa.com).

## SALSA MLPA P054 FOXL2-TWIST1 probemix

Length (nt)	SALSA MLPA probe	Chromosomal position	
		Reference	Gene
64-70-76-82	Q-fragments: DNA quantity; only visible with less than 100 ng sample DNA		
92	Synthetic control fragment at 2q14		
139	<b>ATR probe</b> 2556-L2019		Exon 1
148	<b>FOXC1 probe</b> 2561-L2029		6p25
157	<b>GPR143 (OA1) probe</b> 2974-L2405		Exon 6
166	<b>PITX2 probe</b> 2978-L2409		4q25
175	<b>GPR143 (OA1) probe</b> 2972-L2403		Exon 3
184	<b>FOXL2 probe</b> 1943-L1489		Exon 1
193	<b>TWIST probe</b> 2079-L1598		7p21.2
202	<b>TWISTNB probe</b> 2146-L1642		7p21.2
211	<b>FOXL2 probe</b> 1944-L1490		Exon 1
220	<b>GPR143 (OA1) probe</b> 2975-L2406		Exon 9
229	<b>TWISTNB probe</b> 2147-L1963		7p21.2
238	<b>FOXL2 probe</b> 1945-L1491		Exon 1
247 §	<b>TWIST probe</b> 4916-L4308		7p21.2
256	<b>ATR probe</b> 2557-L2020		Exon 4
265	<b>GPR143 (OA1) probe</b> 2565-L2026		Exon 2
273*	<b>TWIST probe</b> 2080-L2886		7p21.2
283	<b>GPR143 (OA1) probe</b> 3134-L2402		Exon 1
292	<b>TWIST probe</b> 1166-L0722		7p21.2
304	<b>ATR probe</b> 2558-L2021		Exon 22
310	Reference probe 1917-L1461	<i>1q</i>	
319	<b>TWIST probe</b> 1969-L2364		Exon 1
328	<b>GPR143 (OA1) probe</b> 2566-L2027		Exon 4
337	<b>PITX2 probe</b> 2976-L2407		4q25
346 §	<b>TWIST probe</b> 4915-L4301		7p21.2
355	<b>FOXC2 probe</b> 2563-L2024		16q24.3
364±	<b>PITX2 probe</b> 3136-L2887		4q25
373	<b>ATR probe</b> 2560-L2023		Exon 47
382	<b>FOXC1 probe</b> 2562-L2030		6p25
391 §	<b>TWIST probe</b> 4914-L4300		7p21.2
400	<b>GPR143 (OA1) probe</b> 2567-L2028		Exon 8
409	<b>FOXC2 probe</b> 2564-L2025		16q24.3
418	<b>GPR143 (OA1) probe</b> 3135-L2404		Exon 5
427	Reference probe 1240-L0787	<i>8p</i>	
436	Reference probe 2188-L1686	<i>1p</i>	

\* Higher signal as compared to previous lots. Same sequence detected.

± Two nucleotides longer as compared to previous lots. Same sequence detected.

§ New in lot 0905. The 391 bp probe might be less reliable.

**Note: An aspecific fragment of 260 nt was observed in our experiments. Please ignore.** Exon numbering might be different as compared to literature! Please notify us of any mistakes. The identity of the genes detected by the reference probes is available on request: [info@mlpa.com](mailto:info@mlpa.com).

## P054 probes arranged according to chromosomal location

### TWIST1 probes

Length (nt)	SALSA MLPA probe	Twist exon	Distance to next probe
202	TWISTNB probe 2146-L1642		10 Kb
229	TWISTNB probe 2147-L1963		579 Kb
247	TWIST1 probe 4916-L4308	Upstream	1.6 Kb
391	TWIST1 probe 4914-L4300	Upstream	0.1 Kb
346	TWIST1 probe 4915-L4301	Upstream	0.6 Kb
272	TWIST1 probe 2080-L1599	Promotor region	0.3 Kb
319	TWIST1 probe 1969-L2364	Exon 1	0.7 Kb
292	TWIST1 probe 1166-L0722	Exon 2	0.3 Kb
193	TWIST1 probe 2079-L1598	Exon 2	

### ATR & FOXL2 probes probes

Length (nt)	SALSA MLPA probe	ATR & FOXL2 exon	Distance to next probe
139	ATR probe 2556-L2019	Exon 1	16.2 Kb
256	ATR probe 2557-L2020	Exon 4	38.2 Kb
304	ATR probe 2558-L2021	Exon 22	30.8 Kb
373	ATR probe 2560-L2023	Exon 47	3565.0 Kb
184	FOXL2 probe 1943-L1489	Exon 1	0.3 Kb
211	FOXL2 probe 1944-L1490	Exon 1	0.5 Kb
238	FOXL2 probe 1945-L1491	Exon 1	

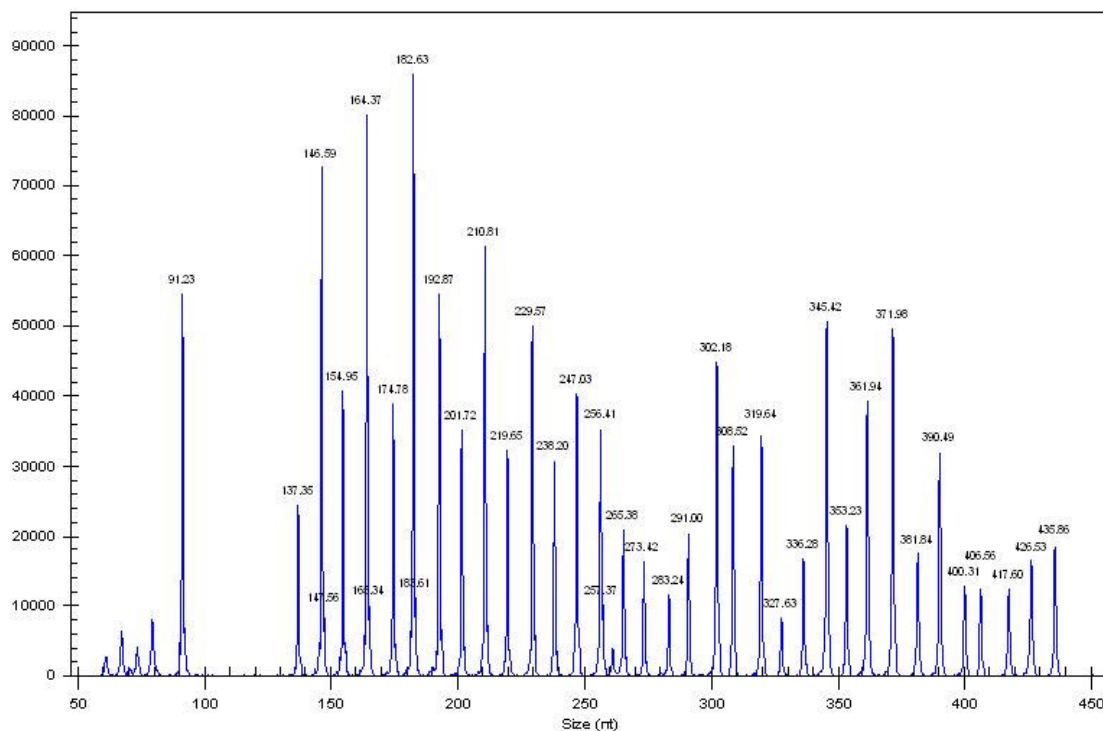
### GPR143 probes

Length (nt)	SALSA MLPA probe	GPR143 (OA1) exon	Distance to next probe
283	GPR143 (OA1) probe 3134-L2402	Exon 1	5.0 Kb
265	GPR143 (OA1) probe 2565-L2026	Exon 2	1.3 Kb
175	GPR143 (OA1) probe 2972-L2403	Exon 3	11.0 Kb
328	GPR143 (OA1) probe 2566-L2027	Exon 4	2.4 Kb
418	GPR143 (OA1) probe 3135-L2404	Exon 5	2.4 Kb
157	GPR143 (OA1) probe 2974-L2405	Exon 6	2.1 Kb
Not present		Exon 7	1.6 Kb
400	GPR143 (OA1) probe 2567-L2028	Exon 8	14.0 Kb
220	GPR143 (OA1) probe 2975-L2406	Exon 9	

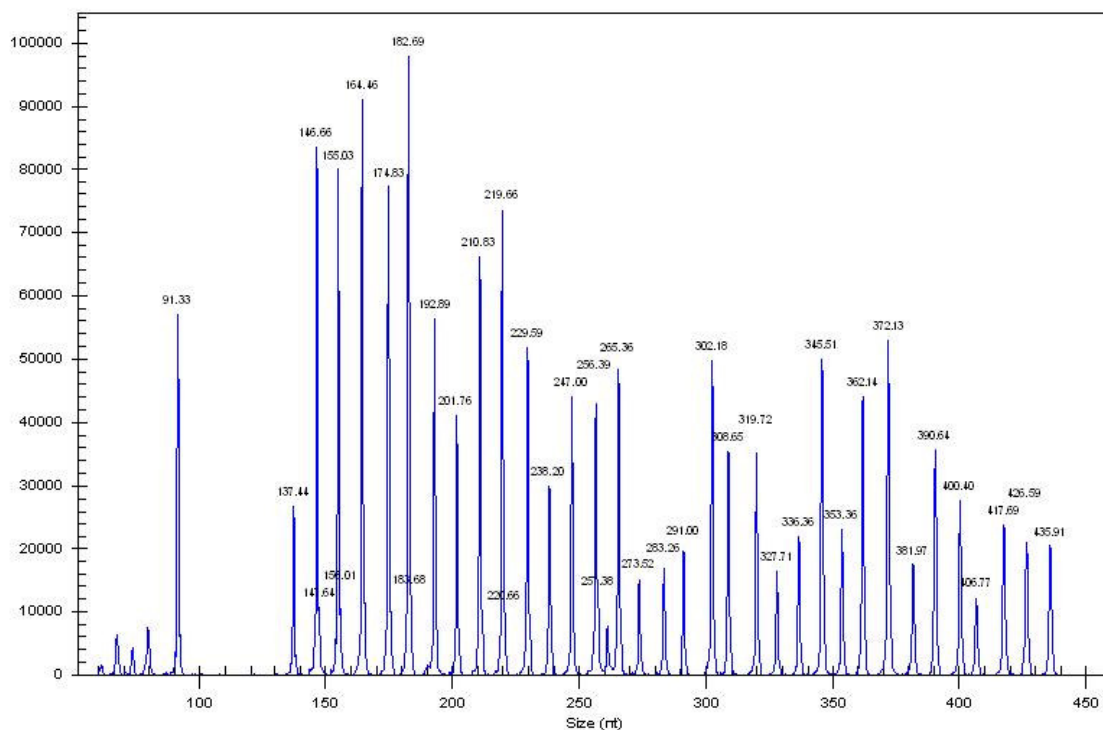
The two FOXC1 probes ( 148 & 382 nt) are located at a distance of 0.6 Kb from each other. The two FOXC2 probes ( 355 & 409 nt) are located at a distance of 0.7 Kb from each other. Three PITX2 probes ( 166, 337 & 364 nt) detect this 19 Kb long gene.

**Note:** Exon numbering might be different as compared to literature!  
Please notify us of any mistakes: [info@mlpa.com](mailto:info@mlpa.com)

## SALSA MLPA kit P054 FOXL2-TWIST1 sample picture



**Figure 1.** Capillary electrophoresis pattern from a sample of approximately 50 ng human male control DNA analyzed with SALSA MLPA kit P054 FOXL2-TWIST1 (lot 0905).



**Figure 2.** Capillary electrophoresis pattern from a sample of approximately 50 ng human female control DNA analyzed with SALSA MLPA kit P054 FOXL2-TWIST1 (lot 0905).