SALSA MLPA probemix P337-B1 TSC2

Lot B1-0216, B1-0114. As compared to version A2 (lot A2-0510), four TSC2 probes were added and one replaced, one flanking probe was added. Two reference probes have been replaced and two removed. In addition, the control fragments have been adjusted (QDX2).

Tuberous Sclerosis (TSC) is an autosomal dominant disorder with high penetrance. Defects in the TSC1 or TSC2 genes are the main cause of TSC. The proteins encoded by these genes are hamartin and tuberin, respectively. TSC has an incidence of roughly 1 in 6000 newborns, and is in most patients caused by *de novo* mutations (sporadic cases) with an absence of family history. TSC causes non-malignant tumour growth in multiple organs including skin, central nervous system and other vital organs. Well-known clinical manifestations include epilepsy, behavioural problems, skin abnormalities and lung- and kidney disease. The majority of cardiac rhabdomyomas is associated with tuberous sclerosis.

Approximately 75% of TSC cases are linked to the TSC2 gene on chromosome 16p13, the remaining are linked to the TSC1 gene on chromosome 9q34. The majority of variations found in these genes are nonsense, missense, frameshift, or splice site mutations, while less than 10% of the TSC cases are due to copy number variation in TSC1 or TSC2.

The TSC2 gene (42 exons) spans ~41 kb of genomic DNA and is located on 16p13.3, 2 Mb from the p-telomere. The P337-B1 probemix contains one probe for each exon of the gene and two probes for exon 1. This probemix furthermore contains two probes for the PKD1 gene, located downstream of TSC2. In addition, 8 reference probes are included in this probemix, detecting several different autosomal chromosomal locations. All TSC2 probes in the P337 probemix differ from the probes included in the P046 probemix and can be used to confirm P046 results.

This SALSA® MLPA® probemix is designed to detect deletions/duplications of one or more sequences in the aforementioned gene in a DNA sample. Heterozygous deletions of recognition sequences should give a 35-50% reduced relative peak height of the amplification product of that probe. Note that a mutation or polymorphism in the sequence detected by a probe can also cause a reduction in relative peak height, even when not located exactly on the ligation site! In addition, some probe signals are more sensitive to sample purity and small changes in experimental conditions. Therefore, deletions and duplications detected by MLPA should always be confirmed by other methods. Not all deletions and duplications detected by MLPA will be pathogenic; users should always verify the latest scientific literature when interpreting their findings. Finally, note that most defects in this gene are expected to be small (point) mutations which will not be detected by this SALSA® MLPA® test.

SALSA® MLPA® probemixes and reagents are sold by MRC-Holland for research purposes and to demonstrate the possibilities of the MLPA technique. They are not CE/FDA certified for use in diagnostic procedures. Purchase of the SALSA® MLPA® test probemixes and reagents includes a limited license to use these products for research purposes.

The use of a SALSA® MLPA® probemix and reagents 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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Related SALSA® MLPA® probemixes

- P046 TSC2: Contains probes for the TSC2 gene, to be used for primary screening of Tuberous Sclerosis.
- P124 TSC1: Contains probes for the TSC1 gene, involved in Tuberous Sclerosis.
- P351/P352: Contain probes for the PKD1 gene, located downstream of TSC2.

References

- Ramandi H. *et al.* (2014). TSC2 Deletions and Duplications: A Descriptive Study in Iranian Patients Affected with Tuberous Sclerosis. *American Journal of Molecular Biology*, 04(03), pp.163-167.
- Lee J.S. *et al.* (2014). Mutational analysis of paediatric patients with tuberous sclerosis complex in Korea: genotype and epilepsy. *Epileptic Disord.* 16:449-55.

Data analysis

The P337-B1 TSC2 probemix contains 53 MLPA probes with amplification products between 124 and 490 nt. In addition, it contains 9 control fragments generating an amplification product smaller than 120 nt: four DNA Quantity fragments (Q-fragments) at 64-70-76-82 nt, three DNA Denaturation control fragments (D-fragments) at 88-92-96 nt, one X-fragment at 100 nt and one Y-fragment at 105 nt. More information on how to interpret observations on these control fragments can be found in the MLPA protocol.

Data generated by this probemix can first be normalised intra-sample by dividing the peak height of each probe's amplification product by the total peak height of only the reference probes in this probemix (block normalisation). Secondly, inter-sample normalisation can be achieved by dividing the intra-normalised probe ratio in a sample by the average intra-normalised probe ratio of all reference samples. Please note that this type of normalisation assumes no changes occurred in the genomic regions recognised by the reference probes.

Data normalisation should 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 blotting, long range PCR, qPCR, FISH.

Note that Coffalyser, the MLPA analysis tool developed at MRC-Holland, can be downloaded free of charge from our website www.mlpa.com.

Many copy number alterations in healthy individuals are described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. For example, a duplication of a complete gene might not be pathogenic, while a partial duplication or a deletion may result in disease. For some genes, certain in-frame deletions may result in a very mild, or no disease. Copy number changes of reference probes are unlikely to be the cause of the condition tested for. Users should always verify the latest scientific literature when interpreting their findings.

This probemix was developed by R. Vijzelaar at MRC-Holland. In case the results obtained with this probemix lead to a scientific publication, it would be very much appreciated if the probemix designer could be included as co-author.

Info/remarks/suggestions for improvement: info@mlpa.com.

Table 1. SALSA MLPA P337-B1 TSC2 probemix

Length (nt)	SALSA MLPA probe	Chromosomal position reference	Chromosomal position TSC2
64-70-76-82	Q-fragments: DNA quantity; only visible with less than 100 ng sample DNA		
88-92-96	D-fragments: Low signal of 88 or 96 nt fragment indicates incomplete denaturation		
100	X-fragment: Specific for the X chromosome		
105	Y-fragment: Specific for the Y chromosome		
124 *	Reference probe 19616-L26275	4p13	
130	Reference probe 09978-L10437	19p13	
136 ±	**TSC2 probe** 11904-L18003		Exon 38
142	**TSC2 probe** 11905-L12726		Exon 19
148 ± ¬	**PKD1 probe** 10960-L16105		Exon 40
153 *	**TSC2 probe** 16721-L19333		Exon 29
160	**TSC2 probe** 11908-L12729		Exon 6
166	**TSC2 probe** 11909-L12730		Exon 24
172	**TSC2 probe** 11910-L12731		Exon 2
178	Reference probe 10107-L10531	8q22	
185	**TSC2 probe** 11911-L12732		Exon 11
190	**TSC2 probe** 14105-L15707		Exon 5
196 ±	**TSC2 probe** 11913-L12734		Exon 37
202 ◍	**TSC2 probe** 13544-L15219		Exon 1
208 ±	**TSC2 probe** 11915-L12736		Exon 33
214	**TSC2 probe** 11916-L18004		Exon 7
220	**TSC2 probe** 11917-L18005		Exon 32
228	Reference probe 06619-L18006	6q24	
232	**TSC2 probe** 11918-L12739		Exon 13
238 ◍	**TSC2 probe** 11919-L12740		Exon 1
244	**TSC2 probe** 13543-L12735		Exon 21
250	**TSC2 probe** 11921-L12742		Exon 16
256	**TSC2 probe** 11922-L12743		Exon 8
263	**TSC2 probe** 11923-L12744		Exon 28
270 * ¬	**PKD1 probe** 10956-L19311		Exon 30
275 ¥	Reference probe 19811-L19312	9q34	
283 ¥	**TSC2 probe** 11925-L19313		Exon 4
290 ¥	**TSC2 probe** 11926-L19314		Exon 26
296 ¥	**TSC2 probe** 11927-L19315		Exon 10
301 ¥	**TSC2 probe** 11928-L19316		Exon 27
308 ¥	**TSC2 probe** 11929-L19317		Exon 15
315 * Ж	**TSC2 probe** 16733-SP0384-L27160		Exon 35
320 ±	**TSC2 probe** 13547-L16100		Exon 36
328	Reference probe 04283-L03687	12q12	
334 *	**TSC2 probe** 20016-L27295		Exon 9
341 ¥	**TSC2 probe** 11932-L19319		Exon 12
347 * ±	**TSC2 probe** 16723-L27370		Exon 39
355	**TSC2 probe** 13545-L12751		Exon 3
364 ±	**TSC2 probe** 11935-L12755		Exon 41
373	**TSC2 probe** 11936-L12756		Exon 14
382	**TSC2 probe** 11937-L12757		Exon 20
391	**TSC2 probe** 13546-L15221		Exon 30
400 *	Reference probe 16567-L19058	11q13	
409 ±	**TSC2 probe** 13552-L12762		Exon 42
417	**TSC2 probe** 13553-L12763		Exon 18
426	**TSC2 probe** 13549-L18016		Exon 17
436	**TSC2 probe** 13551-L12761		Exon 31
445	**TSC2 probe** 13550-L12760		Exon 23
454	**TSC2 probe** 11944-L12764		Exon 25
465	**TSC2 probe** 12803-L12766		Exon 22
472 ±	**TSC2 probe** 12802-L12765		Exon 40
481 *	**TSC2 probe** 16724-L19336		Exon 34
490	Reference probe 10218-L10698	7q22	

± This probe is located within, or close to, a very strong CpG island. A low signal of this probe can be due to incomplete sample DNA denaturation, e.g. due to the presence of salt in the sample DNA.
¬ Flanking probe. Included to facilitate the determination of the extent of a deletion/duplication. Copy number alterations of flanking and reference probes are unlikely to be related to the condition tested.
* New in version B1 (from lot B1-0114 onwards).
¥ Changed in version B1 (from lot B1-0114 onwards). Small change in length, no change in sequence detected.
Ж This probe consists of three parts and has two ligation sites.
ⓐ The significance of exon 1 deletions is not clear as this exon is non-coding and alternative transcript variants using other transcription start sites are known.

Note

- **The TSC2 exon numbering has changed.** From description version 06 onwards, we have adopted the NCBI exon numbering that is present in NM_000548.3. This exon numbering used here may differ from literature!
- The identity of the genes detected by the reference probes is available on request: info@mlpa.com.

Table 2. TSC2 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Exon	Ligation site NM_000548.3	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*107-109 (exon 2)*		
202 ⓐ	13544-L15219	Exon 1	33-34	GGGGGTGCGCCT-TTCTCCGCGTCG	0.0 kb
238 ⓐ	11919-L12740	Exon 1	4 nt after exon 1	GGCGCGGGGTAA-GTGGCGGTCCCC	0.7 kb
172	11910-L12731	Exon 2	220-221	CAGACGGAGTTT-ATCATCACCGCG	1.6 kb
355	13545-L12751	Exon 3	33 nt before exon 3	GTGGCCTGAGCA-CTGGCCCCTTTT	3.0 kb
283 ¥	11925-L19313	Exon 4	27 nt before exon 4	AGAGCACATCCT-CACCGCTGTCCC	1.0 kb
190	14105-L15707	Exon 5	32 nt before exon 5	GCGACGCTGGCA-GGCTCTGCTGAT	1.2 kb
160	11908-L12729	Exon 6	695-696	AGTACATCGCAA-GGATGGTTCAGT	0.7 kb
214	11916-L18004	Exon 7	720-721	GATCTGTCTGCT-GTGCGTCCGGAC	0.6 kb
256	11922-L12743	Exon 8	13 nt after exon 8	TGGGGTTTCTGA-AACTGCTCTGGA	0.3 kb
334 *	20016-L27295	Exon 9	28 nt before exon 9	CTTATGCCTGCC-AGCCCCTGACAC	1.8 kb
296 ¥	11927-L19315	Exon 10	11 nt after exon 10	GGTAAGGCGGTT-TCTGTGTGCAGT	1.8 kb
185	11911-L12732	Exon 11	37 nt before exon 11	AGCAAGCAAGCA-GCTCTGACCCTG	1.3 kb
341 ¥	11932-L19319	Exon 12	1345-1346	CTGGTGGAGAGA-TGTGCGGACCAG	0.6 kb
232	11918-L12739	Exon 13	1463-1464	TGGAGAGATTCT-TCAGGTAGGGGG	0.5 kb
373	11936-L12756	Exon 14	40 nt after exon 14	GCTCAGGGCTAT-TTCTCCGTGGGC	1.2 kb
308 ¥	11929-L19317	Exon 15	1561-1562	GAGGAGCTGATT-AACTCAGTGGTC	1.4 kb
250	11921-L12742	Exon 16	69 nt after exon 16	CTGCATCTGCGT-TGTGTTGGAGTC	4.7 kb
426	13549-L18016	Exon 17	20 nt before exon 17	GCGCCGTGGTGA-GCTGCGTCCTCT	1.1 kb
417	13553-L12763	Exon 18	21 nt before exon 18	TGGCTCTGGCTT-TCACCATCCTCT	0.4 kb
142	11905-L12726	Exon 19	2198-2199	TGCAGTGCTTGA-AGCAGGTGAGTG	0.3 kb
382	11937-L12757	Exon 20	42 nt before exon 20	GCCTCTGTCTCT-AGGGTCCAGAAG	0.7 kb
244	13543-L12735	Exon 21	2424-2425	GCTGACAGCATT-AATCTCTTACCA	1.3 kb
465	12803-L12766	Exon 22	2535-2536	GGCCTTGTCCAT-CTGCAGCGTGGA	1.5 kb
445	13550-L12760	Exon 23	61 nt before exon 23	GAGCAGCCGTGT-TGGCCTTCAGAG	0.4 kb
166	11909-L12730	Exon 24	2796-2797	AGCCATGTGGTT-CATCAGGTGCCG	0.4 kb
454	11944-L12764	Exon 25	7 nt before exon 25	GGTGTGCTCACT-CTGCCAGGGCCT	1.2 kb
290 ¥	11926-L19314	Exon 26	3057-3058	CAGTGTGTCTGA-ACATGTGGTCCG	1.3 kb
301 ¥	11928-L19316	Exon 27	3078-3079	CTCCAGCAGGAT-ACAGACGTCCCT	0.3 kb
263	11923-L12744	Exon 28	3279-3280	TGGCAGGACCAA-AACCTGGCTGGT	0.3 kb
153 *	16721-L19333	Exon 29	3498-3499	GGTCCGTTCCAT-GTCGGGTGAGCC	0.5 kb
391	13546-L15221	Exon 30	34 nt before exon 30	TGCATCAGGTAA-GTGGTGGTCACC	1.7 kb
436	13551-L12761	Exon 31	2 nt after exon 31	TCCAACACAGGT-GAGTGGCATGGC	0.7 kb
220	11917-L18005	Exon 32	3963-3964	CTGCCAAGGACA-GCTGCACAGGAG	1.3 kb
208 ±	11915-L12736	Exon 33	4 nt after exon 33	ACAGCAGGGTGA-GTGTGGCTCAGA	0.4 kb
481 *	16724-L19336	Exon 34	4146-4147	GGAGAAGTCGCT-CCACGCGGAGGA	0.8 kb

Length (nt)	SALSA MLPA probe	Exon	Ligation site NM_000548.3	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
315 * Ж	16733-SP0384-L27160	Exon 35	4672-4673; 24 nt after exon 35	CTGCTGCCCAAT- 27 nt spanning oligo -ATCCGCTGGAGC	0.2 kb
320 ±	13547-L16100	Exon 36	22 nt before exon 36	GTCTGGGGCTCA-GGCAGGGCTCTG	1.0 kb
196 ±	11913-L12734	Exon 37	4798-4799	ATCCTGTCCAAT-GAGCATGGCTCC	0.5 kb
136 ±	11904-L18003	Exon 38	57 nt before exon 38	GCCCCAGTGCAA-GGCACAGAGGGC	1.2 kb
347 * ±	16723-L27370	Exon 39	5110-5111	CAGTTCAACTTT-GTCCACGTGATC	0.2 kb
472 ±	12802-L12765	Exon 40	10 nt before exon 40	CGTGACCACCAA-GTCTCCCCAGAC	0.3 kb
364 ±	11935-L12755	Exon 41	5346-5347	GCTCCGCCACAT-CAAGCGGCTCCG	0.1 kb
409 ±	13552-L12762	Exon 42	5367-5368	CTGCCTTCAGAT-CTGCGAGGAAGC	3.7 kb
		stop codon	*5528-5530 (exon 42)*		
148 ± ¬	10960-L16105	PKD1 Exon 40		AGCACCAGCGAT-TACGACGTTGGC	7.3 kb
270 * ¬	10956-L19311	PKD1 Exon 30		GAAGCCAGAATG-GTGAAAGAACGA	

± This probe is located within, or close to, a very strong CpG island. A low signal of this probe can be due to incomplete sample DNA denaturation, e.g. due to the presence of salt in the sample DNA.
¬ Flanking probe. Included to facilitate the determination of the extent of a deletion/duplication. Copy number alterations of flanking and reference probes are unlikely to be related to the condition tested.
* New in version B1 (from lot B1-0114 onwards).
¥ Changed in version B1 (from lot B1-0114 onwards). Small change in length, no change in sequence detected.
Ж This probe consists of three parts and has two ligation sites.
ɑ The significance of exon 1 deletions is not clear as this exon is non-coding and alternative transcript variants using other transcription start sites are known.

The NM_000548.3 sequence is a reference standard in the NCBI RefSeqGene project.

Note: The TSC2 exon numbering has changed. From description version 06 onwards, we have adopted the NCBI exon numbering that is present in NM_000548.3. Complete probe sequences are available on request: info@mlpa.com. Please notify us of any mistakes: info@mlpa.com.

SALSA MLPA probemix P337-B1 TSC2 sample picture

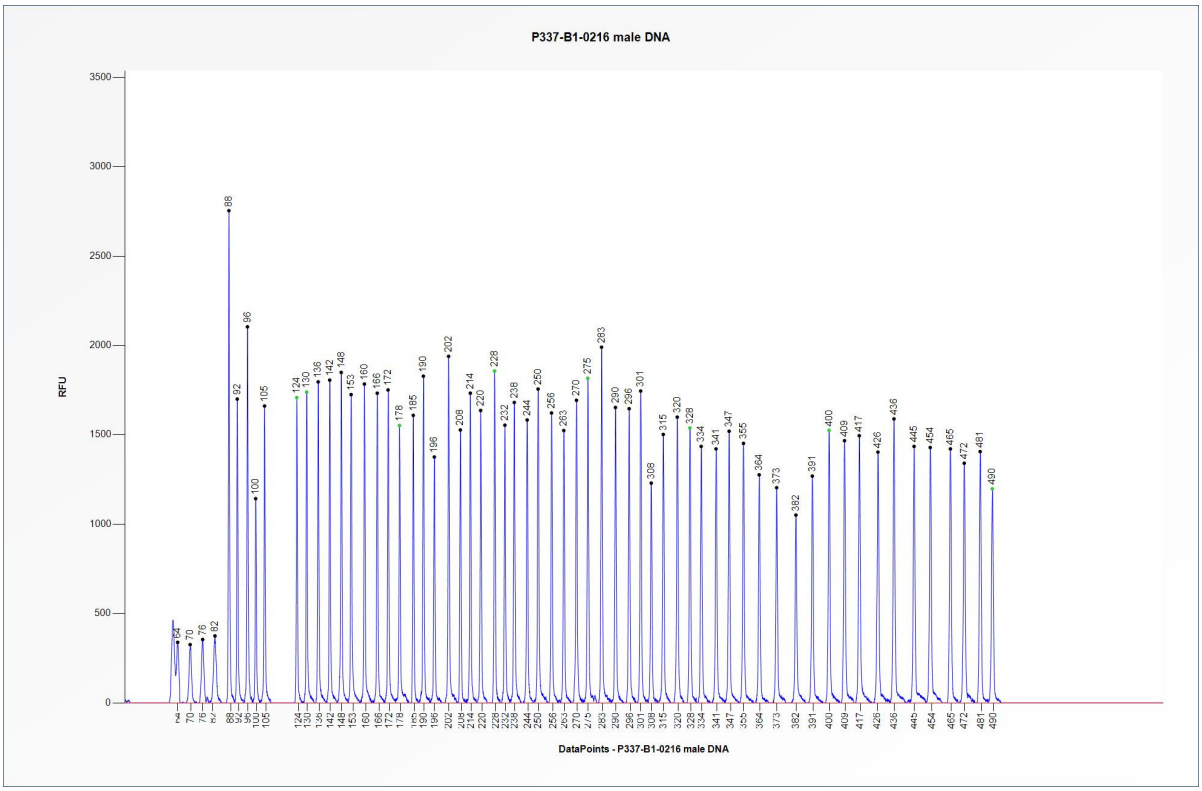

Figure 1. Capillary electrophoresis pattern of a sample of approximately 50 ng human male control DNA analysed with SALSA MLPA probemix P337-B1 TSC2 (lot B1-0216).

Implemented Changes – compared to the previous product description versions.
Version 08 – 02 August 2016 (55) - Product description adapted to a new lot (lot number added, new picture included). - Various minor textual and layout changes. - Warning added in Table 1 and 2: 208 nt probe 11915-L12736. - Warning added below Table 1 and 2 regarding uncertain significance of exon 1 deletions. - References added on page 2. *Version 07 – 01 June 2015 (54)* - The exon numbering of P046 TSC2 has been adjusted according to NM_000548.3 and therefore the comments below Table 1 and 2 have been adjusted. *Version 06 (53)* - Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included). - Exon numbering has been adjusted according to NM_000548.3. *Version 05 (48)* - Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added. *Version 04 (48)* - Remark on RefSeqGene standard and transcript variant added below Table 2. - Various minor textual and layout changes.