Product Description
SALSA® MLPA® Probemix P073-A1 IKBKG

To be used with the MLPA General Protocol.

Version A1
For complete product history see page 10.

Catalogue numbers:
- **P073-025R:** SALSA MLPA Probemix P073 IKBKG, 25 reactions.
- **P073-050R:** SALSA MLPA Probemix P073 IKBKG, 50 reactions.
- **P073-100R:** SALSA MLPA Probemix P073 IKBKG, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit and Coffalyser.Net data analysis software. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman/SCIEX capillary sequencers, respectively (see www.mrcholland.com).

Certificate of Analysis
Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mrcholland.com.

Precautions and warnings
For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mrcholland.com. It is the responsibility of the user to be aware of the latest scientific knowledge of the application before drawing any conclusions from findings generated with this product. Important notes:

1. Many probes in this assay cannot discriminate between the copy number status of *IKBKG* and its pseudogene (see Table 2 and Figure 1). Therefore, demonstration of the presence of e.g. the common IKBKGexon4_10del mutation always requires confirmation.
2. Duplication of the *IKBKG* gene and deletion of its pseudogene are relative frequent polymorphisms in the normal population (~2%).
3. A copy number neutral translocation of exon 4-10 from the *IKBKG* gene to its pseudogene will not be detected by MLPA but will be detected by gap-PCR.
4. Low level mosaic cases are likely not detectable using this probemix.

General information
The SALSA MLPA Probemix P073 IKBKG is a **research use only (RUO)** assay for the detection of deletions or duplications in in the chromosome Xq28 region, including *IKBKG*, which is associated with Incontinentia Pigmenti (IP). Due to the high sequence homology, this assay cannot discriminate copy number status between *IKBKG* and its pseudogene, and the presence of e.g. the common IKBKGexon4_10del mutation always requires confirmation.

IP (OMIM #308300), or Bloch-Sulzberger Syndrome, is an X-linked dominant disorder that is primarily characterized by skin lesions. Initial clinical manifestations start at birth with a vesiculobullous eruption (Stage I), followed by a verrucous stage (Stage II), a hyperpigmentation stage (Stage III), and finally a hypopigmentation stage (Stage IV) usually continuing throughout life. Other characteristics include ocular abnormalities, central nervous system abnormalities, and teeth defects. IP is easy to diagnose when the classical features are present after birth. However, the diagnosis can be difficult in patients with partial or non-classical clinical features. The *IKBKG* gene is the only known gene associated with IP. The absence of a functional *IKBKG* gene is lethal. Therefore, as an X-linked disorder, IP occurs primarily in females. However, male patients with a 47,XXY karyotype or somatic mosaicism have been described.

The majority of patients (>65%) harbour a 11.7 kb deletion, which is generated by a recombination event between two repeats (*MER67B*) located in intron 3 and about 6 kb downstream of *IKBKG* exon 10. This results in the removal of *IKBKG* exon 4 to 10 ("IKBKGexon4_10del"). Additionally, other large deletions of different sizes (including parts of neighbouring genes) and single nucleotide substitutions and insertions have been reported (Conte et al., 2014). Notably, over 60% of IP patients are sporadic carrying a *de novo IKBKG* mutation.

Duplication of (part of) the *IKBKG* gene or deletion of the pseudogene *IKBKGP1* are common polymorphisms throughout populations with a frequency of 1-2% and do not result in any clinical phenotype. About 10% of IP parents carry one of these common polymorphisms, making them important risk factors for IP in offspring (Fusco et al., 2009). More information is available at http://www.ncbi.nlm.nih.gov/books/NBK1472/ and https://www.omim.org/entry/308300.

Apart from IP, the *IKBKG* gene is also involved in X-linked hypohidrotic ectodermal dysplasia and immunodeficiency (HED-ID), which is also known as anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID; http://ghr.nlm.nih.gov/condition/anhidrotic-ectodermal-dysplasia-with-immune-deficiency), and HED-ID with osteopetrosis and lymphedema (OL-HED-ID; http://ghr.nlm.nih.gov/condition/osteopetrosis).

This SALSA MLPA probemix is not CE/FDA registered for use in diagnostic procedures. Purchase of this product includes a limited license for research purposes.

Gene structure and transcript variants:
The *IKBKG* gene (*inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma*; previously named *NEMO*, *Nuclear factor kappaB essential modulator*) spans about 17.2 kb of genomic DNA and is located on chromosome Xq28, close to the q-telomere (about 154.5 Mb from the p-telomere). It contains nine coding exons (exons 2–10) and several alternative noncoding first exons, some of which are located in the neighbouring *G6PD* gene (see Figure 1).

IKBKG is part of a ~36 kb segmental duplication region or low copy repeat (LCR) (Figure 1). LCR1 contains *IKBKG* and LCR2 contains *IKBKG*'s non-functional truncated pseudogene, *IKBKGP1* or *pseudoNEMO*, arranged in an opposite orientation. The two LCRs, separated by a 21.7 kb unique sequence, are derived from a duplication event resulting in >99.5% of identity.

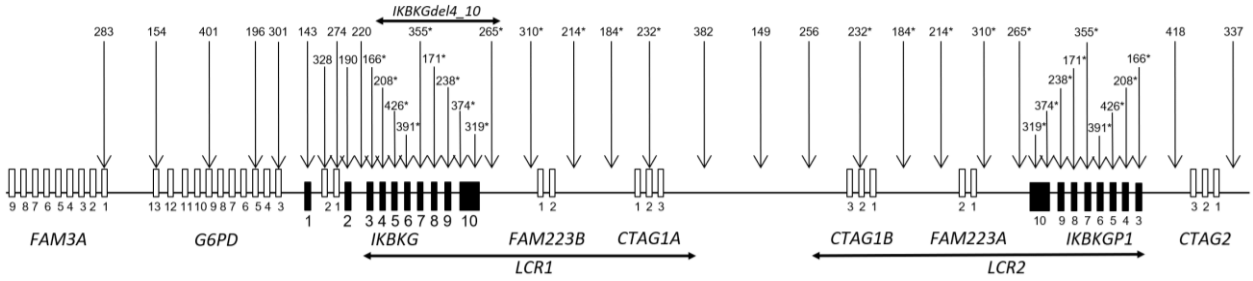

Figure 1. *Schematic representation of the IKBKG locus. Two nearly identical inverted repeat regions are located on chromosome Xq28: LCR1 and LCR2 (horizontal arrows). LCR1 contains, among others, the functional IKBKG gene, while the non-functional pseudogene IKBKGP1 is situated in LCR2. Both regions are separated by a unique ~22 kb sequence. Vertical bars represent exons. The location of target probes in the P073 IKBKG probemix are indicated by vertical arrows, including their length (see also Table 1 & 2). Probes indicated with an asterisk (*) detect both sequences in LCR1 and LCR2; four copies in most females, two copies in most males. Figure is not to scale.*

Entrez Gene shows transcript variants of each gene: http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene
For NM_ mRNA reference sequences: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

Exon numbering
The *IKBKG* and *G6PD* exon numbering used in this P073-A1 IKBKG product description is the exon numbering from the LRG_70 and LRG_148 sequence, respectively. The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the LRG sequences. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

Probemix content
The SALSA MLPA Probemix P073-A1 IKBKG contains 38 MLPA probes with amplification products between 136 and 436 nucleotides (nt). This includes 13 probes for the *IKBKG* gene. Ten of these probes also detect sequences in *IKBKGP1*, hence these probes detect four copies in most healthy female individuals. The probemix further contains seven probes upstream of the *IKBKG* coding sequence, including six probes for the *G6PD* gene. Also, nine probes downstream of the *IKBKG* gene are included, of which four are located in the duplicated region, and therefore detect four copies in most healthy female individuals, and two of which are telomeric of *IKBKGP1*. In addition, nine reference probes are included that detect locations on the X-chromosome. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

This probemix contains nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, and one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mrcholland.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

MLPA technique
The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.com).

MLPA technique validation
Internal validation of the MLPA technique using 16 DNA samples from healthy individuals of the same sex is required, in particular when using MLPA for the first time, or when changing the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all reference probes over the experiment.

Required specimens
Extracted DNA free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference samples
A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from different unrelated individuals who are from families without a history of IP. Reference samples should **NOT** contain the frequent *IKBKG* duplication or *IKBKGP1* deletion. It is recommended to use samples of the same sex to facilitate interpretation. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

Positive control DNA samples

MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. Sample ID number NA19225 from the Coriell Institute have been tested with this P073-A1 probemix at MRC Holland and can be used as a positive control sample to detect a heterozygous deletion of *IKBKG* exon 4-10. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Chromosomal position of copy number alteration*	Altered target gene in P073-A1	Expected copy number alteration
NA19225	Coriell Institute	Xq28	*IKBKG*	IKBKGexon4_10del mutation

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of copy number alteration (CNA) present in this cell line cannot be determined by this P073-A1 IKBKG probemix.

Data analysis

Coffalyser.Net software should be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mrcholland.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

Interpretation of results

The standard deviation of each individual probe over all the reference samples should be ≤0.10 and the final ratio (FR) of each individual reference probe in the patient samples should be between 0.80 and 1.20.

The majority of IP patients carry the common IKBKGexon4_10 mutation. Probes located in this region will therefore detect **four copies** in reference samples, and a ~25% signal reduction of these probes will be observed in patients (see Figure 2). Note that an exon 4_10 deletion in the pseudogene, *IKBKGP1*, will result in a similar pattern! Individuals carrying *IKBKGP1* deletions are, however, without any clinical phenotype.

		ReferenceSamples			AllSamples	
		P073-A1-1015-QT…	P073-A1-1015-QT…	P073-A1-1015-QT…	P073-A1-1015-QT…	P073-A1-1015-QT…
FRSS (n=7)		100%	100%	100%	100%	100%
CAS (n=5)	FMRS	100%	100%	100%	100%	100%
	PSLP - Relati…	OK	OK	OK	OK	OK
	FSLP - Relati…	OK	OK	OK	OK	OK
	RSQ - Refere…	OK	OK	OK	OK	OK
	RPQ - Refere…	OK	OK	OK	OK	OK
Xq (n=29)	FAM3A-1	1	1	1.01	1.02	1
	G6PD-13	0.97	1.02	1.02	1.01	0.98
	G6PD-9	0.99	0.99	1.02	0.99	1.01
	G6PD-5	1	1	0.99	0.97	1
	G6PD-3	1.02	0.98	1	0.99	1.07
	IKBKG-1	0.99	0.99	1.02	0.99	1.01
	G6PD-2	1.01	1	1	0.99	0.97
	G6PD-1	0.99	0.99	1.02	1.03	1.02
	IKBKG-2	0.99	1.02	0.99	1.01	1.02
	IKBKG-3	0.99	0.99	1.02	1	0.98
	IKBKG-3	0.98	1.01	1.01	0.99	0.97
	IKBKG-4	0.98	1	1.02	0.79	0.78
	IKBKG-5	0.98	1.02	1	0.83	0.8
	IKBKG-6	1.06	1.01	0.94	0.76	0.8
	IKBKG-7	0.97	1.01	1.03	0.78	0.75
	IKBKG-8	1.01	1	0.99	0.76	0.75
	IKBKG-9	1	1	1	0.77	0.8
	IKBKG-10	0.97	1.01	1.02	0.77	0.77
	IKBKG-10	1	0.99	1.02	0.79	0.8
	IKBKG-10	0.98	0.98	1.04	0.77	0.78
	FAM223B-Up	0.99	1.02	1	1.06	1.03
	FAM223B-Do…	1.01	0.98	1.01	1.02	1.01
	CTAG1A-Up	1	0.99	1.01	1.01	1.01
	CTAG1A-Down	1.02	0.98	1	1	1
	CTAG1A-Down	1	1	1	1.06	1
	CTAG1A-Down	0.99	1.01	0.99	0.96	0.99
	CTAG1A-Down	0.98	1.02	1	1.05	0.99
	CTAG2-Up	0.99	1.01	1	1.02	1.02
	CTAG2-Down	1	0.98	1.01	1.01	1.01
References…	Reference*	1.03	0.99	0.98	1	1
	Reference*	1.02	1.02	0.97	1.03	1.05
	Reference*	1.04	1.02	0.94	0.98	1.02
	Reference*	1.04	1	0.97	1.02	1.06
	Reference*	1.03	1.03	0.95	1.08	1.04
	Reference*	1.04	0.98	0.99	0.98	1.04
	Reference*	1.03	1.02	0.96	1	1.06
	Reference*	1	0.99	1	1.02	1.04
	Reference*	1.06	1	0.95	1.07	1.07

***Figure 2**. Comparative analysis using Coffalyser.Net software of P073-A1 IKBKG probemix on DNA from 3 female reference samples (left) and 2 female IP patients (right).*

Since the MLPA probes for the most part of the IKBKG gene detect 4 copies in the reference samples, a ~25% reduction is observed in the IP patients carrying the common IKBKGexon4_10 deletion (shaded box).

Note that a similar pattern will be obtained when the common IKBKGP1 deletion is present. IKBKGP1 deletions do not result in any abnormal phenotype and conclusions should not be drawn in the absence of clinical data!

For probes detecting only a single location (outside the LCR regions, see Figure 1 and Table 2), the following cut-off values for the Final ratio of the probes can be used to interpret MLPA results:

Copy Number status: Male samples	**Final ratio**
Normal	0.80 < FR < 1.20
Deletion	FR = 0
Duplication	1.65 < FR < 2.25
Ambiguous copy number	All other values

Copy Number status: Female samples	**Final ratio**
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	1.75 < FR < 2.15
Ambiguous copy number	All other values

Note: The term “dosage quotient”, used in older product description versions, has been replaced by “final ratio” to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

P073 specific note:

- It is important to emphasize that the probes detecting *IKBKG* exon 3 to 10 and further downstream sequences cannot discriminate *IKBKG* and its pseudogene *IKBKGP1* (see Figure 1 and Table 2). A reduced signal of these probes might therefore as well reflect a deletion of the pseudogene, and conclusions should not be drawn in the absence of a clinical diagnosis, and conformation with another technique is required.

Limitations of the procedure

- In most populations, a significant portion of IP cases (20-25%) is caused by small (point) mutations in the *IKBKG* gene, most of which will not be detected by using SALSA MLPA Probemix P073 IKBKG.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.

Confirmation of results

Confirmation of the IKBKGexon4_10 deletion can be done by long range PCR, as described in Bardaro et al. (2004) and Steffann et al. (2004). Copy number changes detected by only a single probe always require confirmation by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polymorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target

sequence. The finding of a heterozygous mutation or polymorphism indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

Copy number changes detected by more than one consecutive probe should be confirmed by another independent technique such as long range PCR, qPCR, array CGH or Southern blotting, whenever possible. Deletions/duplications of more than 50 kb in length can often be confirmed by FISH.

***IKBKG* mutation database**

https://databases.lovd.nl/shared/genes/IKBKG. We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report copy number changes detected by the reference probes, false positive results due to SNVs and unusual results to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P073-A1 IKBKG

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]				
		Reference	*IKBKG*	*IKBKGP1*	*IKBKG* upstream	*IKBKG* downstream
64-105	Control fragments – see table in probemix content section for more information					
136	Reference probe 07103-L14743	Xp22				
143 «	**IKBKG probe** 20297-L26986		**Exon 1**			
149	**CTAG1A probe** 20298-L28165					***CTAG1A* down**
154	**G6PD probe** 19935-L26975				***G6PD* exon 13**	
160	Reference probe 16675-L20191	Xp11				
166	**IKBKG probe** 20299-L27679		**Exon 3**	**Exon 3**		
171	**IKBKG probe** 20300-L27608		**Exon 8**	**Exon 8**		
178	Reference probe 18008-L22348	Xp22				
184	**CTAG1A probe** 20773-L28742					***CTAG1A/B* up**
190	**IKBKG probe** 20782-L28766		**Exon 2**			
196	**G6PD probe** 20038-L26988				***G6PD* exon 5**	
208	**IKBKG probe** 20781-L28765		**Exon 4**	**Exon 4**		
214	**FAM223B probe** 20774-L28743					***FAM223B/A* down**
220	**IKBKG probe** 20304-L27680		**Exon 3**			
226	Reference probe 15561-L17416	Xq21				
232	**CTAG1A probe** 20775-L28744					***CTAG1A*/B exon 2**
238	**IKBKG probe** 20263-L27604		**Exon 9**	**Exon 9**		
256	**CTAG1A probe** 19937-L26978					***CTAG1A* down**
265	**IKBKG probe** 20776-L28745		**Exon 10**	**Exon 10**		
274 «	**G6PD probe** 19938-L27692				***G6PD* exon 1**	
283	**FAM3A probe** 19939-L26980				***FAM3A* exon 1**	
292	Reference probe 07671-L07377	Xp22				
301 «	**G6PD probe** 20783-L27996				***G6PD* exon 3**	
310	**FAM223B probe** 20777-L28746					***FAM223B/A* up**
319	**IKBKG probe** 20301-L14030		**Exon 10**	**Exon 10**		
328 «	**G6PD probe** 19940-L26981				***G6PD* exon 2**	
337	**CTAG2 probe** 19941-L26982					***CTAG2* up**
346	Reference probe 19560-L26139	Xq26				
355	**IKBKG probe** 20265-L27606		**Exon 7**	**Exon 7**		
364	Reference probe 03730-L03190	Xq27				
374	**IKBKG probe** 20266-L27607		**Exon 10**	**Exon 10**		
382 Ж	**CTAG1A probe** 19942-SP0906-L26983					***CTAG1A* down**
391	**IKBKG probe** 12897-L14035		**Exon 6**	**Exon 6**		
401	**G6PD probe** 19943-L26984				***G6PD* exon 9**	
411	Reference probe 17688-L21770	Xq25				
418	**CTAG2 probe** 19944-L26985					***CTAG2* down**
426 Ж	**IKBKG probe** 20633-SP0108-L28339		**Exon 5**	**Exon 5**		
436	Reference probe 06188-L20735	Xq13				

Probes marked in GREY detect sequences in both LCR1 and LCR2, see also Figure 1. Hence, each probe detects four copies in most healthy females and two copies in most healthy males!

[a] See section Exon numbering on page 3 for more information.

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 2. *Xq28* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene/ Exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	MV Location (GRCh38/hg38)	Distance to next probe
283 «	19939-L26980	FAM3A Exon 1	NM_021806.4; 235-236	AGATCGTGAGCC-AACGGGTTCCTG	154515960-154516027	15.5 kb
		G6PD stop codon 1656-1658 (exon 13); NM_001042351.3				
154	19935-L26975	G6PD Exon 13	2100-2101	TGACATTCCTTG-TCACCAGCAACA	154531520-154531587	1.5 kb
401	19943-L26984	G6PD Exon 9	1000-1001	ATGCATCTCAGA-GGTGCAGGCCAA	154533065-154533135	2.1 kb
196	20038-L26988	G6PD Exon 5	526-525, reverse	GCAAGGCCAGGT-AGAAGAGGCGGT	154535210-154535274	0.9 kb
301 «	20783-L27996	G6PD Exon 3	9 nt after exon 3	TGGTAAGTGTGT-CCCACCACTGCC	154536096-154536164	6.2 kb
		IKBKG start codon 259-261 (exon 2); NM_003639.4				
143 «	20297-L26986	IKBKG Exon 1	124-123, reverse; in NM_001099856.6	GAGGGGAAGAGA-CTGGGATTGATA	154542294-154542367	3.8 kb
328 «	19940-L26981	G6PD Exon 2	170-171	CGGGAAGAGCTT-TTCCAGGGCGAT	154546058-154546125	1.4 kb
274 «	19938-L27692	G6PD Exon 1	81-82	GTCCGCCAGGCT-CTGCAGGCCCGC	154547451-154547519	4.6 kb
		G6PD start codon 111-113 (exon 2); NM_001042351.3				
190	20782-L28766	IKBKG Exon 2	327-328	CCGGCAGCAGAT-CAGGACGTACTG	154552032-154552108	3.3 kb
220	20304-L27680	IKBKG Exon 3	822 nt before exon 3	AGGGAGGACCAA-TACCGAGCATCT	154555314-154555382	1.0 kb
166	20299-L27679	IKBKG Exon 3	609-610	CTGAAGAGGCAG-AAGGAGCAGGCT	154556301-154556365	2.3 kb
208	*20781-L28765*	*IKBKG Exon 4*	*749-748 reverse*	*GGCATTCCTTAG-TGGCAGCCTCCA*	154558590-154558657	*2.0 kb*
426 Ж	*20633-SP0108-L28339*	*IKBKG Exon 5*	*107 nt and 131 nt after exon 5*	*GCCTTCTGAGGA-24 nt spanning oligo-GTGGGCCCATTC*	154560639-154560726	*1.0 kb*
391	*12897-L14035*	*IKBKG Exon 6*	*18 nt before exon 6*	*GTTTCTCCAGCA-AAAGCTCCCTTT*	154561642-154561706	*1.2 kb*
355	*20265-L27606*	*IKBKG Exon 7*	*1104-1103 reverse*	*TCCTTCAGCTTA-TCGATCACCTCC*	154562857-154562917	*0.8 kb*
171	*20300-L27608*	*IKBKG Exon 8*	*1289-1290*	*CAGCAAACTGAA-GGCCAGCTGTCA*	154563642-154563705	*0.3 kb*
238	*20263-L27604*	*IKBKG Exon 9*	*1322-1323*	*AAGGATCGAGGA-CATGAGGAAGCG*	154563928-154563998	*0.4 kb*
374	*20266-L27607*	*IKBKG Exon 10*	*1451-1452*	*CTTCTGCTGTCC-CAAGTGCCAGTA*	154564367-154564428	*0.3 kb*
319	*20301-L14030*	*IKBKG Exon 10*	*1773-1774*	*CTGTCTGCTCGA-ACCACTTGCCTC*	154564687-154564751	*0.5 kb*
265	*20776-L28745*	*IKBKG Exon 10*	*140 nt after exon 10*	*TAGCCTTGCCAA-GGAGGAGGTGGG*	154565149-154565223	*4.9 kb*
		IKBKG stop codon 1516-1518 (exon 10); NM_003639.4				
310	20777-L28746	FAM223B Up	1.2 kb before FAM223B	AAACCAAAACGG-ATTCCTCTTCCT	154569989-154570059	5.3 kb
214	20774-L28743	FAM223B Down	3.3 kb after FAM223B	GCACCAAGACTA-TGGACTGAAGTG	154575208-154575280	4.3 kb
184	20773-L28742	CTAG1A Up	5.6 kb before CTAG1A	GGACTGTGTGGT-GCTACCTGAGTG	154579546-154579618	6.8 kb
232	20775-L28744	CTAG1A Exon 2	105 nt after exon 2	AGGCTGCTAGAA-ACCTGGGAACAC	154586364-154586428	5.6 kb
382 Ж	19942-SP0906-L26983	CTAG1A Down	5.2 kb and 5.2 kb after CTAG1A	AAAGGACAGGCA-30 nt spanning oligo-ATTGAGGAGTTT	154591936-154592040	10.6 kb
149	20298-L28165	CTAG1A Down	15.8 kb after CTAG1A	TTCAGGTAAGTA-AAACAACCAAGT	154602562-154602634	5.2 kb
256	19937-L26978	CTAG1A Down	21 kb after CTAG1A	CCTGAACCTAAA-TAGCATCACAAT	154607775-154607852	10.1 kb
232	20775-L28744	CTAG1B Exon 2	105 nt after exon 2	AGGCTGCTAGAA-ACCTGGGAACAC	154617996-154618060	6.8 kb
184	20773-L28742	CTAG1B Up	5.6 kb before CTAG1B	GGACTGTGTGGT-GCTACCTGAGTG	154624807-154624879	4.3 kb
214	20774-L28743	FAM223A Down	3.3 kb after FAM223A	GCACCAAGACTA-TGGACTGAAGTG	154629149-154629221	5.3 kb
310	20777-L28746	FAM223A Up	1.2 kb before FAM223A	AAACCAAAACGG-ATTCCTCTTCCT	154634370-154634440	4.9 kb
		IKBKGP1; NG_001576				
265	20776-L28745	Exon 10	140 nt after NG_001576	TAGCCTTGCCAA-GGAGGAGGTGGG	154639213-154639287	0.5 kb
319	20301-L14030	Exon 10	8654-8655	CTGTCTGCTCGA-ACCACTTGCCTC	154639685-154639749	0.3 kb
373	20266-L27607	Exon 10	8332-8333	CTTCTGCTGTCC-CAAGTGCCAGTA	154640008-154640069	0.4 kb
238	20263-L27604	Exon 9	7905-7906	AAGGATCGAGGA-CATGAGGAAGCG	154640438-154640508	0.3 kb
171	20300-L27608	Exon 8	7616-7617	CAGCAAACTGAA-GGCCAGCTGTCA	154640731-154640794	0.8 kb
355	20265-L27606	Exon 7	6885-6886	TCCTTCAGCTTA-TCGATCACCTCC	154641520-154641580	1.2 kb
391	12897-L14035	Exon 6	5606-5607	GTTTCTCCAGCA-AAAGCTCCCTTT	154642731-154642795	1.0 kb
426 Ж	20633-SP0108-L28339	Exon 5	4604-4605 and 4628-4629	GCCTTCTGAGGA-24 nt spanning oligo-GTGGGCCCATTC	154643711-154643798	2.0 kb
208	20781-L28765	Exon 4	2558-2557 reverse	GGCATTCCTTAG-TGGCAGCCTCCA	154645780-154645847	2.3 kb
166	20299-L27679	Exon 3	265-266	CTGAAGAGGCAG-AAGGAGCAGGCT	154648072-154648136	1.1 kb
		IKBKGP1; NG_001576				
418	19944-L26985	CTAG2 Up	2.7 kb after exon 3	GGGCAGAAGGAG-AACCTGCTCTCT	154649182-154649249	6.7 kb
337	19941-L26982	CTAG2 Down	2.6 kb before exon 1	GAGATGTGAGAA-GATGTCTACGAA	154655888-154655956	

Probes marked in GREY detect sequences in both LCR1 and LCR2, see also Figure 1. Hence, each probe detects four copies in most healthy females and two copies in most healthy males!

Probes marked in ITALIC detect sequences that are ~25% reduced in peak height when the IKBKGexon4_10 deletion is present.

[a] See section Exon numbering on page 3 for more information.
[b] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Related SALSA MLPA probemixes

P285 LRP5	Contains probes for the *NDP*, *DKK1*, *LRP5* and *FZD4* genes.

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- Kawai M et al. (2020). Molecular analysis of low-level mosaicism of the IKBKG mutation using the X Chromosome Inactivation pattern in Incontinentia Pigmenti. *Mol. Genet. Genomic Med*, 8(12), e1531.
- Kim HY et al. (2021). Importance of extracutaneous organ involvement in determining the clinical severity and prognosis of incontinentia pigmenti caused by mutations in the IKBKG gene. *Exp. Dermatol*., 30(5), 676-683. Publication 2

P073 product history	
Version	*Modification*
A1	First release.

Implemented changes in the product description
Version A1-02 – 07 April 2022 (04P) - Product description rewritten and adapted to a new template. - Ligation sites of the probes targeting the *G6PD* and *FAM3A* genes updated according to new versions of the NM_ reference sequences. - Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products. *Version A1-01 - 23 April 2019 (01P)* - Various minor textual and layout changes. - Product description restructured and adapted to a new template. - Several ligation sites of the probes targeting the *G6PD* and *IKBKG* genes were updated. - Capillary electrophoresis pattern pictures removed.

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