Product Description
SALSA® MLPA® Probemixes P351-D1 PKD1 & P352-E1 PKD1-PKD2

To be used with the MLPA General Protocol.

P351 version D1
As compared to version C1, two PKD1 probes have been added, and two PKD1 probes, one TSC2 probe and four reference probes have been replaced. In addition, three PKD1 probes have been changed in length. For complete product history see page 12.

P352 version E1
As compared to version D1, three PKD1 probes and one PKD2 probe have been added, and one PKD2 probe and one reference probe have been replaced. In addition, one PKD1 probe and two PKD2 probes have been changed in length. For complete product history see page 13.

Catalogue numbers:
- **P351-025R:** SALSA MLPA Probemix P351 PKD1, 25 reactions.
- **P351-050R:** SALSA MLPA Probemix P351 PKD1, 50 reactions.
- **P351-100R:** SALSA MLPA Probemix P351 PKD1, 100 reactions.

- **P352-025R:** SALSA MLPA Probemix P352 PKD1-PKD2, 25 reactions.
- **P352-050R:** SALSA MLPA Probemix P352 PKD1-PKD2, 50 reactions.
- **P352-100R:** SALSA MLPA Probemix P352 PKD1-PKD2, 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.

The complete *PKD1* gene is difficult to denature, but the region between *PKD1* exon 31 and *TSC2* exon 42 is even more difficult to denature due to an extremely high GC content. This may cause false positive results in samples containing salt. A low signal of the 88 nt and 96 nt D-fragments provides a warning for incomplete DNA denaturation.

Intended purpose
The SALSA MLPA Probemixes P351 PKD1 and P352 PKD1-PKD2 are in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assays[2] for the detection of deletions or duplications in the *PKD1* and *PKD2* genes, in order to confirm a potential cause for and clinical diagnosis of autosomal dominant polycystic kidney disease (ADPKD). P351 PKD1 can also be used to detect deletions or duplications in *TSC2* exons 36, 38 and 42. Deletions disrupting both *PKD1* and *TSC2* can confirm a potential cause for and clinical diagnosis of *TSC2*/*PKD1* contiguous gene deletion syndrome. Both assays are for use with genomic DNA isolated from human peripheral whole blood specimens, and are also intended for molecular genetic testing of at-risk family members.

The detection of copy number variations (CNVs) in *PKD1* requires the use of both P351 PKD1 and P352 PKD1-PKD2, whereas the detection of CNVs in *PKD2* only requires the use of P352 PKD1-PKD2. CNVs detected with

P351 PKD1 and P352 PKD1-PKD2 should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *PKD1*, *PKD2* and *TSC2* genes are point mutations, none of which will be detected by MLPA. It is therefore recommended to use these assays in combination with sequence analysis.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

These devices are not intended to be used for standalone diagnostic purposes, pre-implantation or prenatal testing, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations.

[1] Please note that these probemixes are for in vitro diagnostic (IVD) use in the countries specified at the end of this product description. In all other countries, the products are for research use only (RUO).
[2] To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

Clinical background
Autosomal dominant polycystic kidney disease (ADPKD), the most common hereditary kidney disease, is characterized by bi-lateral development and expansion of renal cysts, hypertension and a progressive decline in renal function. In ~50% of affected individuals, ADPKD results in end-stage renal disease (ESRD), and 4-10% of ESRD worldwide is due to ADPKD. Although the kidney is the main organ involved, ADPKD is a multisystem disorder with profound extra-renal manifestations, including liver cysts and intracranial aneurysms. There is a substantial intra- and interfamilial variability in the severity of both renal and extra-renal disease manifestations. ADPKD is typically a late-onset disease diagnosed in adulthood, but about 2-5% of the ADPKD patients show a very early onset of the disease (during childhood or even prenatally) and a severe phenotype.

ADPKD is caused by heterozygous pathogenic mutations in either the *PKD1*, *PKD2*, *GANAB* or *DNAJB11* genes (Cornec-Le Gall et al. 2018; Porath et al. 2016). Most patients carry a defect in *PKD1* (~78%) or *PKD2* (~15%), whereas a minority of the cases is explained by defects in *GANAB* (~0.3%) and *DNAJB11* (~0.1%). In ~7% of the cases, the underlying genetic defect is unknown. Patients with a *PKD1* mutation, especially those with truncating mutations, generally have a more rapidly progressive disease with an earlier onset of ESRD than patients with a *PKD2* mutation. It is estimated that ~3% of the *PKD1* and *PKD2* mutations are deletions or duplications (Carrera et al. 2016; Consugar et al. 2008; Schonauer et al. 2020; Xu et al. 2018). More information about ADPKD is available at https://www.ncbi.nlm.nih.gov/books/NBK1246/.

TSC2/*PKD1* contiguous gene deletion syndrome is a disorder in which the phenotypes of tuberous sclerosis complex and ADPKD are combined. Tuberous sclerosis complex is a neurocutaneous disorder that involves abnormalities of the skin, brain, kidney, heart and lungs. When combined with ADPKD, it is characterised by a very early onset of severe polycystic kidney disease, that is diagnosed in utero or in infancy. The *PKD1* gene lies directly adjacent to the *TSC2* gene in a tail-to-tail orientation. Large *PKD1* deletions that also disrupt the adjacent *TSC2* gene result in *TSC2*/*PKD1* contiguous gene deletion syndrome (Consugar et al. 2008).

Gene structure
The *PKD1* gene spans ~47 kilobases (kb) on chromosome 16p13.3 and contains 46 exons. The GenBank genomic DNA sequence is NG_008617.1. There is no LRG sequence available for *PKD1*.

The *PKD2* gene spans ~70 kb on chromosome 4q22.1 and contains 15 exons. The GenBank genomic DNA sequence is NG_008604.1. There is no LRG sequence available for *PKD2*.

The *TSC2* gene spans ~41 kb on chromosome 16p13.3 and contains 42 exons. The *TSC2* LRG_487 is available at www.lrg-sequence.org and is identical to GenBank genomic DNA sequence NG_005895.1.

Transcript variants
For *PKD1*, two transcript variants have been described (https://www.ncbi.nlm.nih.gov/gene/5310). Transcript variant 1 encodes isoform 1 (NM_001009944.3; 14140 nt; coding sequence 210-13121). This sequence is a

reference standard in the NCBI RefSeq project. The ATG translation start site is located in exon 1 and the stop codon is located in exon 46. Transcript variant 2 (NM_000296.4; 14137 nt; coding sequence 210-13118) uses an alternate acceptor splice site and encodes isoform 2, which is one amino acid shorter than isoform 1.

For *PKD2*, one transcript variant has been described encoding the full length protein (NM_000297.4; 5089 nt; coding sequence 100-3006; https://www.ncbi.nlm.nih.gov/gene/5311). This sequence is a reference standard in the NCBI RefSeq project. The ATG translation start site is located in exon 1 and the stop codon is located in exon 15.

For *TSC2*, multiple variants have been described. Transcript variant 1 is the most predominant and encodes isoform 1 (NM_000548.5; 6415 nt; coding sequence 111-5534; https://www.ncbi.nlm.nih.gov/gene/7249). This sequence is a reference standard in the NCBI RefSeq project. The ATG translation start site is located in exon 2 and the stop codon is located in exon 42.

Exon numbering

The *PKD1* and *PKD2* exon numbering used in this P351-D1/P352-E1 PKD1-PKD2 product description is the exon numbering from the NG_008617.1 and NG_008604.1 sequences, respectively. The *TSC2* exon numbering used is the exon numbering from the LRG_487 sequence. 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 NG or LRG sequence. 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 P351-D1 PKD1 contains 43 MLPA probes with amplification products between 135 and 481 nucleotides (nt). The SALSA MLPA Probemix P352-E1 PKD1-PKD2 contains 45 MLPA probes with amplification products between 136 and 490 nt.

The P351-D1 and P352-E1 probemixes contain 29 probes and 16 probes for the *PKD1* gene, respectively. Together, these probemixes cover 41 of the 46 *PKD1* exons. There are two probes upstream of *PKD1* and three probes for *PKD1* exon 15. The P351-D1 probemix also contains three probes for the *TSC2* gene, located just downstream of *PKD1*. Furthermore, the P352-E1 probemix contains 18 probes for the *PKD2* gene. All exons of the *PKD2* gene are covered and there are two probes present for exons 1, 2 and 6. The P351-D1 and P352-E1 probemixes contain 11 reference probes each that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

Each of the probemixes 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 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 probes over the experiment.

Required specimens
Extracted DNA from human peripheral blood, 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 polycystic kidney disease. 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 numbers NA02325, NA10800 and NA00782 from the Coriell Institute have been tested with the P351-D1 and/or P352-E1 probemixes at MRC Holland and can be used as a positive control samples. The quality of cell lines can change; therefore samples should be validated before use.

Sample ID number	Source	Probemix by which CNV is detected	Expected CNV*
NA02325	Coriell Institute	P351/P352	Heterozygous duplication of *PKD1* and *TSC2*
NA10800	Coriell Institute	P352	Heterozygous deletion of *PKD2*
NA00782	Coriell Institute	P352	Heterozygous duplication of *PKD2*

* The whole extent of the CNV present in this cell line cannot be determined by the P351-D1 PKD1 and P352-E1 PKD1-PKD2 probemixes.

Performance characteristics
Approximately 3% of the pathogenic mutations in the *PKD1* and *PKD2* genes in ADPKD patients are large deletions or duplications that can be detected with the P351 PKD1 and P352 PKD1-PKD2 probemixes (https://www.ncbi.nlm.nih.gov/books/NBK1246/; Carrera et al. 2016; Consugar et al. 2008; Schonauer et al. 2020; Xu et al. 2018;). Large deletions that disrupt both *PKD1* and *TSC2* explain 100% of the cases of *TSC2*/*PKD1* contiguous gene deletion syndrome. The analytical sensitivity and specificity for the detection of deletions or duplications in the *PKD1* and *PKD2* genes and in exons 36, 38 and 42 of the *TSC2* gene is very high and can be considered >99% (based on a 2008-2021 literature review).

Analytical performance can be compromised by: SNVs or other polymorphisms in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

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 expected results for *PKD1*, *PKD2* and *TSC2* region specific MLPA probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), or 3 (heterozygous duplication). In rare cases, copy numbers of 0 (homozygous deletion) or 4 (heterozygous triplication/homozygous duplication) may be obtained.

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. When these criteria are fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
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 (Consugar et al. 2008; Hopp et al. 2020). 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 or in or near the *PKD1*, *PKD2* and *TSC2* genes. 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 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.

P351/P352 specific notes:

- No probes are present for *PKD1* exons 4, 8, 17, 28 and 32. This is due to the existence of several *PKD1* pseudogenes that are almost identical to the actual gene. These pseudogenes are also present on chromosome 16.
- Probes detecting *PKD1* exon 1-31 rely for their specificity on a single nucleotide difference between the *PKD1* gene and its pseudogenes. For these probes, an apparent duplication can be the result of a clinically non-significant single nucleotide sequence change in one of the pseudogenes.
- The complete *PKD1* gene is difficult to denature, but the region between *PKD1* exon 31 and *TSC2* exon 42 is even more difficult to denature due to an extremely high GC content. This may cause false positive results in samples containing salt.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *PKD1*, *PKD2* and *TSC2* genes are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P351 PKD1 and SALSA MLPA Probemix P352 PKD1-PKD2.
- 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

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.

ADPKD mutation database

https://pkdb.mayo.edu/. We strongly encourage users to deposit positive results in the ADPKD mutation database. 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 (e.g., a duplication of *PKD2* exons 5 and 7 but not exon 6) to MRC Holland: info@mrcholland.com.

Table 1a. SALSA MLPA Probemix P351-D1 PKD1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*PKD1*	*TSC2*
64-105	Control fragments – see table in probemix content section for more information			
135	Reference probe 00797-L26847	5q		
141 «	**PKD1 probe** 10938-L24677		**Exon 3**	
148 «	**PKD1 probe** 10960-L16105		**Exon 40**	
154 «	**PKD1 probe** 10950-L24689		**Exon 20**	
160 ¥ « ±	**PKD1 probe** 10961-L31500		**Exon 41**	
166	Reference probe 02310-L24631	19p		
172 * «	**PKD1 probe** 22347-L30144		**Exon 1**	
178 «	**PKD1 probe** 10958-L24696		**Exon 38**	
184 «	**PKD1 probe** 10963-L24700		**Exon 43**	
190	Reference probe 08528-L24624	9p		
196 «	**PKD1 probe** 10946-L27456		**Exon 14**	
202 «	**PKD1 probe** 10962-L24699		**Exon 42**	
209 * «	**TSC2 probe** 22214-L31268			**Exon 42**
226 «	**PKD1 probe** 10952-L24690		**Exon 23**	
232 «	**PKD1 probe** 14095-L24702		**Exon 46**	
238 * «	**PKD1 probe** 21754-L31494		**Exon 16**	
244 *	Reference probe 20757-L28659	1q		
251 «	**PKD1 probe** 14096-L24686		**Exon 15**	
258 * «	**PKD1 probe** 21758-L31693		**Exon 30**	
266 «	**PKD1 probe** 10942-L27362		**Exon 10**	
274 «	**PKD1 probe** 10954-L24692		**Exon 27**	
281	Reference probe 08887-L24627	12q		
290 «	**PKD1 probe** 10949-L24688		**Exon 18**	
297 «	**PKD1 probe** 10940-L24678		**Exon 5**	
307 ¥ «	**PKD1 probe** 10944-L31694		**Exon 12**	
316 *	Reference probe 18924-L25193	1p		
322 «	**PKD1 probe** 10955-L24693		**Exon 29**	
335 «	**PKD1 probe** 10964-L27363		**Exon 44**	
343 «	**PKD1 probe** 14097-L24685		**Exon 15**	
352 *	Reference probe 13400-L20982	6q		
361 «	**PKD1 probe** 19919-L24683		**Exon 13**	
371 ¥ «	**PKD1 probe** 22348-L29683		**Exon 11**	
379 «	**PKD1 probe** 10959-L24697		**Exon 39**	
387	Reference probe 07925-L21227	20p		
394 «	**PKD1 probe** 10941-L24679		**Exon 9**	
414 «	**PKD1 probe** 10953-L24691		**Exon 25**	
424 «	**TSC2 probe** 01842-L24628			**Exon 36**
431	Reference probe 15541-L25346	2q		
441 «	**TSC2 probe** 01843-L24629			**Exon 38**
450 «	**PKD1 probe** 19918-L24695		**Exon 35**	
459	Reference probe 01799-L23610	13q		
472 * «	**PKD1 probe** 21755-L30421		**Exon 45**	
481 *	Reference probe 15738-L11546	15q		

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

* New in version D1.

¥ Changed in version D1. Minor alteration, no change in sequence detected.

« 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.

± SNP rs151157369 could influence the 160 nt probe signal (P351-D1). SNP rs528103165 could influence the 153 nt probe signal (P352-E1). In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

Table 1b. SALSA MLPA Probemix P352-E1 PKD1-PKD2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*PKD1*	*PKD2*
64-105	Control fragments – see table in probemix content section for more information			
136	Reference probe 00797-L24120	5q		
142	**PKD2 probe** 10995-L24648			**Exon 2**
148	**PKD2 probe** 11005-L24659			**Exon 12**
153 « ±	**PKD1 probe** 14100-L24666		**Exon 7**	
159 «	**PKD1 probe** 10992-L24674		**Exon 36**	
166	**PKD2 probe** 11004-L24658			**Exon 11**
172	Reference probe 03245-L24643	13q		
178 ¥	**PKD2 probe** 21417-L11672			**Exon 8**
185 «	**PKD1 probe** 19917-L11651		**Exon 6**	
191 « ¤	**PKD1 probe** 11011-L27419		**Exon 31**	
202	**PKD2 probe** 11000-L24654			**Exon 7**
208 * «	**PKD1 probe** 21759-L30691		**Exon 34**	
214	Reference probe 18153-L22663	21q		
220	**PKD2 probe** 10998-L24652			**Exon 5**
226 * «	**PKD1 probe** 21756-L30692		**Exon 24**	
233	**PKD2 probe** 14739-L24653			**Exon 6**
240 * «	**PKD1 probe** 21752-L30143		**Exon 2**	
248 « ¤	**PKD1 probe** 10983-L24667		**Exon 15**	
257 «	**PKD1 probe** 14102-L24664		**Upstream**	
263	**PKD2 probe** 14738-L20398			**Exon 6**
270 «	**PKD1 probe** 10986-L24669		**Exon 21**	
280	Reference probe 10799-L24638	2q		
291 ¥ « Δ	**PKD1 probe** 10984-L32624		**Exon 19**	
297 «	**PKD2 probe** 10994-L24647			**Exon 1**
307	Reference probe 18034-L22721	17q		
315 « Δ	**PKD1 probe** 11009-L24671		**Exon 26**	
325	**PKD2 probe** 11002-L24656			**Exon 9**
334	Reference probe 01918-L24637	1q		
342	**PKD2 probe** 10997-L24651			**Exon 4**
352	**PKD2 probe** 11003-L24657			**Exon 10**
362	Reference probe 06383-L24641	8p		
370 *	**PKD2 probe** 21760-L30426			**Exon 3**
381 ¥	**PKD2 probe** 22213-L24661			**Exon 14**
388 «	**PKD2 probe** 14740-L24646			**Exon 1**
396	Reference probe 00713-L24645	19q		
405 «	**PKD1 probe** 14087-L24675		**Exon 37**	
416 «	**PKD1 probe** 10990-L24673		**Exon 33**	
425	**PKD2 probe** 11008-L24662			**Exon 15**
433	Reference probe 00680-L24640	7q		
444 «	**PKD1 probe** 14103-L24663		**Upstream**	
452 «	**PKD1 probe** 14094-L24670		**Exon 22**	
463	**PKD2 probe** 14741-L24649			**Exon 2**
471	Reference probe 07607-L24644	15q		
478 *	**PKD2 probe** 21578-L30146			**Exon 13**
490 *	Reference probe 20096-L27538	4p		

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

* New in version E1.

¥ Changed in version E1. Minor alteration, no change in sequence detected.

« 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.

± SNP rs151157369 could influence the 160 nt probe signal (P351-D1). SNP rs528103165 could influence the 153 nt probe signal (P352-E1). In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

¤ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

Δ More variable. If the ligation reaction is performed at room temperature, this probe is more prone to bind to homologous sequences. Aberrant results should therefore always be verified.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Table 2. P351-D1/P352-E1 probes arranged according to chromosomal location

Table 2a. *PKD1* and *TSC2*

Length (nt) P351	Length (nt) P352	SALSA MLPA probe	Gene exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
			***PKD1* gene**	**NM_001009944.3**		
			start codon	*210-212 (Exon 1)*		
	444	14103-L24663	Upstream	2.0 kb before exon 1	TCTTCCCTGAAC-CAAACAAGATCT	0.5 kb
	257	14102-L24664	Upstream	1.5 kb before exon 1	CATTGAGACTTG-GTTAATCTGTTT	1.2 kb
172		22347-L30144	Exon 1	281 nt before exon 1 reverse	AGGCGCACGAGC-TATTTTTCACGC	16.7 kb
	240	21752-L30143	Exon 2	142 nt before exon 2 reverse	GTCAGGAGGGGA-CTTTCTGATGGA	0.3 kb
141		10938-L24677	Exon 3	10 nt before exon 3	CCGAATCCCCCG-TCTCTTCCAGGG	1.1 kb
		No probe	Exon 4			
297		10940-L24678	Exon 5	1143-1144	GAGACGGCTCCG-CCGAGGTGGATG	0.5 kb
	185	19917-L11651	Exon 6	1519-1520	GGAGCAGTGTCA-GGCCTGGGCCGG	0.7 kb
	153 ±	14100-L24666	Exon 7	1807-1808	CTGCGAGCTGCA-GCCCGGAGGTGT	0.6 kb
		No probe	Exon 8			
394		10941-L24679	Exon 9	64 nt before exon 9	GAAGTTCGGGTA-GGGGGAGTCTGG	0.9 kb
266		10942-L27362	Exon 10	83 nt after exon 10	GGGTCTGTGCAC-CAGACACACCCA	1.0 kb
371		22348-L29683	Exon 11	2916-2917	TGCCGTGGCTCA-GTGAGGGGGAGC	1.0 kb
307		10944-L31694	Exon 12	11 nt before exon 12	GGCTGACACCAT-TCCCCCCGCAGA	0.4 kb
361		19919-L24683	Exon 13	3242-3243	AACTACAACGTA-ACCGTGGAGCGG	0.4 kb
196		10946-L27456	Exon 14	17 nt before exon 14	TCACTCACTGCG-TCCCACCGCCCC	1.0 kb
	248 ¤	10983-L24667	Exon 15	3851-3852	GAGCTCCGCGGA-CTCAGCGTGGAC	1.3 kb
343		14097-L24685	Exon 15	5177-5176 reverse	GTGCCATCCCTA-ACCACGGCCTGC	1.5 kb
251		14096-L24686	Exon 15	6712-6713	ACAGCGCAACTA-CTTGGAGGCCCA	0.7 kb
238		21754-L31494	Exon 16	7229-7228 reverse	TTCCACACGGTC-AGGCTGAAGGTG	1.4 kb
		No probe	Exon 17			
290		10949-L24688	Exon 18	7553-7554	ACCTTCACGCTC-ACGGTGCTGGGC	0.3 kb
	291 Δ	10984-L32624	Exon 19	7809-7810	GCAGCCTCTCCA-GCTACGGAGCCG	0.4 kb
154		10950-L24689	Exon 20	24 nt after exon 20	TGGGAGGGGACG-TCACATCTGCTG	0.3 kb
	270	10986-L24669	Exon 21	36 nt before exon 21	CGGCCTCCTGCG-CTGCTGACAGCT	0.9 kb
	452	14094-L24670	Exon 22	8296-8297	GGCCATGATGCT-CATCCTGCAGGC	0.7 kb
226		10952-L24690	Exon 23	8416-8417	GGCACCACAGCC-CTCAGAGCTGGG	1.0 kb
	226	21756-L30692	Exon 24	9110-9111	CGCCCAGAGTCA-CTCCAGGGTGCT	0.3 kb
414		10953-L24691	Exon 25	9276-9277	GCGAGGAGGACA-TGGTGTGGCGGA	0.5 kb
	315 Δ	11009-L24671	Exon 26	12 nt after exon 26	GTGAGGGGCGCA-GCGGGGTGGCAG	1.4 kb
274		10954-L24692	Exon 27	86 nt before exon 27	CTTGAGTGCGCA-CAGGCCAAAGCT	0.6 kb
		No probe	Exon 28			
322		10955-L24693	Exon 29	9935-9936	GACGCAGCCCTT-TTGCGCTTCCGG	0.4 kb
258		21758-L31693	Exon 30	10232-10231 reverse	CGGAAGAGAAAA-AGGATGGCCAGG	1.8 kb
	191 ¤	11011-L27419	Exon 31	7 nt after exon 31	CTGAGGTGAGGA-CTCTACTGGGGG	0.5 kb

Length (nt) P351	Length (nt) P352	SALSA MLPA probe	Gene exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		No probe	Exon 32			
	416	10990-L24673	Exon 33	10604-10605	GCCAAATCCTTC-TCAGCATCAGGT	0.2 kb
	208	21759-L30691	Exon 34	10672-10673	AGCCCCTACCCA-AGACACCCACAT	2.9 kb
450		19918-L24695	Exon 35	36 nt before exon 35	CTGCCTCCTGGA-GGCCGGGATGAA	0.3 kb
	159	10992-L24674	Exon 36	10848-10849	AGGGTCTGCGGA-AGCGCCTGCTGC	0.4 kb
	405	14087-L24675	Exon 37	11176-11177	TGCACTCTTCCT-GGCCAAGGAAGA	0.6 kb
178		10958-L24696	Exon 38	11324-11325	CAAAGCGCCATC-AAGCAGGAGCTG	0.4 kb
379		10959-L24697	Exon 39	11388-11389	TCTGGCCATGGA-TGGCCCACGTGC	0.5 kb
148		10960-L16105	Exon 40	11549-11550	AGCACCAGCGAT-TACGACGTTGGC	0.3 kb
160 ±		10961-L31500	Exon 41	11684-11685	GAGCTGGGCCTG-AGCCTGGAGGAG	0.3 kb
202		10962-L24699	Exon 42	11766-11767	CTGTGTTCCTGG-AGCTCACGCGCT	0.4 kb
184		10963-L24700	Exon 43	11949-11950	TCGCCGTGCACT-TCGCCGTGGCCG	0.4 kb
335		10964-L27363	Exon 44	12250-12251	CCAGTGGTCCGT-CTTTGGCAAGAC	0.4 kb
472		21755-L30421	Exon 45	12541-12542	CTGGCGCTACCA-CGCCTTGCGTGG	0.9 kb
232		14095-L24702	Exon 46	13364-13365	ACGGTTTCTAGC-CTCTGAGATGCT	0.9 kb
			stop codon	*13119-13121 (Exon 46)*		
			***TSC2* gene**	**NM_000548.5**		
			stop codon	*5532-5534 (Exon 42)*		
209		22214-L31268	Exon 42	5474-5475	ACACCTGGCTAT-GAGGTGGGCCAG	1.7 kb
441		01843-L24629	Exon 38	5045-5046	GGCAACGACTTT-GTGTCCATTGTC	1.5 kb
424		01842-L24628	Exon 36	4728-4729	AGATCCCATCAT-ACGACACCCACA	
			start codon	*111-113 (Exon 2)*		

Table 2b. *PKD2*

Length (nt) P351	Length (nt) P352	SALSA MLPA probe	*PKD2* exon[a]	Ligation site NM_000297.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
			start codon	*100-102 (Exon 1)*		
	388 «	14740-L24646	Exon 1	114-115	GTGAACTCCAGT-CGCGTGCAGCCT	0.2 kb
	297 «	10994-L24647	Exon 1	266-267	GGAGATCGAGAT-GCAGCGCATCCG	11.6 kb
	142	10995-L24648	Exon 2	738-739	AGCACTAACCGA-GAGAAATACCTT	0.1 kb
	463	14741-L24649	Exon 2	3 nt after exon 2	TGTGCATCTGTA-AGTAGAATATTT	16.7 kb
	370	21760-L30426	Exon 3	873-874	CAGCTCTTCCTA-GACACCCCCGTG	2.0 kb
	342	10997-L24651	Exon 4	979-980	GGCTGTACTGGA-AGATGCAGCCCA	5.0 kb
	220	10998-L24652	Exon 5	1288-1289	TGGATTTGTCAA-GAACAAGAGAGG	3.4 kb
	263	14738-L20398	Exon 6	1505-1506	TGTCACAACTTT-TGATTTCTTCCT	0.1 kb
	233	14739-L24653	Exon 6	1596-1597	CGCATTCACAAA-CTACACTATTTC	5.3 kb
	202	11000-L24654	Exon 7	1720-1721	AGTTTCTGGAAG-ATCAAAATACTT	4.1 kb
	178	21417-L11672	Exon 8	1861-1862	GCCAGCTCTCGA-CAACCATGTCTC	1.9 kb
	325	11002-L24656	Exon 9	2060-2061	GGAAGCTAATCG-AGTTTTGGGACC	3.9 kb
	352	11003-L24657	Exon 10	2176-2177	CTGACTTGGCAC-AGCAGAAAGCTG	3.5 kb
	166	11004-L24658	Exon 11	2303-2304	AGGAGGAGGCAA-GTTAAACTTTGA	0.4 kb
	148	11005-L24659	Exon 12	2403-2404	GATGGAGACCAA-GAACTGACCGAA	2.2 kb
	478	21578-L30146	Exon 13	2570-2569 reverse	CCCTCCTTCTGG-AGCTATGTCCGC	6.9 kb
	381	22213-L24661	Exon 14	2675-2676	CATAGTGTCCAA-GATTGACGCCGT	0.7 kb
	425	11008-L24662	Exon 15	2872-2873	CAGCTTCCCAGA-TCAGTCATGGTT	
			stop codon	*3004-3006 (Exon 15)*		

[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.

± SNP rs151157369 could influence the 160 nt probe signal (P351-D1). SNP rs528103165 could influence the 153 nt probe signal (P352-E1). In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

¤ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

Δ More variable. If the ligation reaction is performed at room temperature, this probe is more prone to bind to homologous sequences. Aberrant results should therefore always be verified.

Important notes

- The complete *PKD1* gene is difficult to denature, but the region between *PKD1* exon 31 and *TSC2* exon 42 is even more difficult to denature due to an extremely high GC content. This may cause false positive results in samples containing salt.
- Probes detecting *PKD1* exon 1-31 rely for their specificity on a single nucleotide difference between the *PKD1* gene and its pseudogenes. For these probes, an apparent duplication can be the result of a clinically non-significant single nucleotide sequence change in one of the pseudogenes.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Related SALSA MLPA probemixes

P046 TSC2	Contains probes for the *TSC2* gene, involved in tuberous sclerosis complex.
P337 TSC2 Confirmation	Contains probes for the *TSC2* gene. Used as confirmation of P046 results.
P341/P342 PKDH1	Contains probes for the *PKHD1* gene, responsible for autosomal recessive polycystic kidney disease.

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P351 product history	
Version	*Modification*
D1	Two PKD1 probes have been added, and two PKD1 probes, one TSC2 probe and four reference probes have been replaced. In addition, three PKD1 probes have been changed in length.
C1	One target probe has been removed, and three reference probes have been replaced.
B2	The 88 and 96 nt control fragments have been replaced (QDX2).
B1	Three extra PKD1 probes have been included.
A1	First release.

P352 product history	
Version	*Modification*
E1	Three PKD1 probes and one PKD2 probe have been added, and one PKD2 probe and one reference probe have been replaced. In addition, one PKD1 probe and two PKD2 probes have been changed in length.
D1	One target probe has been removed, and two reference probes have been replaced.
C1	Four new PKD2 probes have been added and one PKD2 probe has been removed. QDX2 has replaced QDX fragments.
B1	Four extra PKD1 probes have been included.
A1	First release.

Implemented changes in the product description

Version D1/E1-01 – 24 August 2021 (04P)
- Product description completely rewritten and adapted to a new template.
- Product description adapted to new product versions (version numbers changed, changes in Table 1a, Table 1b, Table 2a and Table 2b).
- P351-D1 and P352-E1 are now CE marked.
- Warning added to Table 1a, Table 1b and Table 2a for SNPs influencing the signal of the 160 nt probe 10961-L31500 (P351-D1) and the 153 nt probe 14100-L24666 (P352-E1).
- Warning added Table 1b and Table 2 for sensitivity to experimental variation of the 191 nt probe 11011-L27419 (P352-E1) and the 248 nt probe 10983-L24667 (P352-E1).

Version C1/D1-03 – 03 March 2020 (02P)
- Product description rewritten and adapted to a new template.
- Ligation sites of the probes targeting the *TSC2* gene updated according to new version of the NM_ reference sequence.
- NM_ reference sequence version of the *PKD1* gene updated.

Version C1/D1-02 – 26 April 2019 (01P)
- Ligation sites of the probes targeting the *PKD2* gene updated according to new version of the NM_reference sequence (no change in exon numbering).
- Warnings added below Tables 1 and 2a for probes 10944-L24682, 10984-L24668 and 11009-L24671.
- References using probemixes P351/P352 were updated.

Version C1/D1-01 – 08 March 2018 (01P)
- Product description restructured and adapted to a new template.
- Various minor textual and layout changes.
- Exon numbering *TSC2* adjusted.

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