Product Description

SALSA® MLPA® Probemix P360-B2 Y-Chromosome

To be used with the MLPA General Protocol.

Version B2
For complete product history see page 13.

This SALSA MLPA probemix is for basic research and intended for experienced MLPA users only! This probemix is intended to quantify genes or chromosomal regions in which the occurrence of copy number changes is not yet well-established and the relationship between genotype and phenotype is not yet clear. Interpretation of results can be complicated. MRC Holland recommends thoroughly screening any available literature.

Catalogue numbers:

- **P360-025R:** SALSA MLPA Probemix P360 Y-Chromosome, 25 reactions.
- **P360-050R:** SALSA MLPA Probemix P360 Y-Chromosome, 50 reactions.
- **P360-100R:** SALSA MLPA Probemix P360 Y-Chromosome, 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.

General information
The SALSA MLPA Probemix P360 Y-Chromosome is a **research use only (RUO)** assay for the detection of deletions or duplications in the azoospermia factors (AZF) regions on the Y chromosome, which are associated with spermatogenetic failure in infertile men.

Microdeletions of the Y chromosome are the second most frequent genetic cause of spermatogenetic failure in infertile men after Klinefelter syndrome. These microdeletions are caused by intrachromosomal recombination events between large homologous repetitive sequence blocks (Foresta et al. 2001) and are clustered in three specific regions on the long arm of the Y chromosome, designated as azoospermia factors (AZF) loci: AZFa (~13.1-15.2 Mb from the p-telomere), AZFb (~18.5-22.8 Mb) and AZFc (~23.3-25.5 Mb) (Vogt et al. 1996). The most frequent type is the AZFc region deletion (~80%) followed by AZFa (0.5-4%), AZFb (1-5%) and AZFbc (1-3%) deletions (Krausz et al, 2014). Deletions which are detected as AZFabc are most likely related to abnormal karyotype such as 46,XX male or iso (Y) (Lange et al. 2009).

Inconsistent findings on the clinical effects of AZF-linked duplications (Giachini et al. 2008 and Lin et al. 2007), demonstrate the necessity of further research, for which at present, MLPA offers the most suitable technology.

This probemix contains probes for regions AZFa, AZFb and AZFc. The mix includes three probes which detect a sequence that is present three times across these regions and seven probes which detect a sequence that is present twice. See Table 1 for an overview of the probes and the number of sequences detected. Table 3 details the probes mapped to the AZF regions.

The best characterised AZFc deletion spans the whole AZFc region (about 3.5 Mb), however, partial AZFc deletions are also known. In a recent large study (Rozen et al. 2012) the frequency of four recurrent partial AZFc deletions was studied in five populations. It was found that partial (interstitial) AZFc deletions are more common among individuals with severe spermatogenic failure than in controls. However, it is important to note that certain partial AZFc deletions, such as the 1.6 Mb gr/gr deletion, are also present in high frequency in fertile men (Machev et al. 2004 and Hucklenbroich et al. 2005).

Finally, this probemix contains one probe detecting the *SRY* gene on the p arm of the Y chromosome and 12 reference probes that detect several different autosomal chromosomal locations.

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:
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/

Probemix content
The SALSA MLPA Probemix P360-B2 Y-Chromosome contains 55 MLPA probes with amplification products between 130 and 507 nucleotides (nt). This includes 43 probes spread over the relevant regions of the Y chromosome. In addition, 12 reference probes are included that detect 12 different autosomal chromosomal locations. 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).

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
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 spermatogenetic failure in infertile men. It is required to use male reference samples 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. The quality of cell lines can change; therefore samples should be validated before use.

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

Due to strong homology between large sequence blocks within AZFb and AZFc, some probes included in this probemix detect two or even three target sites (see Table 3). Data interpretation can be complicated and MRC Holland cannot offer further support. We strongly recommend using the P360-B2 data sheet that is available on request: info@mrcholland.com.

In this P360-B2 data sheet, all positions detected by the probes are arranged according to chromosomal location. Probes detecting two or three targets are included two or three times, respectively. For each probe, fill in the probe ratio as calculated by Coffalyser.Net. If a probe detects two or three target sequences, the probe value needs to be filled in for each target sequence separately. Due to the fact that some probes target two or even three targets, it can be difficult to determine which deleted target is actually causing a decreased signal. Using the data sheet can help to determine the possible deletion boundaries of a certain sample. Please see the *Example sheet* in the P360-B2 data sheet for more information.

Determination of microdeletions on the Y chromosome is often performed by the analysis of the presence or absence of a series of sequence-tagged sites (STSs). More information on Y chromosome microdeletions and common sequence-tagged sites (STSs) can be found in the EAA/EMQN best practice guidelines for molecular diagnosis of Y chromosomal microdeletions: state-of-the-art 2013 (Krausz et al. 2014) at http://www.ncbi.nlm.nih.gov/pubmed/24357628. To give an idea about where MLPA probes are located compared to the common STS markers, Tables 3, 6 and 7 also include the STS locations. To determine the exact location of the STS, we used the online database http://breakpointmapper.wi.mit.edu/, which provides regionally targeted catalogues of STSs.

The standard deviation of all probes in the reference samples should be ≤0.10 and the final ratio (FR) of the reference probes in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the number of detected sequences per individual probe (see Table 1) and their respective expected ratios for duplication or deletion status (see Table 4) can be used to interpret MLPA results.

- 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.

Limitations of the procedure

- 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.

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 P360-B2 Y-Chromosome

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)		Number of sequences detected
		Reference	Target region	
64-105	Control fragments – see table in probemix content section for more information			
130	Reference probe 19616-L26704	4p		
136	**EIF1AY probe** 11734-L12517		Yq11.222	1
142	**PPP1R12BP1 probe** 12733-L14796		Yq11.23	1
148	Reference probe 05170-L21820	13q		
154	Reference probe 14206-L16410	1p		
160	**CDY2B probe** 15236-L17486		Yq11.221	1
166	**BPY2 probe** 11739-L13811		Yq11.223	1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)		Number of sequences detected
		Reference	Target region	
172	Reference probe 10922-L25079	9q		
178	**BPY2 probe** 11740-L14251		Yq11.223	1
184	**VCY1B probe** 15238-L17485		Yq11.221	1
190	Reference probe 06378-L05844	6p		
196	Reference probe 08170-L08050	5q		
202	Reference probe 15519-L26914	16q		
208	**KDM5D probe** 11747-L12530		Yq11.222	1
215	**UTY probe** 11812-L13342		Yq11.21	1
220	**CDY2A probe** 20673-L18625		Yq11.221	1
227	**RPS24P1 probe** 15239-L18627		Yq11.21	1
234	**DDX3Y probe** 11816-L12611		Yq11.21	1
239	**CDY2A probe** 15245-L28543		Yq11.221	**3**
245	**KDM5D probe** 11754-L28544		Yq11.222	1
250	**ARSLP1 probe** 11818-L28545		Yq11.21	1
256	**USP9Y probe** 11821-L12616		Yq11.21	1
263	**RBMY1J probe** 11757-L28748		Yq11.223	1
267	**DAZ2 probe** 11758-L28912		Yq11.223	**2**
273	**KDM5D probe** 21365-L31361		Yq11.222	1
279	**SRY probe** 01023-L28750		Yp11.31	1
284	**DAZ2 probe** 12738-L14632		Yq11.223	**2**
291	**CDY2B probe** 11759-L28751		Yq11.222	**2**
295	Reference probe 03796-L20977	21q		
301	**DAZ2 probe** 11761-L28752		Yq11.223	**2**
308	**DDX3Y probe** 13061-L28753		Yq11.21	1
315	**RPS24P1 probe** 20393-L28553		Yq11.21	1
328	**UTY probe** 20392-L28932		Yq11.21	1
336	**USP9Y probe** 11826-L28756		Yq11.21	1
342	**VCY probe** 15243-L28903		Yq11.221	1
348	**UTY probe** 11828-L19232		Yq11.21	1
355	**VCY1B probe** 20394-L18629		Yq11.221	1
362	**CDY1B probe** 15246-L28757		Yq11.223	**2**
370	**USP9Y probe** 15244-L28758		Yq11.21	1
376	**BPY2 probe** 11768-L28759		Yq11.223	**3**
382	Reference probe 12558-L23858	11p		
391	**RBMY2DP probe** 15241-L12617		Yq11.23	1
398	**HSFY1 probe** 15247-L18630		Yq11.222	**2**
403	**VCY1B probe** 11852-L18631		Yq11.221	
410	**HSFY1 probe** 11772-L12555		Yq11.222	1
418	**BPY2 probe** 11773-L12556		Yq11.223	1
427	**RBMY1J probe** 11774-L28902		Yq11.223	1
436	**HSFY1 probe** 12740-L18632		Yq11.222	**2**
445	**KDM5D probe** 11776-L12559		Yq11.222	1
454	Reference probe 08274-L08153	8q		
463	**NLGN4Y probe** 11853-L12650		Yq11.221	1
471	Reference probe 00979-L21316	10p		
486	**BPY2 probe** 15248-L17487		Yq11.223	**3**
499	**EIF1AY probe** 15249-L28507		Yq11.222	1
507	Reference probe 14883-L28906	14q		

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. Reference sequences and gene synonyms

Gene name (according to HUGO)	Location	NCBI reference sequence	Alias gene names
ARSLP1	Yq11.21	NG_000880.5	*ARSEP, ARSEP1*
BPY2	Yq11.223	NM_004678.3 ‡	*BPY2A, VCY2A, VCY2*
CDY1B	Yq11.223	NM_001003894.2	*CDY*
CDY2A	Yq11.221	NM_004825.2	*CDY2*
CDY2B	Yq11.221	NM_001001722.2	*CDY*
DAZ2	Yq11.223	NM_020363.3	*PDP1678, MGC126442*
DDX3Y	Yq11.221	NM_004660.5 ‡	*DBY*
EIF1AY	Yq11.222	NM_004681.4	*EIF-4C*
HSFY1	Yq11.222	NM_152584.1	*HSFY, HSF2L*
KDM5D	Yq11.222	NM_001146705.2	*JARID1D, HYA, HY, SMCY*
NLGN4Y	Yq11.221	NM_014893.5	*KIAA0951, HNL4Y*
PPP1R12BP1	Yq11.23	NG_087935.1	*PPP1R12BP, PPP1R12BPY1*
RBMY1J	Yq11.223	NM_001006117.4	-
RBMY2DP	Yq11.23	NG_087929.1	*RBM, RBMY2*
RPS24P1	Yq11.21	NG_000893.5	*RPS24P*
SRY	Yp11.31	NM_003140.3 ‡	*TDF, SRXX1, SRXY1, TDY*
USP9Y	Yq11.21	NM_004654.4 ‡	*DFFRY, AZFA*
UTY	Yq11.21	NM_001258249.2	*UTY1, KDM6AL, KDM6C*
VCY	Yq11.221f	NM_004679.4 ‡	*BPY1, VCY1, VCY1A*
VCY1B	Yq11.221	NM_181880.2	*VCYB, BPY1B*

‡ These sequences are reference standards in the NCBI RefSeqGene project.

Note: Please notify us of any mistakes. The identity of the genes detected by the reference probes and the complete probe sequences are available on request: info@mlpa.com.

Table 3. P360 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Probe start (hg38)	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
279	SRY probe 01023-L28750	2787442	GCACTGAAAGCT-GTAACTCTAAGT	9418.8 kb
Start of AZFa region*				
315	**RPS24P1 probe** 20393-L28553	12206264	TTACAGAAGGTA-TGTCCTTGCACT	28.2 kb
	sY82 STS marker	12207374		
227	**RPS24P1 probe** 15239-L18627	12234480	TCCCTAGTGCTA-CTGCCTCACTTA	127.6 kb
	sY1064 STS marker	12321375		
250	**ARSLP1 probe** 11818-L28545	12362126	ACCTTCCCAGCA-AGCCGCCTTGAA	154.4 kb
	sY86 STS marker	12495696		
370	**USP9Y probe** 15244-L28758	12516542	ATCATGTGGCAT-TACCTCATTTGC	138.8 kb
	sY85 STS marker	12525880		
336	**USP9Y probe** 11826-L28756	12655385	GAAAGGCAAGGA-CTTTACCTTAAA	130.9 kb
	sY84 STS marker	12678104		
256	**USP9Y probe** 11821-L12616	12786240	TGGAGAAGGCAA-ACTTAGTCCACC	128.8 kb
	sY1324 STS marker	12790267		
	sY1316 STS marker	12791368		
	sY1714 STS marker	12859074		
234	**DDX3Y probe** 11816-L12611	12915001	AAGCAAAGAACA-TGTCAGTGACTA	2.1 kb
308	**DDX3Y probe** 13061-L28753	12917057	AGCCTTCACTCT-TGTTATTGCTTA	331.1 kb
	sY1065 STS marker	13110496		
	sY1182 STS marker	13112000		
End of AZFa region*				
348	**UTY probe** 11828-L19232	13248173	TGCATTATTGCA-GTACTTTCTTCA	54.7 kb

Length (nt)	SALSA MLPA probe	Probe start (hg38)	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
328 +	**UTY probe** 20392-L28932	13302888	ATTGGTCCAGGA-GATTGTGAATGG	301.5 kb
	sY88 STS marker	13492083		
215 +	**UTY probe** 11812-L13342	13604368	AGGATCCTGGAT-ATTCCACTACCA	159.0 kb
342	**VCY probe** 15243-L28903	13763364	TCCCTTTCTACA-CTTAGATCTCTG	161.4 kb
184	**VCY1B probe** 15238-L17485	13924779	GCATATTGAGTA-GATCATTCCTAG	153.7 kb
403	**VCY1B probe** 11852-L18631	14078518	CACGTTCGCTCA-GTTCTCACTGAT	159.0 kb
355	**VCY1B probe** 20394-L18629	14237473	GGAGTAGACCAA-GAGAGGAATATA	209.5 kb
463	**NLGN4Y probe** 11853-L12650	14447009	TTCTGCGTGGCA-TCACAGTCTTCC	3007.7 kb
	sY105 STS marker	17245407		
160	**CDY2B probe** 15236-L17486	17454741	TTCCAGCCAGGA-CACATCTGGAAA	5.1 kb
291 ⋕	**CDY2B probe** 11759-L28751	17459821	CTTCTGGCTGAA-CTGCGGCACCAA	492.7 kb
Start of AZFb region*				
220	**CDY2A probe** 20673-L18625	17952534	GGCTGTTAATGA-ATTCGTTAATGC	0.1 kb
239 φ	**CDY2A probe** 15245-L28543	17952663	ATTTTCTGTTAA-CCTTAGTGTAAA	1.8 kb
	sY1024 STS marker	17954445		
291 ⋕	**CDY2B probe** 11759-L28751	18446112	CTTCTGGCTGAA-CTGCGGCACCAA	970.7 kb
	sY1224 STS marker	18449738		
398 ⋕	**HSFY1 probe** 15247-L18630	18543186	ATTTGATGATGA-AGATTTAGCAGA	84.4 kb
	sY1967 STS marker copy 1	18590923		
436 ⋕	**HSFY1 probe** 12740-L18632	18627607	AAAGAACACATA-CCAATATAGCTG	21.1 kb
	sY1309 STS marker	18640156		
410	**HSFY1 probe** 11772-L12555	18648702	CTGGACTATGGA-TGCAACTTCCGA	80.3 kb
436 ⋕	**HSFY1 probe** 12740-L18632	18692719	AAAGAACACATA-CCAATATAGCTG	84.4 kb
	sY1967 STS marker copy 2	18729004		
398 ⋕	**HSFY1 probe** 15247-L18630	18777144	ATTTGATGATGA-AGATTTAGCAGA	555.3 kb
	sY121 STS marker	18890191		
	sY3199 STS marker	18978781		
245	**KDM5D probe** 11754-L28544	19332437	CCAACAAAGTCT-TACAATTATACT	587.9 kb
445	**KDM5D probe** 11776-L12559	19920317	CTGATTGGAGCA-CTCAGCCTAAAC	78.0 kb
208	**KDM5D probe** 11747-L12530	19998258	GACCAGGTTCAT-GCCAATATATTT	20.0 kb
273	**KDM5D probe** 21365-L31361	20017832	GATCTGAAGTTA-CTGATGAATCTG	390.7 kb
	sY127 STS marker	20408530		
499	**EIF1AY probe** 15249-L28507	20483261	ACTTTCTAAATG-TTCTTGAATGTA	465.4 kb
136	**EIF1AY probe** 11734-L12517	20507396	CCTGATTCTCCA-ATGGCTTCATAG	1771.9 kb
	sY1233 STS marker	20575614		
	sY134 STS marker	21394174		
	sY142 STS marker	21831727		
	sY143 STS marker	21831756		
	sY3010 STS marker	21860648		
427	**RBMY1J probe** 11774-L28902	22279283	TGGCAAATCCAT-AATATTACAACA	6.4 kb
263	**RBMY1J probe** 11757-L28748	22285703	TACAACCAGAGA-TAATGTAAATAG	441.2 kb
	sY2990 STS marker	22357096		
	sY1197 STS marker	22377470		
Start of AZFc region*				
	sY1192 STS marker	22726630		
418	**BPY2 probe** 11773-L12556	22726854	TTTACATGGTAA-ATTGATGTGCTT	0.5 kb
166	**BPY2 probe** 11739-L13811	22727392	TAGGAGAAAATA-ACAAAATAATGA	1.7 kb
178	**BPY2 probe** 11740-L14251	22729077	CACAGAAATATA-TACACTGTTTGA	42.6 kb
	sY1191 STS marker	22729472		
486 φ	**BPY2 probe** 15248-L17487	22771726	TCTTTGTATTCA-TGCCAAGAAACG	49.2 kb
376 φ	**BPY2 probe** 11768-L28759	22820917	TCATATGTCTGA-AGTCAGAACTTG	45.6 kb
	sY153 STS marker copy 1	22866497		
	sY254 STS marker copy 1	23170045		

Length (nt)	SALSA MLPA probe	Probe start (hg38)	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
	sY254 STS marker copy 2	23180885		
	sY1307 STS marker copy 1	23207970		
	sY1189 STS marker	23358832		
	sY1291 STS marker	23358922		
284 ǂ	**DAZ2 probe** 12738-L14632	23375997	GTTCAGCTGGCA-AGCTAGCTGTGC	98.0 kb
301 ǂ	**DAZ2 probe** 11761-L28752	23473986	AGTATATTCCCA-TTCCTAATAATG	242.0 kb
	sY1054 STS marker copy 1	23702572		
267 ǂ	**DAZ2 probe** 11758-L28912	23716012	CAGTGCTTCTGA-ATGATTTTCAGT	196.1 kb
	sY2858 STS marker copy 1	23718095		
	sY1742 STS marker copy 1	23719123		
362 ǂ	**CDY1B probe** 15246-L28757	23912085	CCTTACTGCTTA-AGGCCGTATTTC	193.1 kb
End of AZFb region*				
239 φ	**CDY2A probe** 15245-L28543	24105184	ATTTTCTGTTAA-CCTTAGTGTAAA	300.3 kb
	sY1206 STS marker	24380298		
486 φ	**BPY2 probe** 15248-L17487	24405469	TCTTTGTATTCA-TGCCAAGAAACG	49.2 kb
376 φ	**BPY2 probe** 11768-L28759	24454665	TCATATGTCTGA-AGTCAGAACTTG	760.7 kb
	sY153 STS marker copy 2	24500246		
	sY1307 STS marker copy 2	24822355		
	sY254 STS marker copy 3	24840815		
	sY254 STS marker copy 4	24851663		
376 φ	**BPY2 probe** 11768-L28759	25215367	TCATATGTCTGA-AGTCAGAACTTG	49.2 kb
486 φ	**BPY2 probe** 15248-L17487	25264573	TCTTTGTATTCA-TGCCAAGAAACG	300.3 kb
	sY1206 STS marker copy 1	25289446		
	sY255 STS marker copy 1	25374204		
239 φ	**CDY2A probe** 15245-L28543	25564875	ATTTTCTGTTAA-CCTTAGTGTAAA	193.1 kb
362 ǂ	**CDY1B probe** 15246-L28757	25757938	CCTTACTGCTTA-AGGCCGTATTTC	196.0 kb
267 ǂ	**DAZ2 probe** 11758-L28912	25953979	CAGTGCTTCTGA-ATGATTTTCAGT	242.6 kb
	sY1054 STS marker copy 2	25967161		
301 ǂ	**DAZ2 probe** 11761-L28752	26196541	AGTATATTCCCA-TTCCTAATAATG	98.0 kb
284 ǂ	**DAZ2 probe** 12738-L14632	26294558	GTTCAGCTGGCA-AGCTAGCTGTGC	41.1 kb
End of AZFc region*				
	sY1201 STS marker	26311168		
142	**PPP1R12BP probe** 12733-L14796	26335630	AGCATTTGGAGA-TGCTCCAGAAGA	88.4 kb
391	**RBMY2DP probe** 15241-L12617	26423998	CACTGAATGGAA-AAGTACAGCTGG	
	sY255 STS marker copy 2	26988591		
	sY255 STS marker copy 3	26999439		

* Please note that the borders of the AZF regions have not been described precisely to the nucleotide. The borders of the AZFb region are based on the "classic" AZFb deletion as defined by Vogt et al. (2021) and the extent to which the AZFb regions overlaps the AZFc region has been described differently before in other literature. The borders of the AZFc region are as described by Kuroda-Kawaguchi et al. (2001).

ǂ These probes detect 2 sequences (for a detailed explanation, please see Table 4a).

φ These probes detect 3 sequences (for a detailed explanation, please see Table 4b).

+ The 105 nt Y chromosome quality control fragment detects a sequence located 52 kb after the 328 nt probe and 250 kb before the 215 nt probe.

Note: Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

Table 4. Expected probe signals and ratios

Table 4a. Expected probe signals and ratios for probes detecting 2 sequences

PROBES detecting 2 sequences (Ħ)	Remaining signal	Decrease of signal	Expected ratio
Both targets present	100%	0%	1
Deletion of 1 target site; 1 target remaining	50%	50%	0.5
Deletion of 2 target sites; no target remaining	0%	100%	0

Table 4b. Expected probe signals and ratios for probes detecting 3 sequences

PROBES detecting 3 sequences (ω)	Remaining signal	Decrease of signal	Expected ratio
All 3 targets present	100%	0	1
Deletion of 1 target site; 2 targets remaining	66%	33%	0.66
Deletion of 2 target sites; 1 target remaining	33%	66%	0.33
Deletion of all 3 target sites; no target remaining	0	100%	0

Table 5. Partial AZFc deletions* (based on Rozen et al. and Shahid et al.)

AZFc deletion	STS markers deleted	STS markers <u>not</u> deleted	Deletion size	Frequency[1]	Risk increase for SSF[2]
b2/b3	sY1192 sY1191	sY142 sY1291	1.8 Mb	1:90	No increase
b1/b3	sY1192 sY1191 sY1189 sY1291 **sY254**: 2 copies deleted	sY142 sY1201 **sY254**: 2 copies remaining	1.6 Mb	1:994	2.5 ×
b2/b4	sY1192 sY1191 sY1189 sY1291 **sY254**: all 4 copies deleted	sY142 sY1201 **sY254**: 0 copies remaining	3.5 Mb	1:2320	145 ×
gr/gr	sY1189 sY1291 **sY254**: 2 copies deleted	sY142 sY1206 sY1191 **sY254**: 2 copies remaining	1.6 Mb	1:41	1.9 ×

* Be aware that not all findings are in concordance with Table 6.

1) Frequency in the total male population. The exact frequency of each deletion type varies per population.

2) SSF: Severe Spermatogenic Failure.

Table 6. Expected probe numbers in samples with partial AZFc deletions

Length (nt)	SALSA MLPA probe	Probe start (hg38)	Normal copy number	left: expected remaining copies per deletion type (based on table 5) right: graphical representation of expected extent of the deletion							
				b2/b3		b1/b3		b2/b4		gr/gr*	
	sY1192 STS marker	22726631		-		-		-		+	
418	**BPY2 probe** 11773-L12556	22726854	1	0		0		0		1	
166	**BPY2 probe** 11739-L13811	22727392	1	0		0		0		1	
178	**BPY2 probe** 11740-L14251	22729077	1	0		0		0		1	
	sY1191 STS marker	22729472		-		-		-		+	
486 ω	**BPY2 probe** 15248-L17487	22771726	3	3 or 2	?	2 or 1		1 or 0		2	
376 ω	**BPY2 probe** 11768-L28759	22820917	3	3 or 2	?	2 or 1		1 or 0		2	
	sY153 STS marker copy 1	22866497									
	sY254 STS marker copy 1	23170045				-		-			
	sY254 STS marker copy 2	23180885				-		-			
	sY1307 STS marker copy 1	23207970									
	sY1189 STS marker	23358832				-		-		-	
	sY1291 STS marker	23358922		+		-		-		-	
284 ⋕	**DAZ2 probe** 12738-L14632	23375997	2	2		1 or 2	?	0 or 1		1	
301 ⋕	**DAZ2 probe** 11761-L28752	23473986	2			1 or 2	?	0 or 2		1	
	sY1054 STS marker copy 1	23702572									
267 ⋕	**DAZ2 probe** 11758-L28912	23716012	2			1 or 2	?	0 or 1		1	
	sY2858 STS marker copy 1	23718095									
	sY1742 STS marker copy 1	23719123									
362 ⋕	**CDY1B probe** 15246-L28757	23912085	2			1 or 2	?	0 or 1		1	
239 ω	**CDY2A probe** 15245-L28543	24105184	3			2 or 3	?	1 or 2		2	
	sY1206 STS marker copy 1	24380298									
486 ω	**BPY2 probe** 15248-L17487	24405469	3			2 or 1	?	0 or 1		2	
376 ω	**BPY2 probe** 11768-L28759	24454665	3			2 or 1	?	0 or 1		2	
	sY153 STS marker copy 2	24500246									
	sY1307 STS marker copy 2	24822355									
	sY254 STS marker copy 3	24840815		+		+		-			
	sY254 STS marker copy 4	24851663		+		+		-			
376 ω	**BPY2 probe** 11768-L28759	25215367	3			2		0 or 1	?	2	
486 ω	**BPY2 probe** 15248-L17487	25264573	3			2		0 or 1	?	2	
	sY1206 STS marker copy 2	25289446								+	
	sY255 STS marker copy 1	25374204									
239 ω	**CDY2A probe** 15245-L28543	25564875	3			2 or 3		1 or 2	?	2	
362 ⋕	**CDY1B probe** 15246-L28757	25757938	2			1 or 2		0 or 1	?	1	
267 ⋕	**DAZ2 probe** 11758-L28912	25953979	2			1 or 2		0 or 1	?	1	
	sY1054 STS marker copy 2	25967161									
301 ⋕	**DAZ2 probe** 11761-L28752	26196541	2			1 or 2		0 or 1	?	1	
284 ⋕	**DAZ2 probe** 12738-L14632	26294558	2			1 or 2		0 or 1	?	1	
	sY1201 STS marker	26311168				+		+		+	
142	**PPP1R12BP probe** 12733-L14796	26335630	1			1		1		1	
391	**RBMY2DP probe** 15241-L12617	26423998	1			1		1		1	
	sY255 STS marker copy 2	26988591									
	sY255 STS marker copy 3	26999439									

⋕ These probes detect 2 sequences (for a detailed explanation, please see table 4a).

ω These probes detect 3 sequences (for a detailed explanation, please see table 4b).

* The location of the gr/gr deletion has been confirmed by testing several samples carrying this specific deletion. Please find the normalised results of one example in table 7.

———— Solid line: represents the area that is certainly absent in this deletion type.

? question mark: represents an area of which it is not clear if it is absent or present in this deletion type. (No empirical values are available.)

- - - - - - - - - - Dotted line: represents an area that is also missing in this deletion type, but of which the exact location cannot be established with certainty because there are two identical repeat areas flanking the solid line, either of which could be the deleted area.

\+ An STS marker with a plus sign is expected to be present in this deletion type based on the results in table 5.

\- An STS marker with a minus sign is expected to be absent in this deletion type based on the results in table 5.

Table 7. Normalised results of a positive sample carrying a gr/gr deletion[1]

Length (nt)	SALSA MLPA probe	Probe start (hg38)	Remaining probe ratios with a gr/gr deletion		
			Empirical value	The extent of the gr/gr deletion	Expected remaining probe value, see also Table 6
427	RBMY1J probe 11774-L28902	22279283	~1		1
263	RBMY1J probe 11757-L28748	22285703	~1		1
	sY2990 STS marker	22357096			
	sY1197 STS marker	22377470			
AZFc region:					
	sY1192 STS marker	22726630			**+**
418	**BPY2 probe** 11773-L12556	22726854	**~1**		**1**
166	**BPY2 probe** 11739-L13811	22727392	**~1**		**1**
178	**BPY2 probe** 11740-L14251	22729077	**~1**		**1**
	sY1191 STS marker	22729472			**+**
486 φ	**BPY2 probe** 15248-L17487	22771726	**0.64**		**0.66**
376 φ	**BPY2 probe** 11768-L28759	22820917	**0.68**		**0.66**
	sY153 STS marker copy 1	22866497			
	sY254 STS marker copy 1	23170045			
	sY254 STS marker copy 2	23180885			
	sY1307 STS marker copy 1	23207970			
	sY1189 STS marker	23358832			**-**
	sY1291 STS marker	23358922			**-**
284 Ħ	**DAZ2 probe** 12738-L14632	23375997	**0.54**		**0.5**
301 Ħ	**DAZ2 probe** 11761-L28752	23473986	**0.5**		**0.5**
	sY1054 STS marker copy 1	23702572			
267 Ħ	**DAZ2 probe** 11758-L28912	23716012	**0.48**		**0.5**
	sY2858 STS marker copy 1	23718095			
	sY1742 STS marker copy 1	23719123			
362 Ħ	**CDY1B probe** 15246-L28757	23912085	**0.49**		**0.5**
239 φ	**CDY2A probe** 15245-L28543	24105184	**0.75**		**0.66**
	sY1206 STS marker copy 1	24380298			
486 φ	**BPY2 probe** 15248-L17487	24405469	**0.64**		**0.66**
376 φ	**BPY2 probe** 11768-L28759	24454665	**0.68**		**0.66**
	sY153 STS marker copy 2	24500246			
	sY1307 STS marker copy 2	24822355			
	sY254 STS marker copy 3	24840815			
	sY254 STS marker copy 4	24851663			
376 φ	**BPY2 probe** 11768-L28759	25215367	**0.68**		**0.66**
486 φ	**BPY2 probe** 15248-L17487	25264573	**0.64**		**0.66**
	sY1206 STS marker copy 2	25289446			**+**
	sY255 STS marker copy 1	25374204			

Length (nt)	SALSA MLPA probe	Probe start (hg38)	Remaining probe ratios with a gr/gr deletion		
			Empirical value	The extent of the gr/gr deletion	Expected remaining probe value, see also Table 6
239 Φ	**CDY2A probe** 15245-L28543	25564875	**0.75**		**0.66**
362 Ħ	**CDY1B probe** 15246-L28757	25757938	**0.48**		**0.5**
267 Ħ	**DAZ2 probe** 11758-L28912	25953979	**0.48**		**0.5**
	sY1054 STS marker copy 2	25967161			
301 Ħ	**DAZ2 probe** 11761-L28752	26196541	**0.5**		**0.5**
284 Ħ	**DAZ2 probe** 12738-L14632	26294558	**0.54**		**0.5**
	sY1201 STS marker	26311168			**+**
142	**PPP1R12BP probe** 12733-L14796	26335630	**~1**		**+**
391	**RBMY2DP probe** 15241-L12617	26423998	**~1**		**+**

[1)] Data courtesy of David J. Bunyan, Salisbury NHS Foundation Trust, UK. This data is based on a sample analysed with MLPA probemix P360-B1 Y-Chromosome.

Solid line: represents the area that is certainly absent in this deletion type.

Dotted line: represents an area that is also missing in this deletion type, but of which the exact location cannot be established with certainty because there are two identical repeat areas flanking the solid line, either of which could be the deleted area.

References

- Foresta C et al. (2001). Y chromosome microdeletions and alterations of spermatogenesis. *Endocr Rev*. 22:226-39.
- Giachini C et al. (2008). Partial AZFc deletions and duplications: clinical correlates in the Italian population. *Hum Genet*. 124:399-410.
- Hucklenbroich K et al. (2005). Partial deletions in the AZFc region of the Y chromosome occur in men with impaired as well as normal spermatogenesis. *Hum Reprod*. 20:191-7.
- Krausz C et al. (2014). EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions: state-of-the-art 2013. *Andrology*. 2:5-19.
- Kuroda-Kawaquchi T et al. (2001). The AZFc region of the Y chromosome features massive palindromes and uniform recurrent deletions in infertile men. *Nat Genet*. 29:279-86.
- Lange J et al. (2008). MSY Breakpoint Mapper, a database of sequence-tagged sites useful in defining naturally occurring deletions in the human Y chromosome. *Nucleic Acids Res*. 36:D809-14. (The breakpoint mapper can be found here: http://breakpointmapper.wi.mit.edu/)
- Lin YW et al. (2007). Partial duplication at AZFc on the Y chromosome is a risk factor for impaired spermatogenesis in Han Chinese in Taiwan. *Hum Mutat*. 28:486-94.
- Machev N et al. (2004). Sequence family variant loss from the AZFc interval of the human Y chromosome, but not gene copy loss, is strongly associated with male infertility. *J Med Genet*. 41:814-25.
- Rozen SG et al. (2012). AZFc deletions and spermatogenic failure: a population-based survey of 20,000 Y chromosomes. *Am J Hum Genet*. 91:890-6.
- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res*. 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat*. 28:205.
- Shahid M et al. (2011). Association of Y-chromosome subdeletion gr/gr with the prevalence of Y-chromosome haplogroups in infertile patients. *Eur J Hum Genet*. 19:23-9.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem*. 421:799-801.
- Vogt PH et al. (1996). Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Hum Mol Genet*. 5:933-43.

- Vogt PH et al. (2021). Human AZFb deletions cause distinct testicular pathologies depending on their extensions in Yq11 and the Y haplogroup: new cases and review of literature. *Cell Biosci*. 11:1-13.

Selected publications using SALSA MLPA Probemix P360 Y-Chromosome

- Bunyan DJ et al. (2012). Detection of partial deletions of Y-chromosome AZFc in infertile men using the multiplex ligation-dependent probe amplification assay. *J Reprod Infertil*. 13:174-8.
- Franchim CS et al. (2020). Efficacy of MLPA for detection of Y-chromosome microdeletions in infertile Brazilian patients. *J Assist Reprod Genet*. 37:1251-1259.
- Katsumi M et al. (2014). Microhomology-mediated microduplication in the Y chromosomal azoospermia factor a region in a male with mild asthenozoospermia. *Cytogenet Genome Res*. 144:285-9.
- Liu XH et al. (2014). ART do not increase the risk of Y-chromosome microdeletion in 19 candidate genes at AZF regions. *Reprod Fertil Dev*. 26:778-86.
- Nailwal M et al. (2017). Azoospermia factor C subregion of the Y chromosome. *J Hum Reprod Sci*. 10:256-60.
- Ogiwara Y et al. (2021). Structural and numerical Y chromosomal variations in elderly men identified through multiplex ligation-dependent probe amplification. *J Hum Genet*. 66:1181-1184.
- Saito K et al. (2015). Copy-number variations in Y-chromosomal azoospermia factor regions identified by multiplex ligation-dependent probe amplification. *J Hum Gen.* 60:127-31.
- Vučić N et al. (2022). Copy number variants within AZF region of Y chromosome and their association with idiopathic male infertility in Serbian population. *Andrologia*. 54:e14297.
- Zhou R et al. (2019). Identifying novel copy number variants in azoospermia factor regions and evaluating their effects on spermatogenic impairment. *Front Genet*. 10:427.

P360 product history	
Version	*Modification*
B2	One probe length has been adjusted.
B1	DPY19L2 probes and one probe for *RBMY2CP* and *KDM5D* removed. One USP9Y probe and 6 reference probes replaced. DDX3Y probes and four reference probes added. Several probes changed in length.
A1	First release.

Implemented changes in the product description

Version B2-02 – 30 November 2022 (04P)
- Product description rewritten and adapted to a new template.
- Updated General information.
- Remarks removed from Table 1.
- Gene names updated: ARSEP updated to ARSLP1, BPY1 updated to VCY, changes to aliases in Table 2.
- NM_ reference sequences updated in Table 2 and remark removed for some genes.
- AZF region borders updated in Table 3.
- Added remark to Table 5.
- Changes to Table 6: copy numbers only kept for probes.
- More selected publications added.
- Name of probemix P360 in this product description has been adjusted from P360 Y-Chromosome Microdeletions to P360 Y-Chromosome.

Version B2-01 – 20 March 2019 (01P)
- Product description restructured and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1, 2 and 3s).
- Warning added for probes: 215 nt (11812-L13342); 362 nt (15246-L28757); 445 nt (11776-L12559); 499 nt (15249-L28507). These probes may be more sensitive to experimental conditions. Aberrant results should be treated with caution.

Version 10 – 06 April 2018 (55) - RBMY2CP has been removed from table 2. - Various minor textual changes.

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