

## Finding the genetic cause of mental retardation with MLPA

The number of genes whose defect can result in mental retardation is very large. In some cases, particular phenotypic features suggest the involvement of a specific gene or chromosomal region. Numerous SALSA MLPA kits are available to find the cause of mental retardation with distinct (syndromic) features, such as RETT syndrome, Sotos syndrome and Prader Willi.

Unfortunately, for patients suffering from non-syndromic mental retardation, the genetic cause is found only in a minority of cases. Usually, primary screening of such patients is done by karyotyping or G-banding. When no abnormality is detected by these methods, we suggest screening the patients with the following two SALSA MLPA kits (see figure 1):

- SALSA MLPA kit *P245 Microdeletion syndromes* contains probes for 21 different microdeletion syndromes causing mental retardation. For more information, please see the P245 product description.
- SALSA MLPA kit *P036 Human telomere* contains one probe for each subtelomeric region and is designed to detect deletions/duplications of each subtelomeric region. Several studies have indicated that 3-8 % (see references p.2) of all cases of mental retardation is caused by aberrant copy numbers of subtelomeric regions.

### SALSA MLPA kit P245 Microdeletion syndromes

In case an abnormality is found with the SALSA MLPA kit *P245 Microdeletion syndromes*, we recommend further investigation of the deletion or duplication with one of the microdeletion follow-up kits (see the P245 product description for an overview).

### SALSA MLPA kit P036 Human telomere

The detection of abnormal copy numbers in subtelomeric regions is very complicated. As compared to other regions of the genome, these regions are rich in sequences with variable copy numbers. Copy number changes of sequences within these regions can also occur in unaffected individuals and the effect of a deletion or duplication will depend on the genes present in the affected region. Please note that abnormalities detected by our subtelomeric probemixes will therefore not always be the cause of the mental retardation.

When used correctly, MLPA will exclude the presence of abnormal copy numbers of subtelomeric regions in the majority of samples. In case an abnormality is detected by P036, there are three ways of confirming the result:

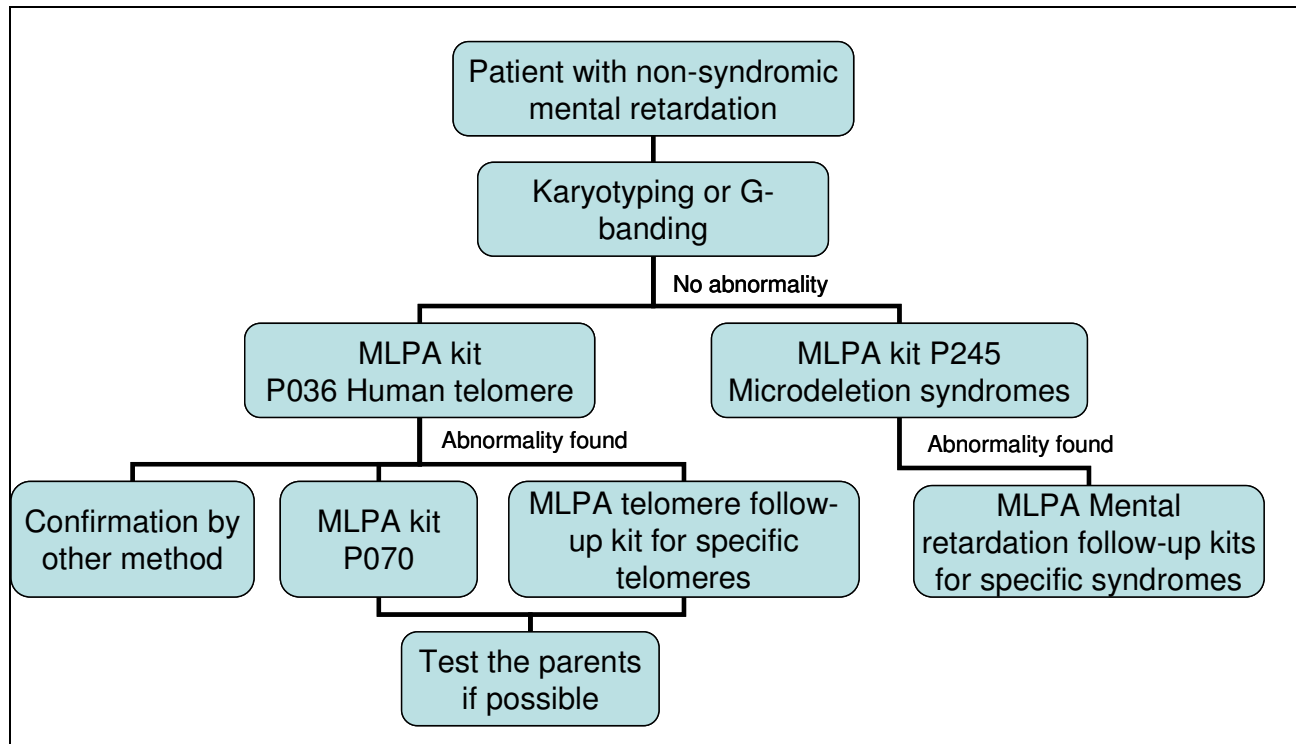
1. Using a SALSA MLPA telomere follow-up kit. Follow-up kits contain more probes per telomere (see table below) and are suitable to examine a specific region more closely.
2. Using SALSA MLPA kit *P069* or *P070 Human telomere* for independent confirmation of the results. Since these probemixes also contain one probe for each subtelomere, they offer a broad subtelomeric screening similar to the P036.
3. Confirmation by another method such as FISH.

Which option to choose depends on the resources available. The advantage of option 2 (broad screening with P069 or P070) is that a single probemix suffices to confirm all P036 probes. However, generally speaking, option 1 will offer the best screening. In most cases where you will find an abnormality with the P036, it will be a single probe that is affected. Using one of the detailed follow-up kits will not only allow you to confirm the actual presence of the aberration, but also to determine the length of a possible deletion/duplication. To make a more detailed screening of all chromosomes easier, a telomere starter kit containing 25 reactions of each of the 12 telomere follow-up kits will be released mid 2008. Also, it is always possible to order 25 reactions probemix only of a follow-up kit (see the overview on the next page).

For all abnormalities detected, we strongly recommend testing the patient's parents to determine whether a copy number aberration found in the patient is truly de novo. De novo deletions or duplications have a high probability of being the cause of the mental retardation. Please be aware that a considerable number of abnormalities detected by a single probe may not have a phenotypic effect, but can be due to a rare polymorphism or a copy number change which is also present in one of the parents. For some chromosome

arms, even a large subtelomeric deletion of more than 1 Mb can be inherited without a phenotypic effect. Some examples are given on the next page.

No method will be capable of detecting all chromosomal aberrations. MLPA will not detect inversions or balanced translocations. Frequent microdeletion syndromes can be detected with the P245 probemix but many rare interstitial deletions will not be detected.



**Figure 1:** Flow scheme suggesting how to test a patient with non-syndromic mental retardation.

### Probes in P036 and P070 MLPA probemixes

Most probes in the P036 and P070 probemixes target well-characterized genes that are located very close to the telomere. The exceptions are the probes for the p-arm of chromosomes 13, 14, 15, 21 and 22, as large parts of these arms are covered by >10 Mb of repeat sequences. For these chromosomes, the probe recognition sequence is therefore situated in one of the first genes following this region (on the q arm, close to centromere).

The development of a perfect MLPA assay for the detection of subtelomeric deletions and duplications is complicated. Genes that are located very close to the telomeres have a higher chance of being polymorphic in copy number (i.e. duplicated/deleted in a small percentage of unaffected individuals, sometimes only in certain populations), whereas situating probes at a larger distance from the telomere may lead to false negatives. Based on feedback from customers, we will try to further improve this P036-D probemix. New versions are therefore expected in the future.

### More specific subtelomeric analysis

For a closer examination of samples in which aberrant copy numbers have been found, the following MLPA kits are available:

Subtelomeric region	SALSA MLPA kit
1p (36 probes)	P147 1p36
2p (13 probes)	P208 telo-6
3p (11 probes)	P208 telo-6
4p (16 probes)	P096 MR2
5p (6 probes)	P096 MR2
6p (12 probes)	P208 telo-6
8p (12 probes)	P208 telo-6
9p (12 probes)	P230 telo-7
10p (11 probes)	P230 telo-7
11p (7 probes)	P230 telo-7
12p (11 probes)	P230 telo-7
"13p" (18 probes)	P163 GJB
"15p" (25 probes)	ME028 PWS/AS
16p (24 probes)	P140 HBA
17p (15 probes)	P249 telo-8
18p (8 probes)	P249 telo-8
19p (9 probes)	P249 telo-8
20p (13 probes)	P249 telo-8
"22p" (30 probes)	P250 DiGeorge

Subtelomeric region	SALSA MLPA kit
1q	P264 telo-9*
2q	P264 telo-9*
3q	P264 telo-9*
4q	P264 telo-9*
5q	P277 telo-10*
6q	P277 telo-10*
7q	P277 telo-10*
8q	P277 telo-10*
9q (13 probes)	P286 telo-11
10q (8 probes)	P286 telo-11
11q (14 probes)	P286 telo-11
12q (9 probes)	P286 telo-11
13q	P291 telo-12*
14q	P291 telo-12*
15q	P291 telo-12*
16q	P291 telo-12*
17q	P320 telo-13*
18q	P320 telo-13*
19q	P320 telo-13*
20q	P320 telo-13*
22q (37 probes)	P188 22q13

\* Expected to be available in the second quarter of 2008

More SALSA MLPA kits for subtelomeric specific analysis are in development.

### Notes on specific telomeres:

- The P036-D control fragments for the **Y-chromosome** (106 and 118 nt) are synthetic probes (the longer probes are "phage M13-derived"). These small fragments seem to have a higher variability in peak area than the other probes and are not close to the Y telomeres. The 298 and 482 probes detect sequences located on both X and Y chromosome close to the telomeres and will thus indicate the combined copy number of X and Y.
- The P036-C/-D and P069 and P070 probes for **11p**, **20p**, and **5q**, detect sequences that have been found to be duplicated in sporadic cases in a normal individual (**11p**: M. Palomares Bralo, Madrid; **20p**: V. Biancalana; Strasbourg; **5q**: K. Mann, London + L. Rooms & S. Seneca, Antwerpen; all personal communication). As deletions of these regions might well have consequences, we will not replace these probes.
- The CAB45 probe for **1p36** which is present in the P036-B version was found to be deleted or duplicated in some healthy families. This CAB45 probe has been replaced in P036-D by a probe for the SCNN1D gene. Although it was tested on a large number of samples in our lab with good results, this probe seems to give variable results in several labs. This 1p probe for the SCNN1D gene will be replaced in the near future.
- The P036-C/D **probe for 2p** has been found to be deleted in several healthy persons in Nijmegen (Erik Sistermans, personal communication.) and Maastricht. We do not know whether this is due to a polymorphism in the Dutch population. In case deletions in healthy persons also occur elsewhere, this probe will be replaced in the future. Please inform us on aberrant results.
- The P036-C/D and P069/P070 **probes for 3p** (CHL1 gene) were found to be duplicated in healthy parents by Joo Wook Ahn (Guy's London) and Eric Sistermans, Nijmegen. In another family, a deletion of the P036-C and P069-P070 probes for 3p was detected in a healthy parent (Joo Wook Ahn, London). According to Dijkhuizen et al (2006; Am.J.Hum.Genet. 140A, 2482-87), defects in the more centromeric CNTN4 and CRBN genes might be more important for the 3p syndrome than CHL1 loss. We recommend the use of the P208 probemix for further analysis of 3p deletions / duplications.
- The P036-C/D **probe for 3q** (BDH gene, 322 nt) was found to be duplicated in a healthy parent by Edwin Reyniers (Antwerpen). This was confirmed by FISH. The P070 probe for 3q, which is located 200 Kb closer to the telomere, did not show this duplication. Apparently, copy number polymorphisms of the BDH gene region do occur in healthy individuals.

- A **3q29** microdeletion syndrome has been described recently: Willatt, L. et al *A. J. Hum. Genet.* 77: 154-160, 2005. Although this syndrome is due to an interstitial deletion, the commonly deleted region is located very close to the 3q telomere. The probe in this P036-D probemix detects a sequence which is within the commonly deleted region. The 3q probe in P070 will not detect this microdeletion. The sequence detected by this P070 probe is located between this interstitial deletion region and the telomere.
- P036 **probe for 4q** (FRG1 gene): The 4q FRG1 probes in P036-B and P036-C are not reliable, due to the presence of SNPs in the hybridising sequence of these probes, which appear to be relatively frequent in some populations. The 4q FRG1 probe in P036-D performed well at MRC-Holland but we have already received reports of high frequency false positive results in several populations. The 4q telomeric region is complicated and very few genes are present. The FRG1 gene is the only well characterized gene in the terminal 2-3 Mb of 4q. As deletions of more than 4 Mb of the telomeric 4q region do not seem to have phenotypic consequences, we will replace this probe by a probe for the FAT gene in the near future. **In case an apparent deletion of 4q by P036 is not confirmed by P069/P070, we recommend to take no further action.** We expect to have a probemix with additional 4q probes available in the second quarter of 2008.
- The P036C/D **probe for 5q** was found to be influenced in one patient by a polymorphism in the first nucleotide after the ligation site by Kathlen Franke (Dresden). Please report similar findings. Probes that are reported more often to be influenced by polymorphisms will be replaced.
- The P036-B **probe for 9q** was found to give variable results by two laboratories. It has been replaced by an EHMT1 probe (closer to the telomere) in the P036-C/D probemix.
- The "**13p**", "**14p**", "**15p**", "**21p**" and "**22p**" **probes** detect sequences that are in the q-arm, close to the centromere. The p-arms of these chromosomes do not contain well characterized genes.
- The P036-C/D **probe for "15p"** (MKRN3 gene, 234 nt) detects a sequence which is close to the centromere on the 15q arm. This region is deleted in **some** Prader-Willi/Angelman syndrome patients. The PWS/AS critical region (SNRPN gene) is located at a distance of 1.3 Mb towards the q-telomere. We recommend to use the ME028 probemix in order to further characterize copy number changes of the MKRN3 region.
- The sequence detected by the P036-C/D **probes for 22q** has only one mismatch with a related sequence on chromosome 2. Sufficient for us to generate a chr. 22q specific MLPA signal. However, when this region is to be sequenced, primer design is complicated. Only 4 mismatches are present in the 350 nt region that contains this probe sequence. The P188 probe set contains many probes close to the 22q13 telomere and can be used to confirm or further characterize 22q13 deletions.

Please notify us of any mistakes: [info@mlpa.com](mailto:info@mlpa.com). All sequences are available on request: [info@mlpa.com](mailto:info@mlpa.com).