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정신지체의 원인인 여러 미세결실증후군의 선별검사로 이용되는 Multiplex Ligation-Dependent Probe Amplification로 형광동소교잡법의 비교 평가

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Comparing Two Diagnostic Laboratory Tests for Several Microdeletions Causing Mental Retardation Syndromes: Multiplex Ligation-Dependent Amplification vs Fluorescent *In Situ* Hybridization

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Background : Microdeletion syndromes not detectable by conventional cytogenetic analysis have been reported to occur in approximately 5% of patients with unexplained mental retardation (MR). Therefore, it is essential to ensure that patients with MR are screened for these microdeletion syndromes. Mental retardation syndrome multiplex ligation-dependent probe amplification (MRS-MLPA) is a new technique for measuring sequence dosages that allows for the detection of copy number changes of several microdeletion syndromes (1p36 deletion syndrome, Williams syndrome, Smith-Magenis syndrome, Miller-Dieker syndrome, DiGeorge syndrome, Prader-Willi/Angelman syndrome, Alagille syndrome, Saethre-Chotzen syndrome, and Sotos syndrome) to be processed simultaneously, thus significantly reducing the amount of laboratory work.

Methods : We assessed the performance of MLPA (MRC-Holland, The Netherlands) for the detection of microdeletion syndromes by comparing the results with those generated using FISH assays. MLPA analysis was carried out on 12 patients with microdeletion confirmed by FISH (three DiGeorge syndrome, four Williams syndrome, four Prader-Willi syndrome, and one Miller-Dieker syndrome).

Results : The results of MLPA analysis showed a complete concordance with FISH in 12 patients with microdeletion syndromes.

Conclusions : On the basis of these results, we conclude that MLPA is an accurate, reliable, and cost-effective alternative to FISH in the screening for microdeletion syndromes. *(Korean J Lab Med 2009;29:71-6)*

Key Words : *Prader-Willi syndrome, DiGeorge syndrome, Williams syndrome, Miller-Dieker syndrome*

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INTRODUCTION

Microdeletion syndromes, which are not visible by conventional cytogenetic analysis, have been reported to occur in approximately 5% of patients with unexplained mental retardation (MR) [1, 2]. FISH, which has been used as a com-

plementary cytogenetic method, has an established role in the diagnosis of unsolved cases of MR and multiple anomalies. The ability of FISH to detect cryptic chromosomal rearrangements exceeds the resolution of the usual cytogenetic banding techniques. However, FISH is still an expensive and labor-intensive assay, requiring practitioners to anticipate a specific microdeletion syndrome in order to determine what kind of FISH testing is needed for patients.

Multiplex ligation-dependent probe amplification (MLPA; MRC-Holland, Amsterdam, The Netherlands) is a new technique for the detection of deletions and/or duplications in various genetic diseases [3, 4]. For microdeletion syndromes, the MLPA kit allows the detection of copy number changes of several chromosomal regions (1p36, 7q11.23, 17p11.2, 17 p13.3, 22q11.21, 15q11.2, 20p12, 7p21.1, and 5q35.3). Thus, MLPA can significantly shorten the process by investigating various chromosomal regions at the same time. In this study, we introduced MLPA as a diagnostic test and then evaluated its performance compared to FISH.

MATERIALS AND METHODS

1. Materials

We tested 12 patients with microdeletion syndromes confirmed by FISH: three cases of DiGeorge syndrome, four cases of Williams syndrome, four cases of Prader-Willi/Angelman syndrome, and one case of Miller-Dieker syndrome.

2. Methods

DNA was extracted from whole blood using a QIAamp DNA Mini Kit (Qiagene, Hilden, Germany), according to the manufacturers' instruction. Extracted DNA was first denatured and hybridized with MLPA probes for 12 hr, then ligation reaction and PCR were performed. The MLPA probe, P064B MR1, is composed of 43 probes and 5 control fragments. 43 probes consist of 7 probes for 1p36 deletion syndrome, 6 probes for Williams syndrome, 5 probes for Smith- Magenis syndrome, 7 probes for Miller-Dieker syndrome, 6 probes for DiGeorge syndrome, 5 probes for Prader-Willi/Angelman syndrome, 2 probes for Alagille syndrome, 3 probes for Sotos syndrome, and 2 probes for Saethre-Chotzen syndrome (Table 1). The PCR products were analyzed by a 3130 *xl* ABI sequencer (Applied Biosystems, Foster City, CA, USA). Analysis was done using Genemarker, version 1.6 software (Softgenetics, State College, PA, USA). The ratio of the probes' peak heights was determined by comparing the probes' peak heights obtained on the patient samples to those obtained on the two normal control samples. FISH was done per the manufacturer's instructions using specific microdeletion probes of Qbiogene (MP Biomedicals, Solon, OH, USA) and Vysis (Abbott, Downers Grove, IL, USA). The probes used in the FISH study were DiGeorge TUPLE region probe/22qter control (Qbiogene), PW/AS SNRPN region probe/PML control (Qbiogene), Williams-Beuren critical region probe (Qbiogene), and LSI LIS1 Miller-Dieker microdeletion probe (Vysis; Fig. 1).

Table 1. Characteristics of MLPA probes used for detection of several microdeletion syndromes

Microdeletion syndrome	Chromosomal position	No. of probes	Target genes
1p36 deletion syndrome	1p36.33	7	*TNFRSF18, TNFRSF4, SCNN1D, GNB1, SKI, FLJ10782, TP73*
Williams syndrome	7q11.23	6	*FZD9, STX1A, ELN, LIMK1, CYLN2*
Smith-Magenis syndrome	17p11.2	5	*TACI/TNFRSF13B, LRRC48, LLGL1, PRPSAP2, MFAP4*
Miller-Dieker syndrome	17p13.3	7	*HIC1, METT10D, PAFAH1B1, ASPA, TRPV1*
DiGeorge syndrome	22q11.21	6	*CTCL1, CDC45L, CLDN5, ARVCF, FLJ14360, SNAP29*
Prader-Willi /Angelman syndrome	15q11.2	5	*MKRN3, NDN, UBE3A, GABRB3*
Alagille syndrome	20p12.2	2	*JAG1*
Saethre-Chotzen syndrome	7p21.2	2	*TWIST, TWISTNB*
Sotos syndrome	5q35.3	3	*NSD1* exon4, *NSD1* exon12, *NSD1* exon 17

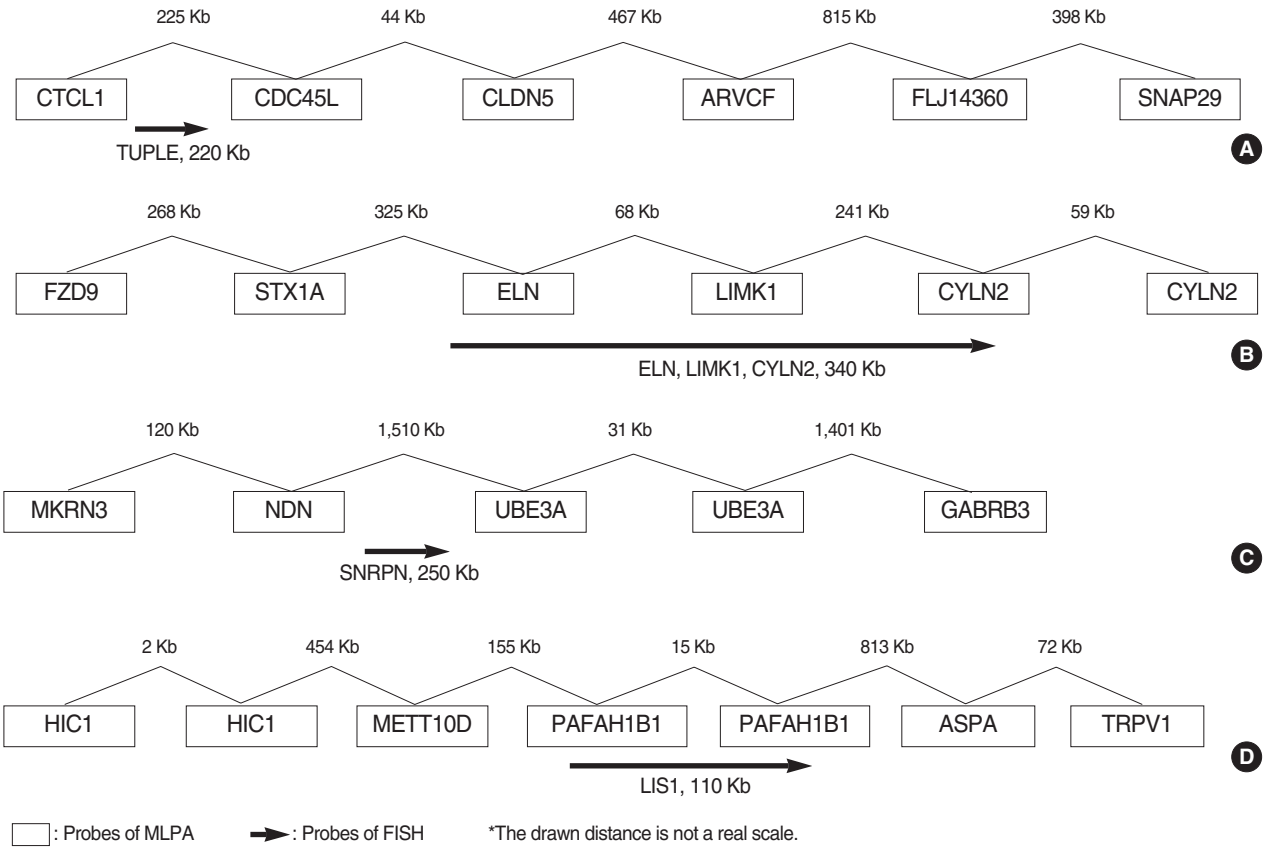

Fig. 1. The positions of FISH probes compared to MLPA probes. (A) DiGeorge syndrome, (B) Williams syndrome, (C) Prader-Willi/Angelman syndrome, (D) Miller-Dieker syndrome.
Abbreviation: MLPA, multiplex ligand-dependent probe amplification.

RESULTS

MLPA showed a perfect concordance with the FISH results. The deleted genes were *CTCL1*, *CDC45L*, *CLDN5*, *ARVCF*, *FLJ14360*, and *SNAP29* for DiGeorge syndrome; *FZD9*, *STX1A*, *ELN*, *LIMK1*, and *CYLN2* for Williams syndrome; *MKRN3*, *NDN*, *UBE3A*, and *GABRB3* for Prader-Willi syndrome; and *HIC1*, *METT10D*, *PAFAH1B1*, *ASPA*, and *TRPV1* for Miller-Dieker syndrome. *SNAP29*, which was the most telomeric gene among the genes investigated in DiGeorge syndrome, was not deleted in one patient with DiGeorge syndrome. In MLPA, the peak heights of the deleted genes were 40-60% of those of normal controls (Fig. 2).

DISCUSSION

Mental retardation affects approximately 2% to 3% of the population [5, 6]. But the underlying cause of MR is determined in only about 20% of patients [2, 5]. Our poor understanding of its origins impedes the provision of effective treatments, preventive measures, and adequate genetic counseling.

As chromosomal aberrations are the most common known cause of MR, several new methods based on FISH, PCR, and array techniques have been developed over recent years. According to Rauch et al., who analyzed the diagnostic yield of various genetic approaches in 1,170 patients with unexplained developmental delay or MR, a conventional cytogenetic diagnosis was made in 16%, a microdeletion syndrome in 5.3%, and subtelomeric screening revealed 1.3% of the causes [5]. Therefore, targeted FISH analysis and subtelomeric FISH screening identified chromosomal abnormalities in an additional 6.6% of causes. Targeted analysis would be more helpful for those patients who are easily missed by clin-

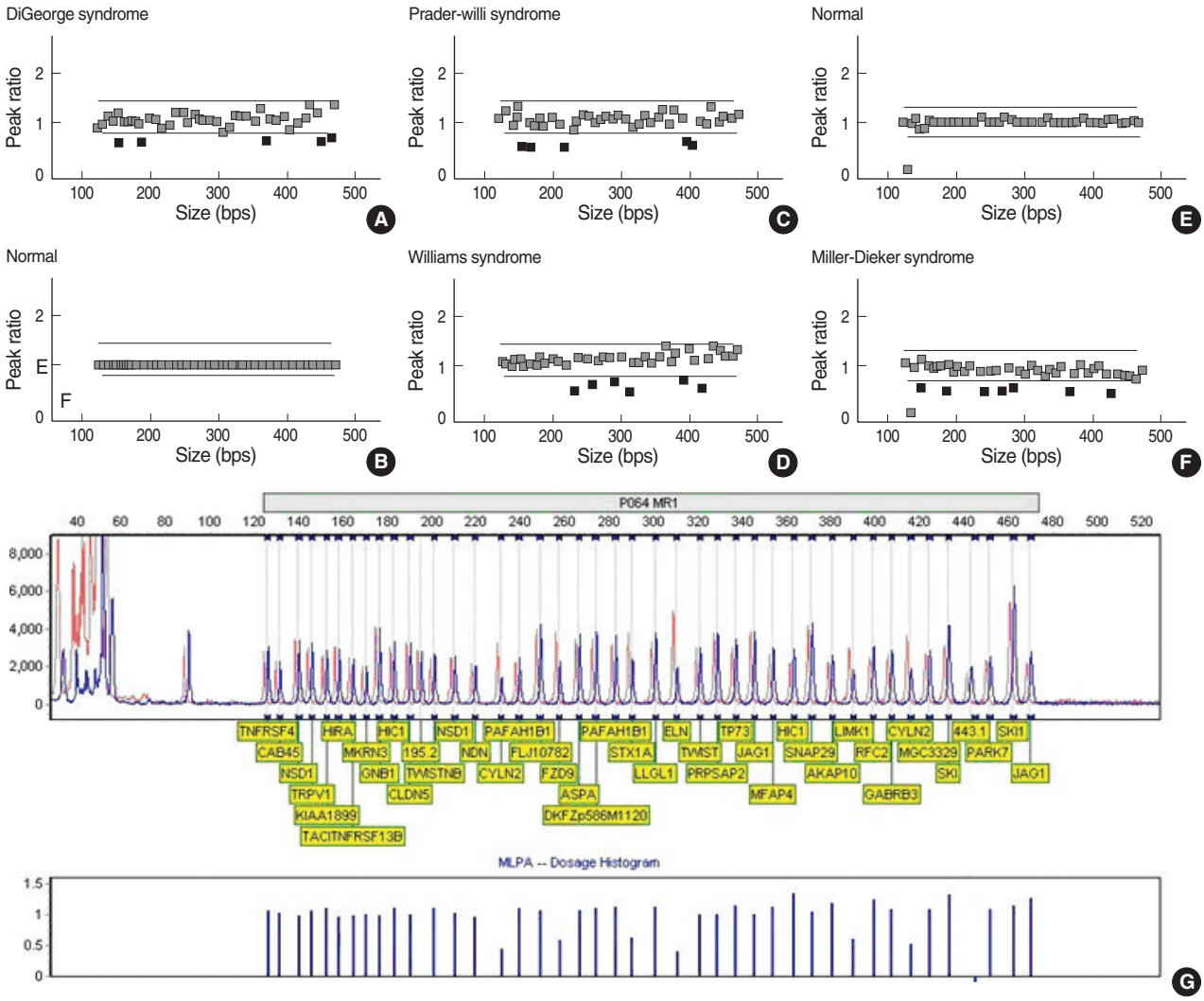

Fig. 2. MLPA results were analyzed employing Genemarker version 1.6 software. The red dot indicates a probe of peak ratio less than 0.75 compared with normal control. The green dot indicates a probe of peak ratio 0.75-1.3 compared with normal control. (A) MLPA results of a patient with DiGeorge syndrome showed the deletions in five probes for *CTCL1, CDC45L, CLDN5, ARVCF*, and *FLJ14360* genes. (B) MLPA results of normal control. (C) MLPA results of a patient with Prader-Willi/Angelman syndrome showed the deletions in five probes of *MKRN3, NDN, UBE3A*, and *GABRB3* genes. (D) MLPA results of a patient with Williams syndrome showed the deletions in six probes of *FZD9, STX1A, ELN, LIMK1*, and *CYLN2* genes. (E) MLPA results of normal control. (F) MLPA results of Miller-Dieker syndrome showed the deletions in seven probes of *HIC1, METT10D, PAFAH1B1, ASPA*, and *TRPV1* genes. (G) MLPA dosage histograms of Williams syndrome. Abbreviation: MLPA, multiplex ligation-dependent probe amplification.

icians due to less characteristic phenotypes. Microarray-based comparative genomic hybridization (array CGH) screens the entire genome and accelerates the identification of novel cytogenetic abnormalities, but the resolution of array CGH can vary depending on the format and design of the array [7]. Therefore, CGH cannot detect some of the microdeletion syndromes with an untargeted gene locus. MRS-MLPA allows testing for a number of micro-deletions/duplications in a single assay, filling a gap between single locus techniques and array techniques. The main drawbacks of FISH are its failure to detect small deletions and duplications and the fact that it is easily affected by the quality of the metaphase spreads. In contrast, MLPA is less time-consuming and capable of detecting smaller, atypical deletions and duplications in microdeletion syndromes. In addition, MLPA is a technically uncomplicated molecular approach and a

higher throughput screening tool for microdeletions in patients with MR.

Although we only tested DiGeorge, Prader-Willi/Angelman, Williams, and Miller-Dieker syndromes, our study has proven that MLPA is a reliable technique for the screening of microdeletion syndromes with a relatively low cost compared to FISH. Although rare microdeletion syndromes (1p36 deletion, Smith-Magenis, and Sotos syndromes) were not tested in our study, it has been shown by others that MLPA can be successfully used for detection of these cases [1]. In the case of 1p36 deletion syndrome, i.e., the most common terminal deletion syndrome with the diversity both in types and sizes of cytogenetic anomalies, more careful interpretation is needed for MLPA. For example, one of the patients who was reported to carry a small (<1 Mb) interstitial 1p36 deletion showed reduced ratios for two of eight probe targeting 1p36 [1]. For detection of Sotos syndrome, MLPA with three probes targeting the NSD1 gene in 5q35.3 not only detected a deletion case but also a duplication case [1]. Because the majority of patients with Saethre-Chotzen and Alagille syndromes have point mutations, there have been no reports for these syndromes detected by MLPA thus far.

In MLPA analysis, we must be cautious about the possibility for misinterpreting polymorphisms present in the probe annealing site as a deletion [8]. Regarding the detection of clinically insignificant familial polymorphisms, many studies have concentrated on the copy number variation (CNV) in the human genome [9-11]. Redon et al. identified a total of 1,447 CNVs >1 Kb in size, demonstrating that CNV may involve as much as 12% of the human genome [12]. For the interpretation of MLPA results, especially in the singular deletion and duplication findings, the currently expanding CNV database would be an important tool [3].

It has been reported that the extent of the 22q11 deletions varies, although 87% of the patients with DiGeorge syndrome have a common 3 Mb deleted region [13]. One case of DiGeorge syndrome in our study showed that the most telomeric gene, *SNAP29*, which was 398 Kb apart from the next probe (*FLJ14360*) was not deleted, and this result was reassuring that MLPA has the capacity for accurately estimating the extent of the deletion.

The sensitivity of MLPA and the incidence of false negative results are not known. This is of a particular importance when clinical laboratories replace FISH with MLPA. There is only one report regarding the use of the MRS-MLPA as a routine screening method in the diagnosis of patients with MR of unknown etiology followed by confirmation of the abnormalities detected by MRS-MLPA with high resolution CGH or real time PCR [1]. In this paper, we have evaluated MLPA for diagnostic testing and it showed a complete concordance with FISH. MRS-MLPA combined with routine cytogenetic studies represents an attractive first test in a clinical algorithm for MR and reduces a remarkable diagnostic delay for the patients.

요 약

배경 : 전통적인 염색체 분석으로 관찰되지 않는 미세결실증후군이 특발성 정신지체의 5%를 차지한다. 따라서 특발성 정신지체 환자에서 특정 미세결실증후군을 배제하는 것이 매우 중요하다. 정신지체증후군(mental retardation syndrome)-multiplex ligation dependent probe amplification (MRS-MLPA)는 유전자의 수적 변화를 검출하는 새로운 기술로서 여러 미세결실증후군(1p36 deletion syndrome, Williams syndrome, Smith-Magenis syndrome, Miller-Dieker syndrome, DiGeorge syndrome, Prader-Willi/Angelmann syndrome, Alagille syndrome, Saethre-Chotzen syndrome, Sotos syndrome)에 대한 검사를 동시에 시행할 수 있어 검사실 업무를 상당히 줄일 수 있다.

방법 : MLPA (MRC-Holland, The Netherlands)의 미세결실증후군의 검출능력을 형광동소교잡법 결과와 비교하였다. 형광동소교잡법 결과 미세결실증후군으로 판정된 12명(3 DiGeorge sydndrome, 4 Williams Syndrome, 4 Prader-Willi/Angelman Syndrome, 1 Miller-Dieker syndrome) 환자에서 MLPA 분석을 시행하였다.

결과 : 형광동소교잡법상 미세결실증후군을 보인 12명의 환자에서 모두 MLPA 검사결과는 형광동소교잡법 결과와 일치하였다.

결론 : MLPA 분석법은 정확하고, 신뢰할만한 결과를 보여주었으며, 특발성 정신지체 환자에서 미세결실 증후군을 선별하는데 있어, 형광동소교잡법의 대체검사로 저렴하게 이용될 수 있을 것으로 사료되었다.

REFERENCES

1. Kirchhoff M, Bisgaard AM, Bryndorf T, Gerdes T. MLPA analysis for a panel of syndromes with mental retardation reveals imbalances in 5.8% of patients with mental retardation and dysmorphic features, including duplications of the Sotos syndrome and Williams-Beuren syndrome regions. Eur J Med Genet 2007;50:33-42.
2. Hunter AG. Outcome of the routine assessment of patients with mental retardation in a genetics clinic. Am J Med Genet 2000;90:60-8.
3. Sellner LN and Taylor GR. MLPA and MAPH: new techniques for detection of gene deletions. Hum Mutat 2004;23:413-9.
4. Rusu C, Sireteanu A, Puiu M, Skrypnyk C, Tomescu E, Csep K, et al. MLPA technique-principles and use in practice. Rev Med Chir Soc Med Nat Iasi 2007;111:1001-4.
5. Rauch A, Hoyer J, Guth S, Zweier C, Kraus C, Becker C, et al. Diagnostic yield of various genetic approaches in patients with unexplained developmental delay or mental retardation. Am J Med Genet A 2006;140:2063-74.
6. Flint J and Knight S. The use of telomere probes to investigate submicroscopic rearrangements associated with mental retardation. Curr Opin Genet Dev 2003;13:310-6.
7. Shaffer LG and Bejjani BA. A cytogeneticist's perspective on genomic microarrays. Hum Reprod Update 2004;10:221-6.
8. Wehner M, Mangold E, Sengteller M, Friedrichs N, Aretz S, Friedl W, et al. Hereditary nonpolyposis colorectal cancer: pitfalls in deletion screening in MSH2 and MLH1 genes. Eur J Hum Genet 2005; 13:983-6.
9. Armour JA, Palla R, Zeeuwen PL, den Heijer M, Schalkwijk J, Hollox EJ. Accurate, high-throughput typing of copy number variation using paralogue ratios from dispersed repeats. Nucleic Acids Res 2007;35:e19.
10. White SJ, Vissers LE, Geurts van Kessel A, de Menezes RX, Kalay E, Lehesjoki AE, et al. Variation of CNV distribution in five different ethnic populations. Cytogenetic Genome Res 2007;118:19-30.
11. Janssen B, Hartmann C, Scholz V, Jauch A, Zschocke J. MLPA analysis for the detection of deletions, duplications and complex rearrangements in the dystrophin gene: potential and pitfalls. Neurogenetics 2005;6:29-35.
12. Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD, et al. Global variation in copy number in the human genome. Nature 2006;444:444-54.
13. Shaikh TH, Kurahashi H, Saitta SC, O'Hare AM, Hu P, Roe BA, et al. Chromosome 22-specific low copy repeats and the 22q11.2 deletion syndrome: genomic organization and deletion endpoint analysis. Hum Mol Genet 2000;9:489-501.