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Development and Application of DNA Array (GD-700) for Congenital Anomaly Syndromes

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Abstract

The recent development of the Comparative Genomic Hybridization (CGH) method enables the comprehensive analysis of the fine structure of human chromosomal abnormalities. The causes of many congenital anomaly syndromes in newborns and children are difficult to be determined. Therefore, it is significantly important to clarify the syndromes and to apply the CGH method for a practical diagnosis.

Professor Inazawa of Tokyo Medical and Dental University established a well reproducible CGH method that enables quantitative analysis of the change in the copy number of the genome. Furthermore, his team prepared the Genome Disorder Array (GD-700) with BAC DNA for congenital anomaly syndromes. From 2005, FUJIFILM has collaborated with Prof. Inazawa, has proven this GD-700 to be practical, and developed an original analysis method "Dual Hybridization method" this year. In this paper, we introduce the design of this array and its original method.

1. Introduction

The conventional analysis of genomic aberration leading to congenital anomaly syndromes is carried out mostly by chromosomal test. As the Comparative Genomic Hybridization (CGH) method has been recently developed, it is now possible to analyze anomalies in the microscopic structure of a human chromosome[1)]. Causes of many congenital anomaly syndromes in newborns and young children are unknown. To determine the causes and diagnose with the CGH method are very important to this field of medical care.

Professor Inazawa at Tokyo Medical and Dental University has established a quantitative analysis method that detects loss or gain in a single copy of a chromosome with high reproducibility[2)]. The professor also made Genome Disorder Array (GD-700) for diagnosis of congenital anomaly syndromes. FUJIFILM started joint research with Professor Inazawa's team in 2005. We have worked with Professor Inazawa, who conducted practicality test on more than 500 cases, and the consortium for practical application of array CGH diagnosis method, which is led by, Professor Inazawa and BML, Inc. At the same time, we have developed Dual Hybridization, our original analysis method suitable for clinical examination. As a result, we have found a new examination system suitable for clinical use based on GD-700 and Dual Hybridization.

This paper reports on the product design and performance of the GD-700 and on the Dual Hybridization, the new analysis method (Fig. 1).

Fig. 1 The appearance of "GD-700".

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2. Launching GD-700

2.1 Design Policy of GD-700

The chromosomal test has been said to be difficult to simplify. It requires such processes as cell culturing, specimen preparation and microscopic observation using G-banding, which are time and effort consuming. It also requires a highly skilled technician to identify a chromosomal anomaly. Using GD-700 with the Dual Hybridization, copy number anomalies of microscopic chromosomes can be detected simply and accurately.

The GD-700 uses the highly reliable RP-11[3], a BAC (Bacterial Artificial Chromosome) library widely used in the Human Genome Project across the world. Specifically, it employs 712 BAC clones that have regions relating to congenital anomaly diseases registered in the human genome database. Using the resource, it is possible to analyze anomalies in genome copy number at once including 30 types of microdeletion/microduplication syndromes, 41 subtelomeres (excluding the p-arms of 13, 14, 15, 21, 22 and Y chromosomes) and 42 pericentromeres. It is significant to be able to analyze subtelomeres with the G-700. Subtelomeres, difficult to analyze with the conventional chromosomal test, is drawing attention as a possible cause of mental retardation (Table 1).

2.2 Production Method

As for the 712 BAC clones comprising the GD-700, we have confirmed that stable signals are obtained in the FISH (Fluorescence in situ hybridization) method and that there is no mishybridization to other chromosomes. We isolated high-purity BAC DNA from the selected BAC clones using the plasmid purification column. We amplified the BAC DNA in the adapter ligation PCR technique to make probe DNA. The 712 probe DNAs are spotted onto a glass slide for DNA array (made by Matsunami Glass Ind., Ltd.) using a non-contact inkjet spotter GENESHOT (made by NGK Insulators, Ltd.) to ensure they are all aligned and uniform in concentration, diameter, shape, and position. Through these processes, we have succeeded in making a BAC array that enables stable detection of genome copy number anomalies[4] (Fig. 2).

Table 1 The list of congenital anomaly syndromes tested by "GD-700".

Syndrome	DNA	Deletion/duplication	Remarks
van der Woude syndrome	*IRF6*	1q32-q41 anomaly (deletion)	
Mowat-Wilson syndrome	*ZFHX18*	2q22.3 anomaly (deletion)	
BPES syndrome	*FOXL2*	3q22.3 deletion	
4p- syndrome	*Multiple*	4p16.3 deletion	
5p- syndrome	*Multiple*	5p15.3-p15.2 deletion	
Sotos syndrome	*NSD1*	5q35 deletion	5q35 duplication syndrome
Saethre-Chotzen syndrome	*TWIST1*	7p21.1 deletion	
Williams syndrome	*ELN*	7q11.23 deletion	7q11.2 duplication syndrome
Langer-Giedion syndrome	*EXT1,TRPSI*	8q24.11-q24.13 deletion	
Beck with-Wiedeman syndrome	*IGF2*	11p15.5 deletion/ duplication	
WAGR syndrome	*WT1,PAX6*	11p13 deletion	
Potocki-Shaffer syndrome	*ALX4,EXT2*	11p11.2 deletion	
Pallister-Killian syndrome	*Multiple*	i(12p) duplication	
Prader Willi syndrome	*SNRPN*	15q11-q13 deletion	15q11-q13 duplication syndrome
Angelman syndrome	*UBE3A*	15q11-q13 deletion	
Rubinstein-Taybi syndrome	*CREBBP*	16p13.3 deletion	
Miller-Dieker syndrome	*LIS1*	17p13.3 deletion	
Charcot-Marie-Tooth 1A	*PMP22*	17p12 duplication	HNPP
Smith-Magenis syndrome	*RAI1*	17p11.2 deletion	Potocki-Lupski syndrome
Neurofibromatosis I	*NF1*	17p11.2 deletion	
Diamond-Blackfan syndrome	*RPS19*	19q13.2 anomaly (deletion)	
Alagille syndrome	*JAG1*	20p12.2 deletion	
Down syndrome	*Multiple*	21q22 duplication	
Cat eye syndrome	*Multiple*	22q11.2 duplication	
22q11.2 deletion syndrome	*TBX1*	22q11.2 deletion	22q11.2 duplication syndrome
X-linked ichthyosis	*STS*	Xp22.31 deletion	
Kallmann syndrome type I	*KAL1*	Xp22.31 deletion	
Duchenne Muscular Dystrophy	*DMD*	Xp21.2 deletion/ duplication	
Pelizaeus–Merzbacher disease	*PLP1*	Xq22.2 deletion/ duplication	
MECP2 duplication syndrome	*MECP2*	Xq28 duplication	

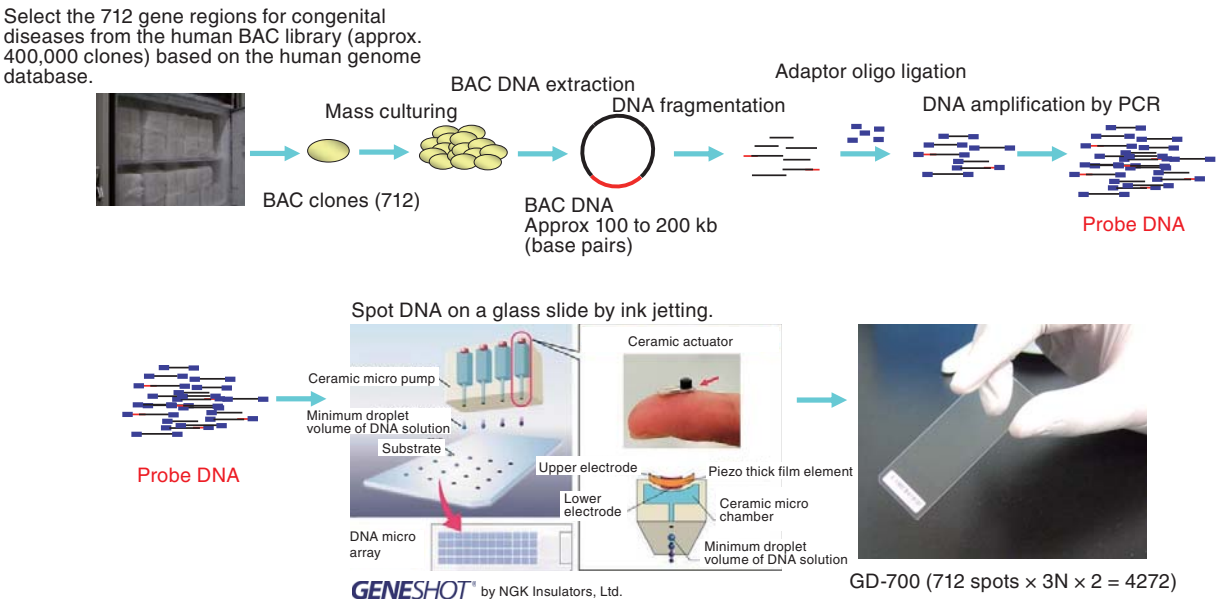

Fig. 2 The flow of "GD-700" production.

3. Development of Analysis Method

3.1 Overview

Fig. 3 shows the principle of a typical array CGH method (one color method). The test sample and the reference sample are labeled with fluorescent molecules (it must have been confirmed that the samples have no genetic aberrations). Each sample is hybridized to the probes on the BAC array. The fluorescence intensities of hybridized DNA on the array are measured to calculate the ratio of test to reference samples in fluorescence intensity. The result shows whether there is deletion or amplification in the sample genome (Fig. 3).

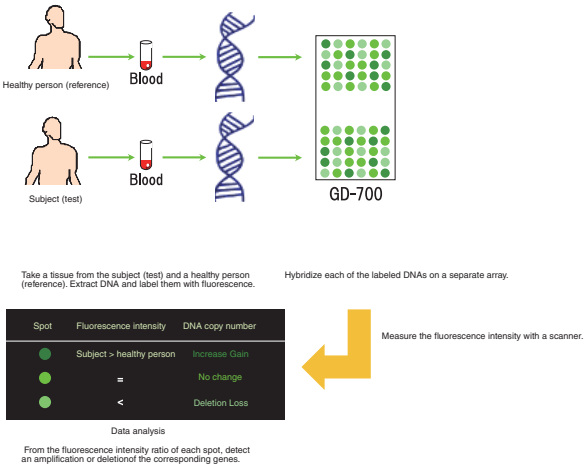

Fig. 3 The principle of CGH array method.

In the conventional one-color method, the test and reference samples have to be hybridized separately on different arrays. That means the test result may vary depending on hybridization condition: for example, composition of the hybridization liquid, temperature, agitation condition, and the amount of immobilized DNA in the probes. We have developed a new array CGH, Dual Hybridization, less subject to the hybridization conditions.

3.2 Principle of Dual Hybridization

In the Dual Hybridization, each of the test and reference samples is mixed with florescence-labeled internal standard DNA before hybridized on a CGH array. As the internal standard DNA, we used one in a quantity large enough to be hybridized to all the probe DNAs on the CGH array.

The fluorescence intensities of the hybridized test and reference probe DNAs and those of the internal standard DNAs are measured. The fluorescence intensity is corrected by dividing the test (reference) fluorescence intensity by that of the internal standard DNA. The ratio of the corrected test and reference fluorescence intensities is calculated. This correction solved the problem of varying data due to hybridization on separate arrays, which is inherent in the conventional one-color method (Fig. 4).

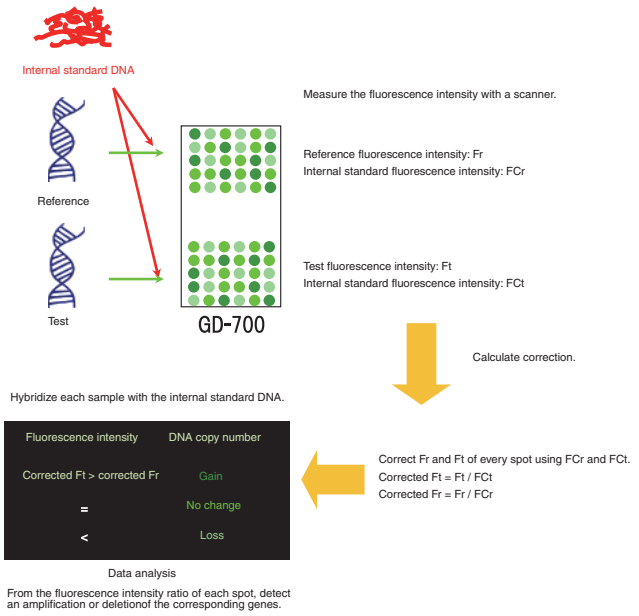

Fig. 4 The principle of Dual Hybridization.

4. Evaluation of GD-700

To evaluate the GD-700, we have carried out sex chromosome comparison (fundamental evaluation) using standard genomes available on the market (male/female by Promega KK) and Dual Hybridization using genomes with chromosome anomalies (positive sample).

4.1 Evaluation Using Standard Genomes

In the evaluation using standard genomes, we set the two criteria below. (1) The fluorescence intensity ratios of the autosomes shall fall within the range from 0.75 to 1.25. Even if there are some data outside the range, they shall not be continuous. (2) When a male genome is used as the reference and a female as the test sample, the X-chromosome, a sex chromosome, shall be different from autosomes. There shall be a difference of 0.5 or more between the X-chromosome average and the autosome average. As a result of the evaluation (n = 4), we have confirmed that both criteria were met concerning all the samples.

4.2 Evaluation Using Chromosomally Aberrant Genomes

For this evaluation, we used six DNA samples with congenital anomaly diseases and three DNA samples with miscarriage and stillbirth.

The DNA samples with congenital anomaly disease had known abnormal chromosome regions: Sample No. 1 (2p25.3 Gain/5p15 Loss), No. 2 (15q26.3 Gain), No. 3 (22q13.31 Loss), No. 4 (16p13.3 Gain), No. 5(22q11.21 Gain), No. 6 (2p25.3 Gain/10p15.3 Loss). We carried out array CGH analysis of those samples in the Dual Hybridization to see whether the regions would be correctly detected. All the regions were detected properly.

All the DNA samples with miscarriage and stillbirth had a trisomy: trisomies 13, 16 and 21. All the trisomies were detected (Fig. 5 and Fig. 6).

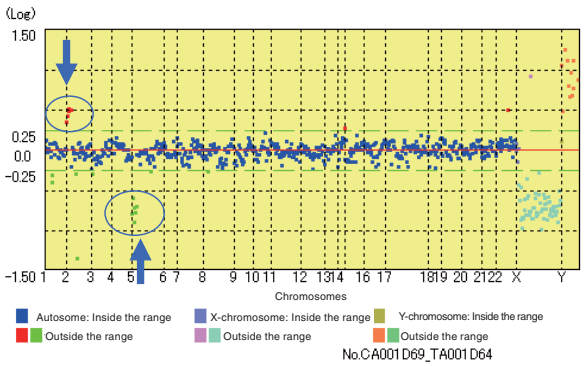

Fig. 5 The result of sample No.1 (2p25.3 Gain/5p15 Loss).

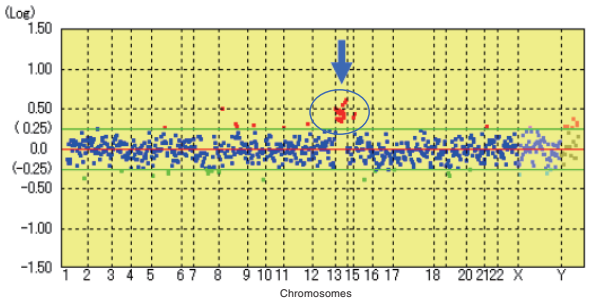

Fig. 6 The result of sample No.7 (13q Gain (trisomy)).

In both evaluations, chromosome anomalies are clearly detected. The combination of the GD-700 and the Dual Hybridization is effective for chromosomal testing (Table 2).

Table 2 The results of positive samples.

Detection of congenital anomaly disease samples		
Sample No.	Abnormal chromosome region	Result
No.1	2p25.3 Gain/5p15 Loss	○ Succeeded
No.2	15q26.3 Gain	○ Succeeded
No.3	22q13.31 Loss	○ Succeeded
No.4	16p13.3 Loss	○ Succeeded
No.5	22q11.21 Gain	○ Succeeded
No.6	2p25.3 Gain/10p15.3 Loss	○ Succeeded
Detection of miscarriage and stillbirth samples		
Sample No.	Abnormal chromosome region	Result
No.7	13q Gain (Trisomy 13)	○ Succeeded
No.8	16q Gain (Trisomy 16)	○ Succeeded
No.9	21q22 Gain (Trisomy 21)	○ Succeeded

* The symbols for abnormal chromosome regions: Example 2p25.3 Gain(Loss)
Chromosome No. (p: long arm, q: short arm) | Sub-band in chromosome | Gain: amplification, Loss: deletion

5. Conclusion

We have succeeded in commercializing the GD-700, a new array for congenital anomaly syndrome detection and developing the Dual Hybridization, the analysis method for the array. We have demonstrated that samples with known abnormal chromosome regions are accurately detected using this array and the method. We believe this method and the array will be widely used and replace or complement the conventional chromosome testing technology. And, they will be applied as genome analysis and diagnosis tools for congenital anomaly diseases of unknown cause, mental development disorders, autism and cancers, behind which chromosome and genome anomalies lie.

6. Acknowledgement

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