Application of KaryoLite™ BACs on Beads™ assay for prenatal diagnosis to detect chromosome aneuploidy in amniotic fluid cells

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ABSTRACT

Prenatal diagnosis (PND) is a technique to detect chromosome abnormalities in fetus before birth. The techniques currently used for PND are conventional karyotyping and fluorescent *in situ* hybridization (FISH). Both techniques still have limitations, e.g., a high error rate of detection. The purpose of this study was to evaluate the efficiency of KaryoLite BACs-on-Beads (KL-BoBs™) as a routine diagnostic assay in a clinical application for PND by comparison with conventional karyotyping. A total of 37 amniotic fluid cell samples from 18 patients were used to validate KL-BoBs™ technique. The results of the KL-BoBs™ were 100% concordant with karyotyping for trisomy 18, trisomy 21, triple X, normal XX and normal XY. In conclusion, our results indicated that KL-BoBs™ had high concordance with conventional karyotying for prenatal diagnosis to detect chromosome status in amniotic fluid cells. Moreover, using an appropriate reference could reduce the coefficient of variation (CV) and increase accuracy of interpretation.

Keywords: prenatal diagnosis; KaryoLite™ BACs on Beads™; conventional karyotyping; amniotic fluid cell

INTRODUCTION

Prenatal diagnostic (PND) is a technique to detect chromosome abnormalities in fetus before birth. PND was used to plan for possible abnormalities with the birth process in order to decide whether to continue a pregnancy. Moreover, PND was used for finding conditions that may affect future pregnancies. The gold standard technique for invasive prenatal diagnosis was conventional karyotyping. It can detect all aneuploidies and large chromosome rearrangement. The failure rate was 5%–25% (Carp *et al*., 2001; Menasha *et al.*, 2005; Robberecht *et al*., 2009). Molecular cytogenetic technique, fluorescence *in situ* hybridization (FISH) was applied for diagnosis of submicroscopic imbalance. The limitations of FISH include overlapping FISH signals, hybridization failure, non-specific hybridization, difficulties in signal interpretation, considerable reported FISH error rates ranging from 3.7% to 50% (Baart *et al.*, 2007; Magli *et al.*, 2007).

Currently, a new technology called KaryoLite™ BACs on Beads™ (KL-BoBs™) which was modified from comparative genomic hybridization was developed to detect DNA copy number gains and losses. This assay contains low resolution coverage of all chromosomes and provides dosage information about the proximal and terminal regions of each chromosome arm. Additionally, there are 3 to 4 beads per chromosome and each bead contains 3 neighboring bacterial artificial chromosome probes to broaden the hybridization target region. Aneusomy

detection with the KL-BoBs™ assay is performed by comparison of the test sample to reference DNA samples. It was used for screening aneuploidy in prenatal sample and products of conception (POCs) by comparing with conventional cytogenetic analysis, FISH and aCGH in previous studies (Baxter and Adayapalam, 2013; Grati *et al*., 2013; Paxton *et al*., 2013;; Sheath *et al*., 2013). The failure rate of KL-BoBs™ was 2% and false positive and false negative rates were <1%. The mosaic detection threshold was 50% (Grati *et al*., 2013). The advantage of this assay was that chromosome abnormalities may be detected long before discriminatory prenatal sign and parents can be informed early of the fetal karyotype to plan for further management of the family. In this study, we performed an evaluation of KL-BoBs™ by comparing the results with conventional karyotyping.

MATERIALS AND METHODS

Sample preparation

A total of 37 amniotic fluid (AF) cell samples were obtained from routine service of Human Genetic Laboratory, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University. Cytogenetic findings were known in all samples. AF samples were divided into 5 groups: trisomy 18 (n=6), trisomy 21 (n=11), triple X (n=6), 46, XX (n=7) and 46,XY (n=7).

After AF samples were cultured for 2 days, trypsin was used to digest the sample and culture media with fetal calf serum was added to stop trypsin digestion. Then, cell morphology including size and cell distribution was evaluated using a stereo zoom microscope (Motic, Hong Kong). AF cells were transferred into 0.2 ml microcentrifuge tube (Figure 1) and stored at –80°C until use.

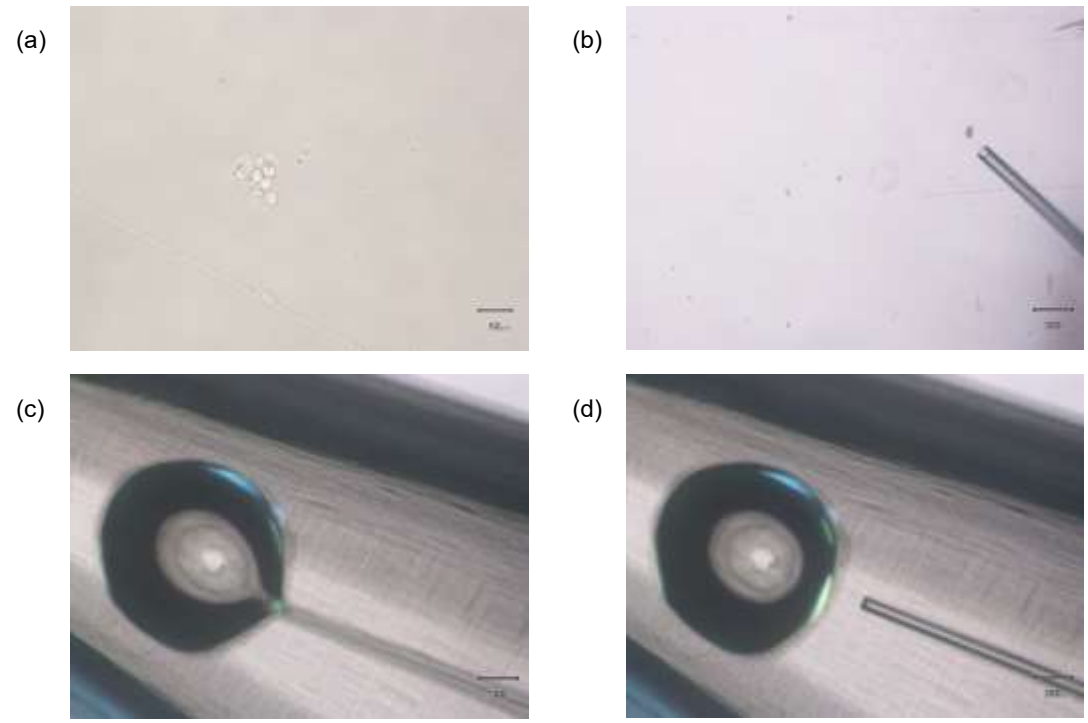

Figure 1 Case 57 AF-0111 (46, XX): amniotic fluid (AF) cells preparation (a) Morphology of AF cells by stereo zoom microscope, (b) AF cells were washed and (c-d) AF cells were transferred into a microcentrifuge tube.

Whole genome amplification (WGA) and amplicon assessment

DNA from isolated single AF cells was amplified using picoplex WGA kit (Rubicon Genomics, USA) to amplify approximately 1 million-fold to produce 2 to 5 microgram DNA within 3 hours. To verify amplified DNA from WGA, the amplicons were loaded into 1.2% agarose gel and run at 100V for 30 min. The WGA kit generates a pool of amplicons with average size of 500-bp amplified products. Nanodrop (Delaware, USA) was used to measure the amount of amplified DNA. The final concentration of amplified products should be 800 to 1,100 ng/µl.

KaryoLite BoBs (PerkinElmer, Finland)

Amplified DNAs were labeled with biotin-dNTP. NucleoFast 96 purification plate (Macherey-Nagel, Germany) and vacuum manifold pumps were used to purify labeled DNA. Biotin-labeled samples were hybridized to the beads in TriNest shaking incubator (PerkinElmer, Finland). Post hybridization was done and reporter mix was added to each plate well. Streptavidin-phycoerythrin quantifies the molecular capture interaction that occurs at the microsphere surface. The plate was loaded into Luminex 200 instrument system and analyzed by xPonent 3.1 software (Luminex, USA). The results were interpreted following the interpretation guidelines of the manufacturer.

RESULTS

Validation of whole genome amplification (Picoplex kit) and KL-BoBs™ technique

Whole genome amplification efficiency was approximately 94.59 % (35/37 samples). There were two unamplifiable samples. One was a single cell AF with trisomy 18 which had inadequate DNA amount because of short culture period (only 1 day culture) (Figure 2). We simultaneously used cultured cells for more than 1 day from 12 single cell AF samples and preformed whole genome amplification. These 12 samples were trisomy 18, trisomy 21, triple X and 46, XY and the amplified products were detected, whereas the 1 day cultured single AF cell was incompletely amplified (data not shown). The other unamplifiable sample was a single AF cell with triple X that the DNA band was not present (Figure 2). This may be due to absence of cell in the tube caused by human error.

We have validated KL-BoBs™ in 37 AF cell samples with known chromosome abnormality: trisomy 18 (n=7), trisomy 21 (n=7), triple X (n=7), 46, XX (n=8) and 46, XY (n=8). We found that when using genomic DNA reference, the coefficient of variation (CV) was higher than 10% and chromosome abnormalities were detected in some markers (Figure 3a). This problem may be caused by using an inappropriate reference. Therefore, the reference was adjusted by using pooled genomic DNA from 6 males and 6 females, and subjected to WGA. The result indicated that the problem was solved and provided a reference with < 8% CV. In Figure 3b, it clearly showed the gain of chromosome 18 (all dots were out of green lines which were normal range) but other chromosomes revealed signals in normal range. In figure 3a of chromosome 18 markers, some dots of chromosome 18 showed gain signals but some did not. The other chromosomes were normal but there were fluctuations of signals from the normal range. When comparing results from a single cell (Figure 4a) and group cells (Figure 4b), we found that both showed the same results of the gain of chromosome 21, while other chromosomes showed clear results of normal range in group cells rather than a single cell. Results of the KL-BoBs™ were 100% concordant with karyotyping for trisomy 18, trisomy 21, triple X, normal XX and normal XY.

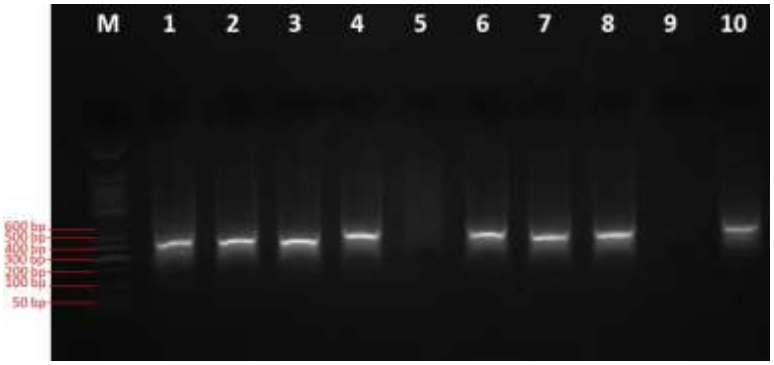

Figure 2 Representative results of WGA products on a 1.2 % agarose gel. WGA products from AF cultured cells with abnormal chromosome variations in both single cells and group cells. The size of the DNA fragments was approximately 500 bp. M = DNA marker, lane 1 = A single cell of amniotic fluid cell (47,XX,+21), lane 2 = Group cells of amniotic fluid cell (47,XY,+18), lane 3 = Group cells of amniotic fluid cell (47,XX,+21), lane 4 = A single cell of amniotic fluid cell (47,XXX), lane 5 = A single cell of amniotic fluid cell (47,XY,+18), lane 6 = Group cells of amniotic fluid cell (47,XXX), lane 7 = Group cells of amniotic fluid cell 47,XY,+18), lane 8 = Group cells of amniotic fluid cell (47,XX,+21), lane 9 = A single cell of amniotic fluid cell (47,XXX), and lane 10 = Group cells of amniotic fluid cell (47,XX,+18).

DISCUSSION

We aimed to evaluate the efficiency of WGA and KL-BoBs™ as a routine diagnostic assay in a clinical setting. Thirty-seven amniotic fluid cell samples from 18 patients were processed in the validation study. We found that amplification rate was ~95% and amplification efficiency was at least 90% for each marker as previously recommended (Harton *et al.*, 2010). The amplification failure due to human error and inappropriate source of DNA caused the samples unsuitable for further analysis was found in 2 out of 37 samples (5.4%). When comparing results of KL-BoBs™ with karyotyping method, KL-BoBs™ results were 100% concordant with karyotyping for trisomy 18, trisomy 21, triple X, normal XX and normal XY and that were consistent with previous studies in products of conception (POCs) sample (Baxter and Adayapalam, 2013; Sheath *et al.*, 2013). However, Paxton *et al*. reported that KL-BoBs™ assay was 76.6% concordant with karyotyping and 78.3% concordant with SNP microarray in POCs sample. Discordant results arose from false negative in polyploidy, structural abnormality, maternal cell contamination and mosaicism (Paxton *et al.*, 2013). Grati *et al.* reported that the mosaic detection threshold was 50% (Grati *et al.*, 2013). The accuracy of detection of KL-BoBs™ depends on the coefficient of variation. It was reduced by using reference from the same kind of DNA to increase the accuracy of interpretation.

When the coefficient was <8 %, loss and gain of chromosome regions was clearly detected as recommended by the manufacturer's analysis guidelines.

The KL-BoBs™ assay provides a higher resolution analysis of 24 chromosomes and faster turnaround time when compared with conventional karyotyping. Other advantages include ease of interpretation, high efficiency, requirement of small amount of input DNA, and high throughput of up to 92 samples per run. The limitation of this technique was inability to detect low level of mosaicism or maternal cell contamination, polyploidy and structural abnormality.

(a) (b)

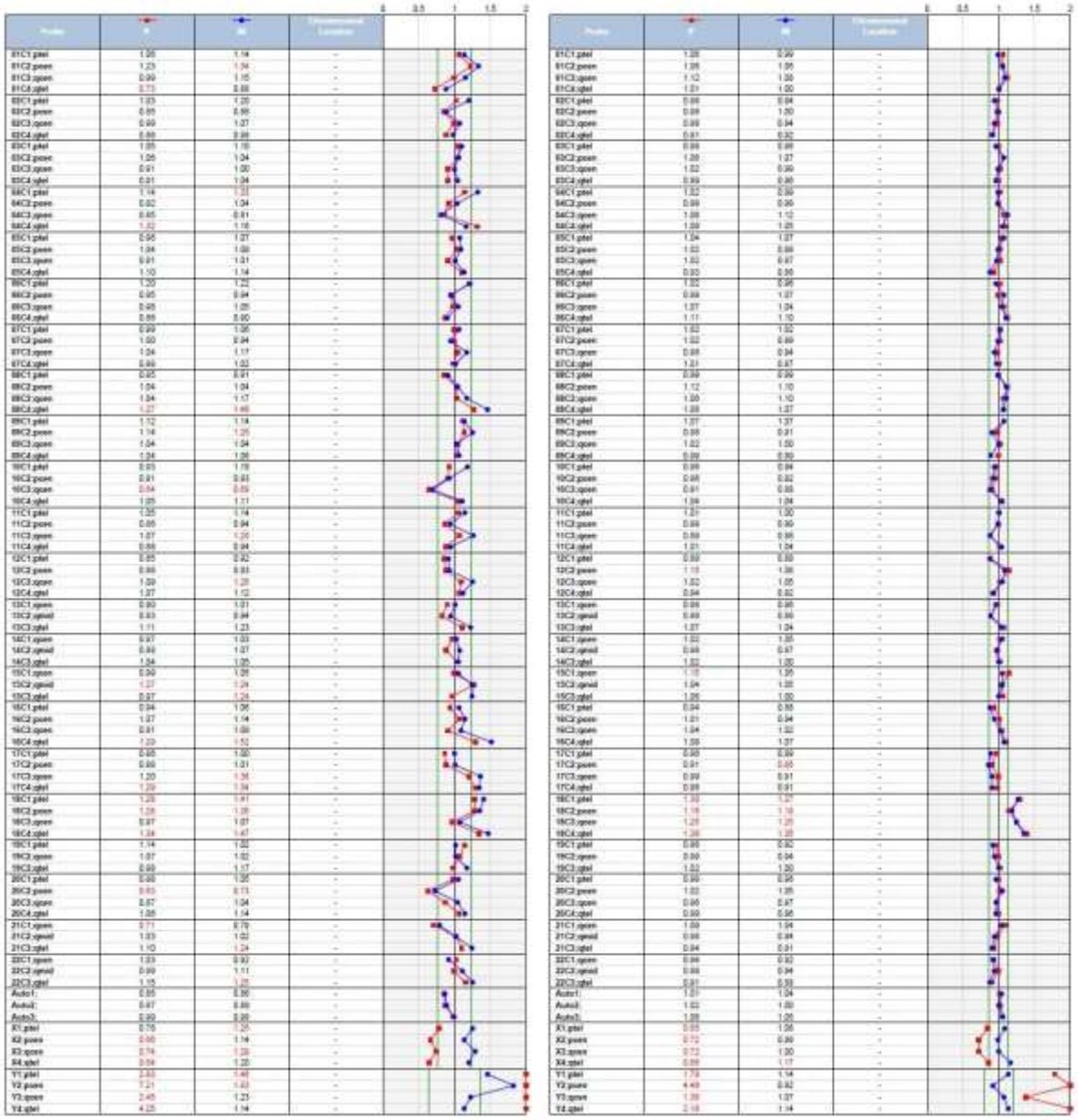

Figure 3 In a case with trisomy 18, XY, comparison of the KL-BoBs™ result using different references was demonstrated. (a) genomic DNA reference (CV = 11.87 %) and (b) WGA DNA from pooled reference samples (CV = 6.59 %). Blue dots represent the proportion of tested DNA compared with male reference DNA. Red dots represent the proportion of tested DNA compared with female reference DNA. Green lines are normal range of the signals.

Although the data were obtained from a limited number of AF cell samples (n = 37) and patients (n = 18), the present study was a preliminary result to use KL-BoBs™ for prenatal diagnosis. In addition, the limitation of this study was that chromosomal genomic balance abnormalities were undetectable by KL-BoBs™ and karyotyping method is still the golden standard for chromosome abnormality detection in prenatal diagnosis.

However, the application of KL-BoBs™ was

(a) (b)

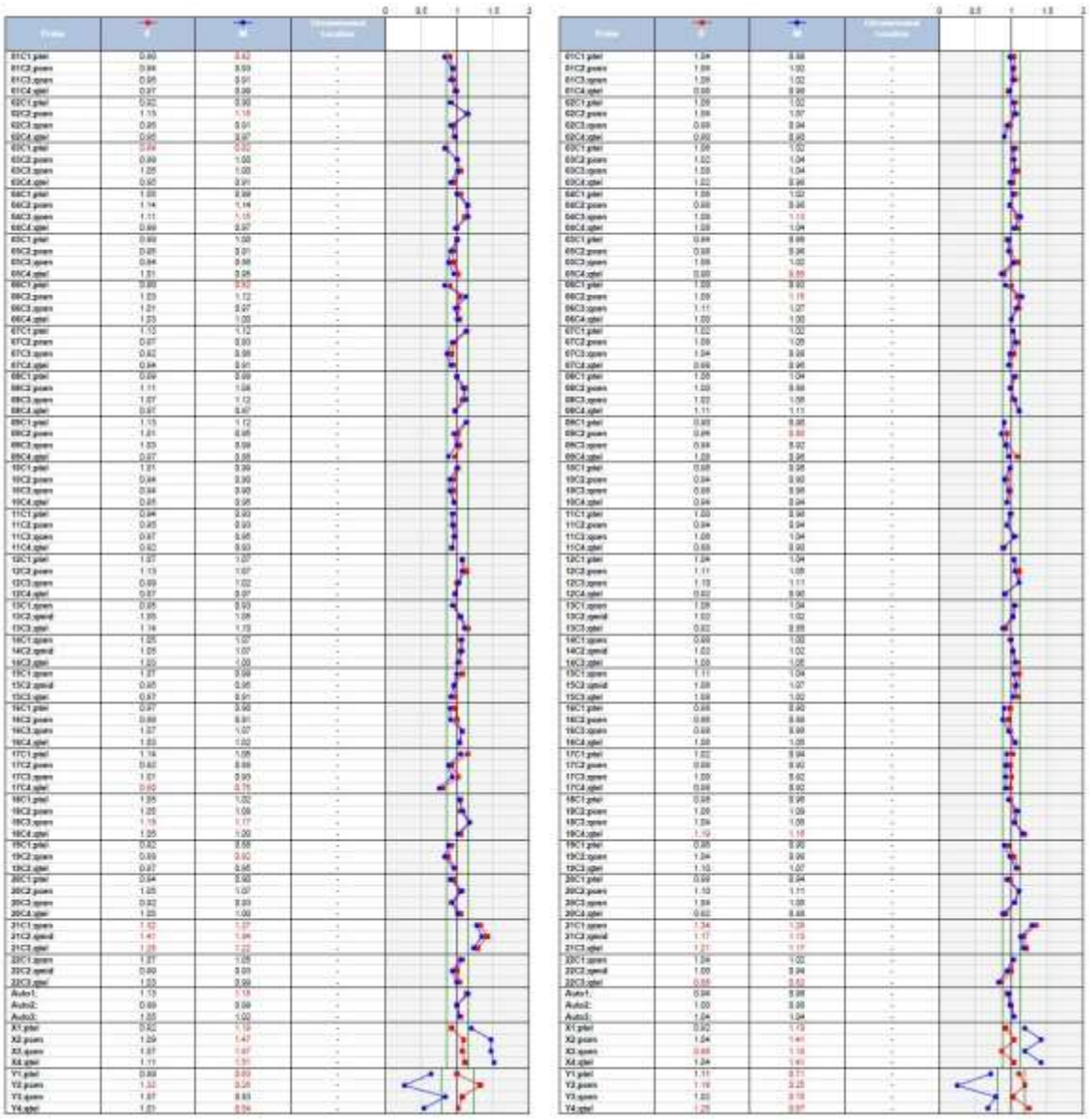

Figure 4 In a case with trisomy 21, XX comparison of the KL-BoBs™ result between different cell amount was demonstrated. (a) a single cell (CV = 7.64 %) and (b) group cells (CV = 6.38 %). Blue dots represent the proportion of tested DNA compared with male reference DNA. Red dots represent the proportion of tested DNA compared with female reference DNA. Green lines are normal range of the signals.

used to screen chromosomal abnormalities in prenatal diagnosis and postnatal diagnosis (Baxter and Adayapalam, 2013; Grati *et al.*, 2013; Paxton *et al.*, 2013; Sheath *et al.*, 2013). Furthermore, it was applied for preimplantation genetic diagnosis (PGS) as an alternative choice for the clinician and patients in a near future.

CONCLUSION

The purpose of this study was to evaluate the efficiency of KaryoLite™ BACs-on-Beads™ (KL-BoBs™) as a routine diagnostic assay in a clinical setting for prenatal diagnosis. The amniotic fluid samples were cultured and conventional karyotyping was tested in parallel. Cultured amniotic fluid

cells were tested by KL-BoBs™ for detection of chromosome status and the results were compared with those of conventional karyotyping. In conclusion, our results indicated that the results from KL-BoBs™ had 100% concordance with conventional karyotyping to detect chromosome abnormalities for prenatal diagnosis in amniotic fluid cells.

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