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Application of digital polymerase chain reaction technology for noninvasive prenatal test

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Recently, noninvasive prenatal test (NIPT) has been adopted as a primary screening tool for fetal chromosomal aneuploidy. The principle of NIPT lies in isolating the fetal fraction of cell-free DNA in maternal plasma and analyzing it with bioinformatic tools to measure the amount of gene from the target chromosome, such as chromosomes 21, 18, and 13. NIPT will contribute to decreasing the need for unnecessary invasive procedures, including amniocentesis and chorionic villi sampling, for confirming fetal aneuploidy because of its higher positive predictive value than that of the conventional prenatal screening method. However, its greater cost than that of the current antenatal screening protocol may be an obstacle to the adoption of this innovative technique in clinical practice. Digital polymerase chain reaction (dPCR) is a novel approach for detecting and quantifying nucleic acid. dPCR provides real-time diagnostic advantages with higher sensitivity, accuracy, and absolute quantification than conventional quantitative PCR. Since the groundbreaking discovery that fetal cell-free nucleic acid exists in maternal plasma was reported, dPCR has been used for the quantification of fetal DNA and for screening for fetal aneuploidy. It has been suggested that dPCR will decrease the cost by targeting specific sequences in the target chromosome, and dPCR-based noninvasive testing will facilitate progress toward the implementation of a noninvasive approach for screening for trisomy 21, 18, and 13. In this review, we highlight the principle of dPCR and discuss its future implications in clinical practice.

Key words: Prenatal diagnosis, Polymerase chain reaction, Noninvasive prenatal diagnosis, Aneuploidy, Chromosome aberration.

Introduction

Prenatal diagnosis is the process checking for the presence of disease or condition in a fetus or embryo before birth, including congenital birth defects, genetic disorders, and chromosome abnormalities. According to a recent Korean national statistics report, congenital birth defects occur in about 3% of the total newborns, and about 20% of congenital malformations in children are due to chromosomal abnormality [1,2]. Among the chromosomal abnormalities, it was reported that Down syndrome (trisomy 21) occurs in 1 in 800 [3,4], Edward syndrome (trisomy 18) in 1 in 6,000 [5], and Patau syndrome (trisomy 13) in 1 in 10,000 fetal subjects [6]. Moreover, birth defects from Down syndrome comprise about 26% of all congenital birth defects [7,8]. As maternal age increases, fetal chromosomal aneuploidy becomes more prevalent [9]. The prevalence of fetal aneuploidy has rapidly increased over the last decade in Korea because of the increasing age of marriage and childbearing (Table 1).

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Table 1. Congenital abnormalities of newborn infants (number per each year)

Variable	2005	2006	2007	2008	2009	2010	2011
Total	18,244	18,911	21,327	23,478	25,082	30,446	36,069
Congenital abnormality of the nervous system	455	464	478	597	582	734	851
Congenital abnormality of the eyes, ears, face, and neck	2,595	2,345	2,918	2,910	2,811	3,333	3,502
Congenital abnormality of the circulatory system	6,199	6,387	6,961	6,889	7,060	7,813	8,476
Congenital abnormality of the respiratory system	277	309	349	778	634	745	784
Cleft lip and cleft palate	453	505	427	478	507	685	753
Congenital abnormality of the digestive system	2,212	2,959	3,868	4,864	5,957	8,341	11,114
Congenital abnormality of reproductive organs	586	624	768	858	1,021	1,258	1,395
Congenital abnormality of the urinary system	1,063	1,205	1,232	1,303	1,405	1,809	1,804
Congenital musculoskeletal deformities	3,569	3,392	3,443	3,959	4,163	4,641	5,992
Other congenital abnormalities	548	499	604	585	689	848	1,107
Nonclassified chromosome abnormality	287	222	279	257	253	239	291

Data on the number of infants per congenital abnormality were from the National Health Insurance Service (Korea, 2013.05.28).

Prenatal diagnostic methods to confirm fetal chromosomal abnormalities are carried out in an invasive way. There are several methods specialized for certain periods of pregnancy, including chorionic villi sampling (CVS), amniocentesis, and cordocentesis, which are performed at 10-12 weeks [10-12], 15-20 weeks [13,14], and 18-20 weeks [15] of pregnancy, respectively. Although those invasive tests are required to confirm the positive prenatal screening test, confirmatory tests carry related risks of maternal and fetal complication, including fetal loss.

To reduce the invasive confirmatory procedures, more accurate and less invasive prenatal screening tests have been developed. Lo et al. [16] reported in 1997 that fetal cell-free DNA (cfDNA) is present in maternal blood. With the advances in experimental technologies, it has been possible to coextract the fetal compartment of cfDNA in maternal plasma and to measure the amount of fetal cell-free nucleic acids according to the gene dosage.

Recently, NIPT with next-generation sequencing (NGS), which uses fetal cfDNA from maternal blood, has been adopted as a primary screening test for fetal chromosomal aneuploidy. NGS-based NIPT has been reported to have >99% sensitivity and specificity in detecting trisomy 21 (T21). In addition, the positive predictive value of NGS-based NIPT for screening T21 is around 80% in the general population. NIPT will contribute to decreasing the need for unnecessary invasive procedures, including amniocentesis and CVS, for confirming fetal aneuploidy. However, NGS has several limitations. First, it is more expensive than the current antenatal screening protocol, which could hinder the adoption of this innovative technique in clinical practice. Second, there is a risk of screening failure due to the limited amount of fetal DNA fraction for analysis. Third, NGS is time consuming—it requires 1 week before results are obtained.

Digital polymerase chain reaction (dPCR) is a novel approach for detecting and quantifying nucleic acid. dPCR provides real-time diagnostic advantages with higher sensitivity, accuracy, and absolute quantification than conventional quantitative PCR. It has been suggested that dPCR will decrease the cost by targeting specific sequences in the target chromosome, and dPCR-based noninvasive testing will facilitate progress toward the implementation of a noninvasive approach for screening for T21, T18 and T13.

Digital PCR

The dPCR is a novel method for the precise quantification of nucleic acid. Conventional PCR is referred to as the first generation of PCR. The first-generation PCR allows qualitative analysis by using agarose gel electrophoresis. However, quantitative analysis with conventional PCR is difficult. To complement the disadvantage of conventional PCR in quantitative analysis, the second generation of PCR, quantitative PCR (qPCR, real-time PCR) was introduced. The qPCR allows quantitative and qualitative analyses of target genes by monitoring the amplification of a targeted DNA molecule during PCR. However, qPCR has disadvantages such as varying results depending on PCR efficiency. dPCR is a new application for the detection and quantitative analysis of nucleic acids [17]. The concept of dPCR was introduced to overcome the limitation of qPCR. Unlike the relative analysis of qPCR that needs a standard curve for the measurement of target molecules, dPCR incorporates absolute quantification by counting the total

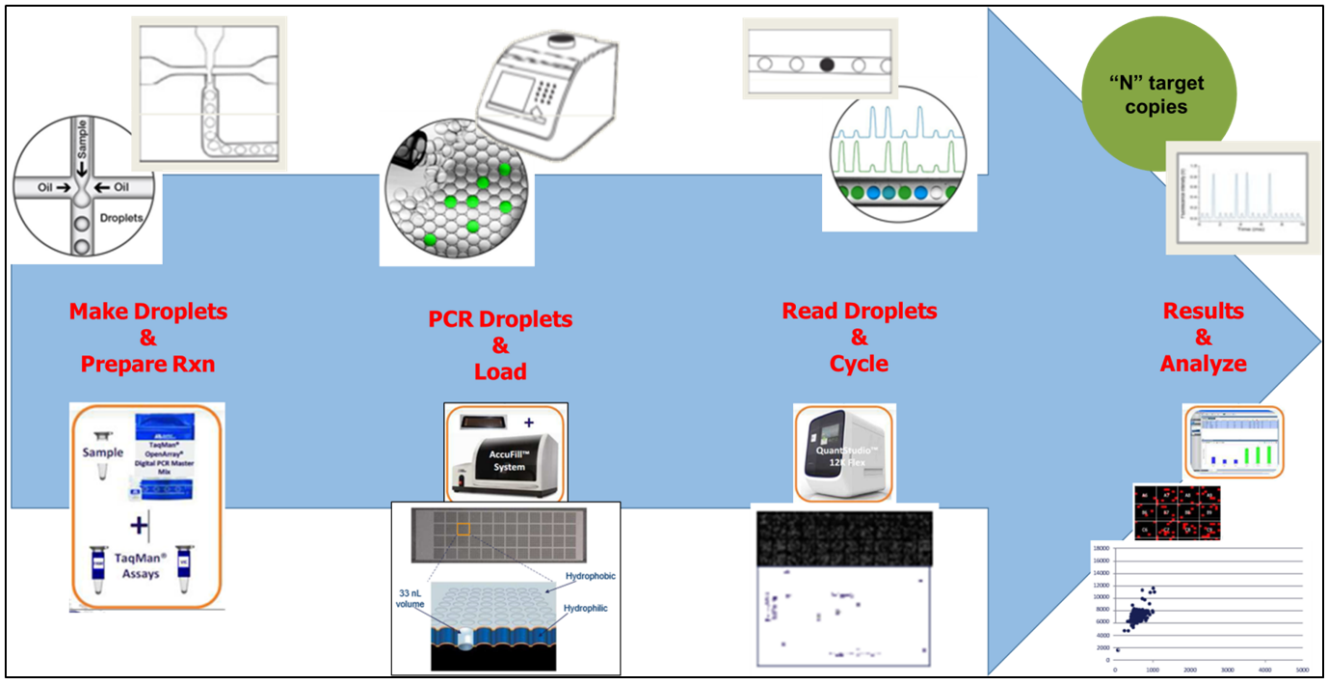

Fig. 1. An experimental method for digital polymerase chain reaction (PCR). Rxn, reaction.

number of target molecules in a digital format. It allows a more sensitive detection and accurate quantitative analysis of the target nucleic acid molecules [18-21].

In the dPCR procedure, the template DNA is diluted and divided into a large number of reaction compartments, allowing one target molecule to be located per two reaction compartments [22]. With the successful distribution of target molecules, positive amplification signals in each compartment reflect the amplification of a single target molecule. In consequence, absolute quantification of a target molecule can be applied in a digital format by counting each reaction compartment as either "1" or "0" for positive or negative signal, respectively. Analyzing the proportion of the "1" signal out of the total reactions, the concentration of the nondiluted original target molecule can be estimated. Increasing the number of reaction compartments improves the precision of quantification, thus enabling the resolution of small concentration differences [23].

There are two different platforms for dPCR; a droplet-based and a chip-based technique. Between the two platforms, the droplet-based approach has four steps for a stable experiment. Those steps consist in droplet formation, PCR reaction for each droplet, followed by droplet reading and result analysis. Once the droplet is formed, each droplet acts as a single well so that the PCR reaction occurs independently in each droplet. The chip-based platform consists in reaction preparation and automatic loading of the reaction mixture to the chip where the PCR reaction will be carried out in each well of the chip plate. The amplified reaction is then analyzed by counting the wells, followed by statistical analysis. On the basis of the results of analysis, it can be applied to various methods (Fig. 1).

Newly emerging dPCR areas have targeted a relatively small segment of the market [24]. However, the annual growth rate of dPCR technology is projected to increase to 52%, and its excellence has been verified during the introductory period. The market demand is increasing steadily as new applications, such as rare mutation detection and NIPT, are consistently suggested. Bio-Rad, Fluidigm, Life Technology, and Rain Dance Technologies entered the field of dPCR market in 2012 (Table 2). Moreover, many companies performing qPCR-based techniques and manufacturing qPCR equipment are relatively active. However, with the high price reaching 136,000 US dollars (USD), the wide use of such installations is difficult to expect, especially with the impact of economic recession [25].

NIPT and dPCR

As we described previously, it is necessary to develop a method for prenatal chromosome screening that can reduce the risk to the fetus related to the confirmatory procedure. Such a method should have relatively simple experimental requirements at a reasonable cost. Various types of NGS methods are being

Table 2. Digital polymerase chain reaction technology

Variable	QuantStudio 3D system	RainDrop system	QX200 system
Company	Thermo Scientific	RainDance Technologies	Bio-Rad
Partitions	20,000	10,000,000	20,000
Core technology description	Well type	Picoliter droplet	Nanoliter droplet
Product configurations	Closed platform	Open source platform	Closed platform
Chemistry	Probes and EvaGreen	Probes and EvaGreen	Probes and EvaGreen
Sample type	DNA, RNA, microRNA, cell-free DNA	DNA, RNA, microRNA, cell-free DNA	DNA, RNA, microRNA, cell-free DNA
Capacity	1 Sample/chip	8 Lanes/chip	96-Well plate
Multiplexing	Possibility	Possibility	Possibility
Run time (hr)	3.5	7	5.5

Table 3. Comparison between Next-generation sequencing (NGS) and digital polymerase chain reaction (dPCR) for noninvasive prenatal test (NIPT)

	NGS	dPCR
Purpose	NIPT	NIPT
Experiment time	>1 day	<7 hr
Target	Whole genome	Target site
Analysis	Sequence analysis	Copy number analysis
Experiment	Complex (various step)	Simple (three steps)
Cost	High	Low

developed for NIPT [26,27]. The NIPT approach with dPCR may provide comparable sensitivity and specificity at a reduced cost. This novel technique will contribute to making NIPT the primary screening test in the future (Table 3).

NIPT for the fetal aneuploidy requires technologies that provide stable, error-free, and secure information without losing genetic information of a fetal gene present in small amount in the pregnant woman's blood plasma. However, cfDNA analysis without any knowledge of the percentage of the fetal genetic material present in the blood or plasma of a pregnant woman can present problems with the reliability of results [28]. Separating fetal-derived genes to the complete exclusion of maternal-derived genes that are present in large amounts in the blood of pregnant women has technical difficulties and cost issues. Furthermore, in the course of amplifying the sample of blood or plasma of pregnant women, background signals due to the amplification of maternal-derived gene lead to a low reliability of the analysis of fetal-derived genes, and are likely to represent false-positive results [29]. Individual institutions incur high expenses for tests and experience difficulties in the interpretation of results.

Lo et al. [30] introduced dPCR for screening fetal T21 in 2007. The authors demonstrated the feasibility of dPCR in determining the allelic imbalance of a single nucleotide polymorphism in *PLAC4* mRNA of chromosome 21. In addition, they detected T21 prenatally by using a nonpolymorphism-based approach with dPCR. However, the sample that the authors used contained 25% of fetal DNA in maternal plasma, which was a relatively large amount. Fan et al. [31] applied microfluidic dPCR to diagnose fetal aneuploidy prenatally. By applying 12,765 digital array microfluidic chips, dPCR analysis accurately identified all fetal aneuploidies in the analyzed samples. In 2009, the estimated cost was only 400 USD. Although the authors used amniotic fluid and CVS samples for the study, they suggested NIPT with dPCR as an attractive tool for prenatal diagnosis because of its robustness and simplicity. Evans et al. [32] conducted power analysis. The authors developed a power equation and calculated the number of wells that were important for the experimental cost. With the model that was adopted in the study, 2,609 wells would be sufficient to achieve a 99% detection rate with a 1% false-positive rate. The authors demonstrated that dPCR is a potentially cheaper method for the diagnosis of T21.

dPCR has been adopted not only for the diagnosis of fetal aneuploidy but also for fetal rhesus D (RHD) genotyping, paternal *CFTR* (cystic fibrosis transmembrane conductance regulator) mutation, and fetal hemophilia, which is a bleeding disorder with X-linked inheritance and fetal RHD genotyping [33-36]. dPCR showed greater sensitivity than qPCR in identifying fetal sex and in RHD genotyping [37].

One of other genetic applications of dPCR is gene expression analysis, such as regulatory RNA molecules affecting basic cellular processes by microRNA [38]. The sensitivity of dPCR enables the detection of the methylation pattern in pathological states of pregnancy [39]. Moreover, gene copy number alterations and rare allele detection were also achieved by using

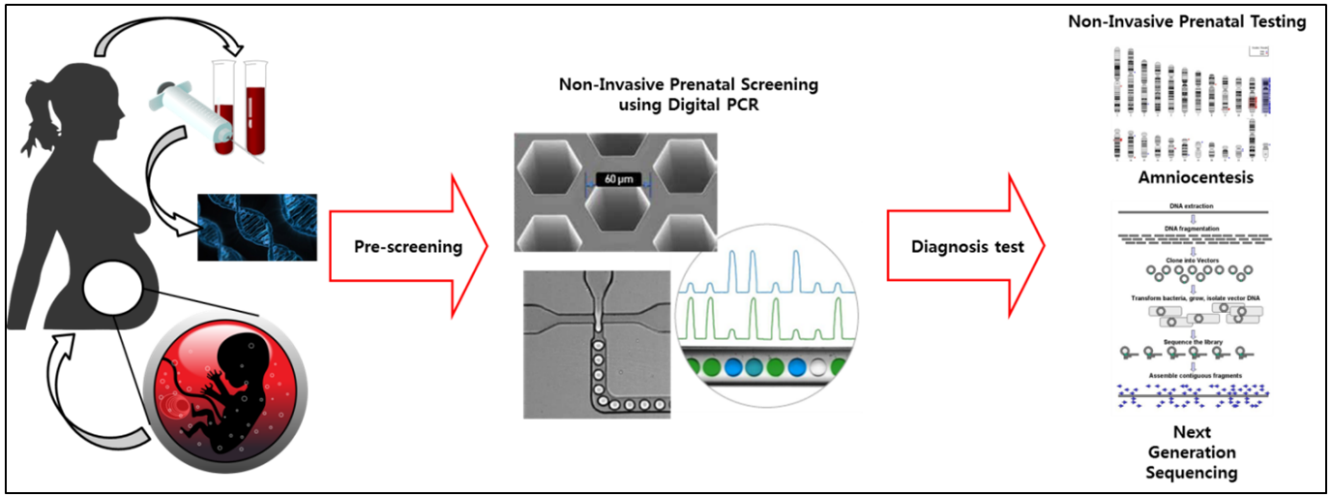

Fig. 2. Application of noninvasive prenatal test by using digital polymerase chain reaction (PCR).

dPCR [40].

dPCR is expected to satisfy the demand for prenatal screening with its accurate test results and relatively cheap cost. Moreover, it has a different competitive power from NGS as an early screening technology that can select patients who need further amniotic fluid testing. Because of the increasing demands for reduced experimental costs, NIPT with dPCR has attracted attention. Compared with NIPT with NGS, NIPT with dPCR would be more cost-effective and less time consuming.

Many NGS methods are being developed for noninvasive prenatal chromosomal screening; however, although these methods need a separate technique for diagnosis, there are limitations in performing these tests in individual hospitals owing to the high costs and difficulty in interpreting the results. Through its many advantages of real-time diagnosis, high sensitivity, and digitization of test results, dPCR has made it possible to develop a variety of diagnostic technologies without the use of a sequencing method.

NIPT with dPCR has several limitations. Unlike NIPT with NGS, the specific target detection of dPCR uses a single probe set; therefore, the sensitivity of each target detection has to be validated individually, which also limits the provision of big data for large-scale analysis. Moreover, unlike NGS, the dPCR approach cannot detect low-grade mosaicism. Furthermore, it is unable to detect chromosomal structural abnormalities such as balanced translocation. Lastly, there are few reports that demonstrate the feasibility of dPCR for detecting fetal aneuploidy antenatally.

Further studies on NIPT with dPCR as an ancillary diagnostic screening tool but not as a substituent, which could aid in increasing the detection rate of NIPT, are being actively performed. Recently, the cost of ancillary diagnosis is expected to be lowered by increasing the number of samples that need to be applied simultaneously, as well as through the development of the dPCR technique (Fig. 2).

Future Perspective

Studies on noninvasive prenatal screening with dPCR, which can quantitatively analyze a very small amount of nucleic acid, involve collecting and analyzing characteristic genetic information from fetal genetic material existing in small amounts in maternal blood and serum.Through these studies, it is anticipated that the method can be applied not only in noninvasive prenatal chromosomal screening but also in the early diagnosis of genetic diseases caused by abnormalities in gene copy number (microdeletions and repetitions). Moreover, by analyzing characteristic fetal gene markers, it is expected that the problem of being unable to detect numerical abnormalities in sparse fetal target genes, due to the abundant background maternal genetic signals when performing dPCR, can be solved. Therefore, through its simple technological applicability, relatively cheap cost, and accurate test results, dPCR is anticipated to be able to replace NGS methods, which are costly and difficult to interpret. Finally, the development of a technology that has great socioeconomic impact can be successfully achieved.

dPCR symbolizes the synergy that results from research in combined academic fields that create new knowledge and compensates for gaps in individual academic areas. The basic

technology is helpful for developing noninvasive methods that can replace invasive methods such as amniocentesis, and that can greatly reduce the risk to the mother and fetus. The large processing capability of dPCR is consistent with the need for a lowered sample volume or simultaneous multiple target detection in prenatal diagnostic screenings. It provides accurate diagnostic information, improves the patients' clinical outcome, and reduces patient management costs. Significant biomarkers can be identified through the development of the noninvasive prenatal screening method of maternal peripheral blood with dPCR, as an ancillary screening before the precise diagnosis. Ultimately, we expect that this method can help mitigate the national health expenditure for patient welfare by preventing unnecessary diagnostic costs. However, there are few studies that evaluate the feasibility of dPCR in detecting fetal aneuploidy. In addition, the cost-effectiveness of dPCR for NIPT should be demonstrated. Further studies are warranted to evaluate the feasibility and cost-effectiveness of the dPCR approach for NIPT.

References

1. Bili C, Divane A, Apessos A, Konstantinos T, Apostolos A, Ioannis B, et al. Prenatal diagnosis of common aneuploidies using quantitative fluorescent PCR. Prenat Diagn 2002;22:360-5.
2. Tepperberg J, Pettenati MJ, Rao PN, Lese CM, Rita D, Wyandt H, et al. Prenatal diagnosis using interphase fluorescence in situ hybridization (FISH): 2-year multi-center retrospective study and review of the literature. Prenat Diagn 2001;21:293-301.
3. Cuckle HS, van Lith JM. Appropriate biochemical parameters in first-trimester screening for Down syndrome. Prenat Diagn 1999;19:505-12.
4. Hayes A, Batshaw ML. Down syndrome. Pediatr Clin North Am 1993;40:523-35.
5. Bianchi D, Larrabee P, Johnson K; assignees. Prenatal diagnosis using cell-free fetal DNA in amniotic fluid. US 10/577,341. 2004, Oct 29.
6. Rasmussen SA, Wong LY, Yang Q, May KM, Friedman JM. Population-based analyses of mortality in trisomy 13 and trisomy 18. Pediatrics 2003;111:777-84.
7. Antonarakis SE, Lyle R, Dermitzakis ET, Reymond A, Deutsch S. Chromosome 21 and down syndrome: from genomics to pathophysiology. Nat Rev Genet 2004;5:725-38.
8. Desai SS. Down syndrome: a review of the literature. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1997;84:279-85.
9. Green RF, Devine O, Crider KS, Olney RS, Archer N, Olshan AF, et al; National Birth Defects Prevention Study. Association of paternal age and risk for major congenital anomalies from the National Birth Defects Prevention Study, 1997 to 2004. Ann Epidemiol 2010;20:241-9.
10. Goldberg JD, Golbus MS. Chorionic villus sampling. Adv Hum Genet 1988;17:1-25.
11. Wapner RJ, Jackson L. Chorionic villus sampling. Clin Obstet Gynecol 1988;31:328-44.
12. Wegner RD, Toennies H. Chorionic villi sampling. In: Wegner RD, ed. Diagnostic cytogenetics. Berlin: Springer, 1999:231-47.
13. Alfirevic Z, Mujezinovic F, Sundberg K. Amniocentesis and chorionic villus sampling for prenatal diagnosis (Review). Cochrane Database Syst Rev 2009;1:1-142.
14. Mujezinovic F, Alfirevic Z. Procedure-related complications of amniocentesis and chorionic villous sampling: a systematic review. Obstet Gynecol 2007;110:687-94.
15. Tongsong T, Wanapirak C, Kunavikatikul C, Sirirchotiyakul S, Piyamongkol W, Chanprapaph P. Cordocentesis at 16-24 weeks of gestation: experience of 1,320 cases. Prenat Diagn 2000;20:224-8.
16. Lo YM, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, et al. Presence of fetal DNA in maternal plasma and serum. Lancet 1997;350:485-7.
17. Pinheiro LB, Coleman VA, Hindson CM, Herrmann J, Hindson BJ, Bhat S, et al. Evaluation of a droplet digital polymerase chain reaction format for DNA copy number quantification. Anal Chem 2012;84:1003-11.
18. Benn P, Cuckle H, Pergament E. Non-invasive prenatal testing for aneuploidy: current status and future prospects. Ultrasound Obstet Gynecol 2013;42:15-33.
19. Kubista M, Andrade JM, Bengtsson M, Forootan A, Jonák J, Lind K, et al. The real-time polymerase chain reaction. Mol Aspects Med 2006;27:95-125.
20. Lo YM, Tein MS, Lau TK, Haines CJ, Leung TN, Poon PM, et al. Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis. Am J Hum Genet 1998;62:768-75.
21. Eisenstein BI. The polymerase chain reaction. A new method of using molecular genetics for medical diagnosis. N Engl J Med 1990;322:178-83.
22. Vogelstein B, Kinzler KW. Digital PCR. Proc Natl Acad Sci U S A 1999;96:9236-41.
23. Dube S, Qin J, Ramakrishnan R. Mathematical analysis of copy number variation in a DNA sample using digital PCR on a nanofluidic device. PLoS One 2008;3:e2876.
24. Day E, Dear PH, McCaughan F. Digital PCR strategies in the development and analysis of molecular biomarkers for personalized medicine. Methods 2013;59:101-7.

25. Chiu RW, Lo YM. Non-invasive prenatal diagnosis by fetal nucleic acid analysis in maternal plasma: the coming of age. Semin Fetal Neonatal Med 2011;16:88-93.
26. Kotsopoulou I, Tsoplou P, Mavrommatis K, Kroupis C. Non-invasive prenatal testing (NIPT): limitations on the way to become diagnosis. Diagnosis 2015;2:141-58.
27. Twiss P, Hill M, Daley R, Chitty LS. Non-invasive prenatal testing for Down syndrome. Semin Fetal Neonatal Med 2014;19:9-14.
28. Vaiopoulos AG, Athanasoula KC, Papantoniou N, Kolialexi A. Review: advances in non-invasive prenatal diagnosis. In Vivo 2013;27:165-70.
29. Evans MI, Kilpatrick M. Noninvasive prenatal diagnosis: 2010. Clin Lab Med 2010;30:655-65.
30. Lo YM, Lun FM, Chan KC, Tsui NB, Chong KC, Lau TK, et al. Digital PCR for the molecular detection of fetal chromosomal aneuploidy. Proc Natl Acad Sci U S A 2007;104:13116-21.
31. Fan HC, Blumenfeld YJ, El-Sayed YY, Chueh J, Quake SR. Microfluidic digital PCR enables rapid prenatal diagnosis of fetal aneuploidy. Am J Obstet Gynecol 2009;200:543.e1-7.
32. Evans MI, Wright DA, Pergament E, Cuckle HS, Nicolaides KH. Digital PCR for noninvasive detection of aneuploidy: power analysis equations for feasibility. Fetal Diagn Ther 2012;31:244-7.
33. Svobodová I, Pazourková E, Hořínek A, Novotná M, Calda P, Korabečná M. Performance of droplet digital PCR in non-invasive fetal RHD genotyping - Comparison with a routine real-time PCR based approach. PLoS One 2015;10:e0142572.
34. Debrand E, Lykoudi A, Bradshaw E, Allen SK. A non-invasive droplet digital PCR (ddPCR) assay to detect paternal CFTR mutations in the cell-free fetal DNA (cffDNA) of three pregnancies at risk of cystic fibrosis via compound heterozygosity. PLoS One 2015;10:e0142729.
35. Tsui NB, Hyland CA, Gardener GJ, Danon D, Fisk NM, Millard G, et al. Noninvasive fetal RHD genotyping by microfluidics digital PCR using maternal plasma from two alloimmunized women with the variant RHD(IVS3+1G>A) allele. Prenat Diagn 2013;33:1214-6.
36. Tsui NB, Kadir RA, Chan KC, Chi C, Mellars G, Tuddenham EG, et al. Noninvasive prenatal diagnosis of hemophilia by microfluidics digital PCR analysis of maternal plasma DNA. Blood 2011;117:3684-91.
37. Sillence KA, Roberts LA, Hollands HJ, Thompson HP, Kiernan M, Madgett TE, et al. Fetal sex and RHD genotyping with digital PCR demonstrates greater sensitivity than real-time PCR. Clin Chem 2015;61:1399-407.
38. Ma J, Li N, Guarnera M, Jiang F. Quantification of plasma miRNAs by digital PCR for cancer diagnosis. Biomark Insights 2013;8:127-36.
39. Li M, Chen WD, Papadopoulos N, Goodman SN, Bjerregaard NC, Laurberg S, et al. Sensitive digital quantification of DNA methylation in clinical samples. Nat Biotechnol 2009;27:858-63.
40. Hindson BJ, Ness KD, Masquelier DA, Belgrader P, Heredia NJ, Makarewicz AJ, et al. High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. Anal Chem 2011;83:8604-10.