

Next generation sequencing as a new detection strategy for maternal cell contamination in clinical prenatal samples

Xiaojie Lin¹, Huafeng Li¹, Ling Liu², Lingyin Kong³, Yan Mao³, Wen Li³, Yingying Xia³, Bo Liang⁴, Yanli Zhang¹

¹Women and Children's Hospital of Linyi City, China

²Pingyi Maternal and Child Health Hospital of Linyi City, China

³Basecare Medical Device Co., Ltd., China

⁴School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, China

ABSTRACT

Objectives: The maternal cell contamination in chorionic villus or amniotic fluid presents a serious preanalytical risk for prenatal misdiagnosis. The following study presents and validates a novel process for identifying MCC by detecting short tandem repeat markers on Ion Proton system. Initially, MCC testing was performed during the detection of chromosomal abnormalities so as to improve the detection efficiency and accuracy of this method.

Material and methods: More than 70 STR loci were selected to establish the detection progress. Capillary electrophoresis was used to compare the next generation sequencing detection results, as well as to identify the optimal STR on Ion Proton system. Evaluation criteria for maternal cell contamination were set, and the automated data analysis was performed. The detection sensitivity was validated via 4 groups with mixed samples and different proportions.

Results: Consequently, twenty-three clinical samples were tested to evaluate the detection accuracy. In addition, 14 reliable STR loci, which were stably detected in more than 25 samples, were found. The detection sensitivity in maternal cell contamination was no less than 20%, while its accuracy reached 100% in clinical samples.

Conclusions: Finally, we established and validated a novel detection procedure for maternal cell contamination in clinical prenatal samples using next generation sequencing. This procedure allowed us to simultaneously perform prenatal testing and MCC testing. Unlike the traditional capillary electrophoresis, this method is rapid, highly sensitive, and suitable for wide range of clinical applications.

Key words: STR, NGS, maternal cell contamination, prenatal diagnosis

Ginekologia Polska 2018; 89, 6: 326–334

INTRODUCTION

The detection of chromosomal abnormalities in prenatal diagnosis based on high-throughput sequencing could contribute to explaining the etiology of miscarriage and fetal abnormalities. This approach has gradually replaced karyotype analysis as the main technique in detecting in clinical chromosomal abnormalities [1]. The detection accuracy of this approach has shown to be highly correlated with the quality of clinical specimens. The quality of clinical speci-

mens is directly related with the accuracy of high-throughput sequencing detection, while the presence of maternal cell contamination in chorionic villus, miscarriage tissue or amniotic fluid samples poses a serious analytical risk for prenatal misdiagnosis [2]. According to existing literature, the proportion of the maternal cell contamination (MCC) in amniotic fluid samples is as high as 25% [3]. However, contamination of clinical samples with maternal cells can be a potential source of error when applying high sensitivity de-

Corresponding author:

Yanli Zhang

Women and Children's Hospital of Linyi City, Shandong Province, 1 Qinghenan Road, Bingheyuan, Linyi, Shandong, 276000 Linyi, China

e-mail: lysfybjy@163.com

tection technologies, such as high-throughput sequencing technology. Therefore, it is of great importance to accurately verify the potential presence of MCC in prenatal samples.

At present, the routine detection of MCC is performed by capillary electrophoresis with fluorescent labeled microsatellites. Short tandem repeats, consisting of 2–6 repeating bases, are found in the genome of both, humans and mammals. They are highly polymorphic, and are used as an important biomarker in forensic identification [4, 5]. The short tandem repeat (STR) that has a very high degree of polymorphism, can be used for the detection of MCC by distinguishing whether the same detection STR allele peak appears in maternal sample [6, 7] However, the long detection period makes this method unsuitable for clinical applications. It is also inconvenient since it requires for large amounts of clinical testing, especially, when compared to next generation sequencing used for detection of chromosomal abnormalities from miscarried tissue. The method developed in the present study can be used to simultaneously detect MCC and miscarried tissue chromosomal abnormalities using next generation sequencing (NGS) system. It is eminently suitable for clinical application due to the low cost, fast applicability and high sensitivity. In the present study, we developed a method for detection of MCC by identifying STR markers using Ion Proton system. The validation of this method in clinical samples proved it suitable for MCC detection, thus providing a valuable reference for MCC in prenatal diagnosis.

MATERIAL AND METHODS

Sample collection

Different samples were collected for diverse experiments in Women and Children's Hospital of Linyi City. This study was approved by the Medicine Ethics Committee of Women and Children's Hospital of Linyi City. All the DNA were extracted using TIANamp Genomic DNA Kit (DP304), and were quantified using Qubit ds DNA HS Assay kit (Q32854). First, 6 samples were randomly collected from healthy individuals so as to investigate the eligibility and assessment of detecting STR using Ion Proton System. These samples were also used for detecting consistency among NGS-STR and CE-STR. Then, few artificial preparation samples were used to examine the sensitivity of NGS-STR. Consequently, four group samples were collected, and they were obtained from mother-son or mother-daughter. Mixtures of DNA samples from two families were prepared in mixture ratios of 1:9, 1:4 and 3:7 (mother: child). A total of 23 clinical samples were used for the detection of chromosomal abnormalities. Three positive samples revealed maternal cell contamination in chromosome abnormalities, while the 20 negative samples showed no chromosome abnormalities.

Process of testing

Sample detection was done according to four steps: primer design, amplification, sequencing and data analysis.

Primer design

The selected STR markers were collected from STR database TPMD, while the DNA sequence information of target markers were collected from GenBank (<http://www.ncbi.nlm.nih.gov/genbank>). All STR markers were highly polymorphic. Primers for PCR amplification were designed using Primer5 software such that they were located in conservative region. The amplicon size of the STR markers was < 180 bp because of the reads limits on Ion Proton system.

PCR amplification

The DNAs from collected and prepared samples were used as templates for the PCR reaction. All the microsatellites were amplified by Premix Taq (TaKaRa Taq Version 2.0, R004A). PCR reaction was performed in a 20 μ L reaction volume containing 10 ng of template DNA, 10 μ L of 10 \times Premix Taq mix, 0.5 mM of each primer, and nuclease-free water. The optimal TM amplification was calculated using the following formula: (TM of forward primer + TM of reverse primer)/2–2.5. The primer extension was cycled for 30 cycles with 15 s at 95°C, 15 s at optimal TM, and 15 s at 72°C.

The amplification products were quantified and mixed at an equivalent amount per STR product. The total mixed products were purified by the Agencourt Ampure XP Reagent (Beckman, A63882), which was added to 1.5 \times volume. The purified STR products were eluted with nuclease-free water.

Sequencing

Library preparation was performed using the Ion Plus Fragment Library Kit Each (Life Technologies, 4471252) with no more than 300 ng of purified PCR products, following the manufacturer's instructions; in addition, no fragmentation was required. The DNA concentration of the libraries was estimated using the Qubit ds DNA HS Assay kit (Life Technologies, Q32854) according to the manufacturer's instructions. Finally, the library was sequenced on Ion Proton System (Life Technologies) using the Ion Pi Hiq Seq 200 Kit 8 Init 8 Init (Life Technologies, A26772). The sample loading was about 50 pmol per library, and 0.5M of raw reads were obtained as expected.

NGS data analysis

The sequencing analysis was done according to previously described approach [8]. Some parameters were adjusted according to the Ion Proton System. The redundant sequences were filtered by filter rate so as to obtain the exact alleles.

Capillary Electrophoresis

The fluorescence primers for capillary electrophoresis were synthesized according to the primer sequences in next generation primer sequencing, while the STR products with fluorescence were sent for testing using Applied Biosystems 3730xl DNA Analyzer. Output data were analyzed using GeneMapper Software Version 4.0 (Applied Biosystems).

RESULTS

The feasibility of detecting STR on Ion Proton System

The read lengths on Ion Proton System P1 chip were not longer than 275bp, leading to shorter DNA fragment lengths. Nevertheless, most STRs have longer length than the reading length, varying from 100bp to 300bp, with repeats of 2bp mostly. In theory, the alleles in uncontaminated samples were no longer than 2, nevertheless more than 2 alleles were detected in an uncontaminated sample while the STR repeats in 2bp. Moreover, we took into

consideration the redundant peaks, where stutters were in the majority. Stutter is a minor PCR product produced in STR amplification, which is one repeat shorter or longer than its corresponding allele. A proposed mechanism for stuttering is slipped strand mispairing during PCR, resulting in either the insertion or deletion of one repeat unit on the new strand [9]. We found that the redundant peaks were high in 2bp repeats STR reads, and sometimes they reached 50% as shown in Figure 1, thus leading to incorrect interpretation of the results. When the STR repeats were 3bp or 4bp, then the amplicon reads were sequenced with relatively low rate and number of redundant peaks, while the rate of the redundant peaks was no more than 10% in this process. The filter rate of 10% means that the redundant reads account for 10% of total allele reads. We can filter out these stutters and redundant reads through different ratios, while the ratio varies from different STR. The STR detecting on Proton has two essential requirements after evaluation: STR length no longer than 180bp, and repeats in 3bp or 4bp. The better

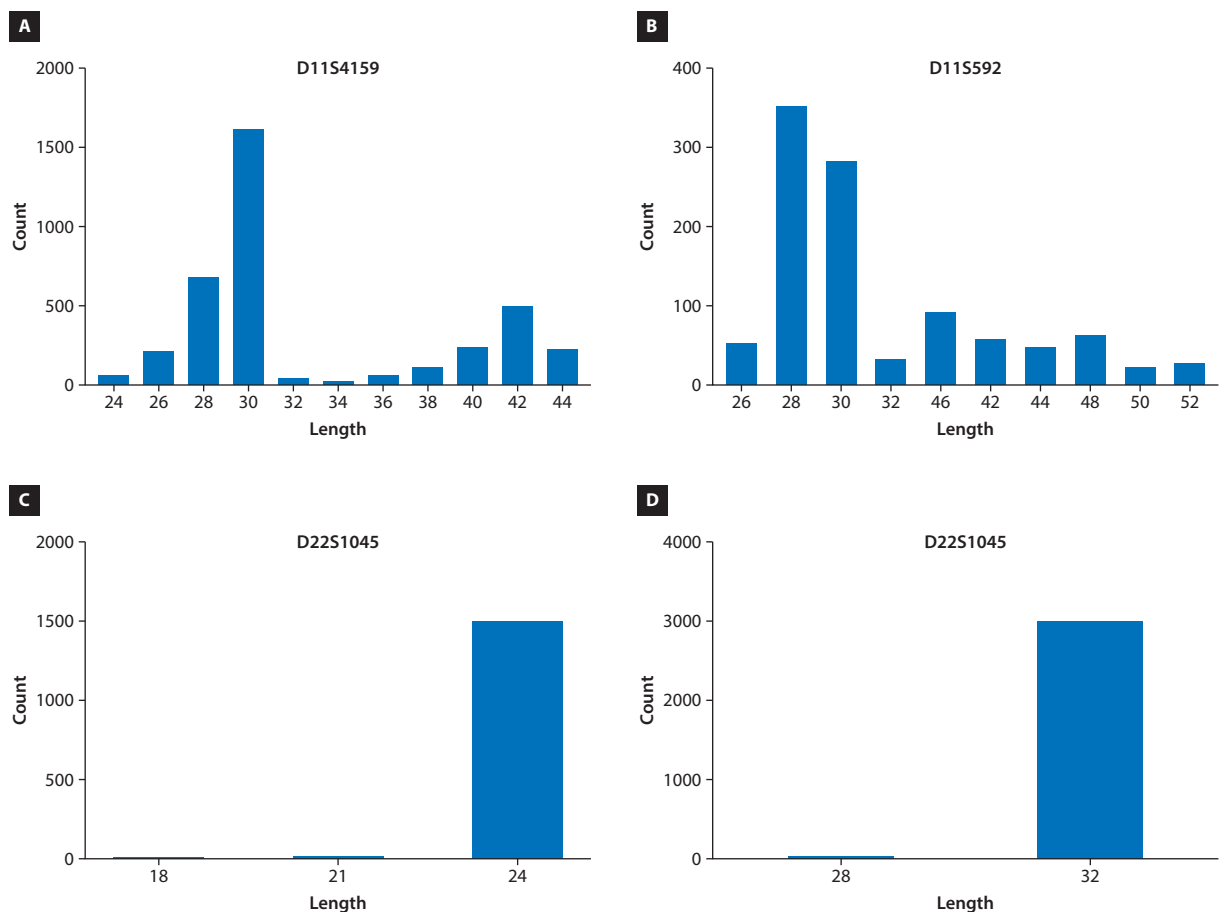


Figure 1. Sequencing results in STR loci of different types x-axis represents the repeat length of the corresponding STR loci alleles, while the y-axis is the number of reads. D11S4159 and D11S925 in figure 1A/1B are STR loci of 2bp repeat, while D22S1045 in figure 1C has 3bp repeat, TPOX in figure 1D has 4bp repeat. It is obvious that D11S4159 and D11S925 have multiple alleles, which result in the failed interpretation of true alleles. However, we can get the clear peaks in D22S1045 and TPOX after the filtering of stutter

detecting effect depends on the short STR length, while the total number of reads for long length STR is incredibly low because of the limitations related to read length.

Selection and assessment of STR

According to the specific requirements for detection of STR using Ion Proton System, 16 highly polymorphic STRs which met the qualifications were selected in the present study. Eight single samples from 4 family groups were used for the verification experiment; 14 STR loci with high stability were found (Fig. 2 shows the NGS results from 14 STR loci in normal human samples). The selection standard for STR loci was based on the sequencing quality. One of the requirements was that the sequencing read number was > 100 for each allele. The most important factor was the redundant peak rate,

which was no more than 10%. Thus, we could obtain low spare peak rates that in turn could be filtered in sequencing analysis.

Detecting consistency among NGS-STR and CE-STR

In consideration of the international golden standard, i.e. using capillary electrophoresis for STR testing, we further examined a comparison between capillary electrophoresis and NGS in detecting STR, aiming at validating the accuracy of STR testing on Ion Proton system. The data in Table 1 show the difference between two detection methods. Briefly, we find that the STR detection by next generation sequencing is more accurate compared to capillary electrophoresis approach. The testing in capillary electrophoresis has 1–2bp bias in STR length, because of the deviation from platform

Table 1. Consistency detection among NGS-STR and CE-STR

STR	Detecting Method	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
D6S1017	CE	153/161	161/172	169/172	161/172	161	161/169
	NGS	151/159	159/171	167/171	159/171	159	159/167
D12S391	CE	114/118	122/126	114/122	118	118/122	114
	NGS	115/119	123/127	115/123	119	119	115
D17S1301	CE	152/156	144/148	148/156	148/152	156/160	153/157
	NGS	154/158	146/150	150/158	150/154	158/162	154/158
GATA165B12	CE	127/140	131	127/131	132/136	131/139	127/131
	NGS	129/141	133	129/133	133/137	133/141	129/13
GATAD172D05	CE	122/126	106/126	122/130	122	106/126	122
	NGS	120/124	108/128	124/132	124	124/128	124
DXS6804	CE	114	114/126	114/118	114	114/126	122
	NGS	115	115/127	115/119	115	115/127	123
DXS7133	CE	112/116	111	111	111/115	111/115	115
	NGS	114/118	114	114	114/118	114/118	118
DXS9902	CE	176/180	172	172	172/176	180	176/180
	NG	176/180	172	172	172/176	180	176/180
D22S1045	CE	155/158	152/155	155	139/152	152	152/158
	NGS	153/156	150/153	153	138/150	150	150/156
D8S1179	CE	179/183	175/183	179	171/180	185/193	171/180
	NGS	181/185	177/185	181	173/181	185/193	172/181
TH01	CE	170	162/170	170	158/170	162	162/170
	NGS	170	162/170	170	158/170	162	162/170
TPOX	CE	113	113/125	124	113/125	112	112/125
	NGS	114	114/126	126	114/126	114	114/126
D16S539	CE	150/154	150/158	150/162	150/162	150/162	162/166
	NGS	149/153	149/157	149/161	149/161	149/161	161/165
D13S1492	CE	133/141	129/150	137/150	–	–	–
	NGS	133/141	129/149	137/149	–	–	–

The data in Table 1 shows high consistency among NGS and CE in detecting STR. The CE data represent the detected STR loci length, while the NGS data show the assemble length which combined detected repeat length with non-repeat length theoretically of the STR loci. The differences in allele length at one sample show high uniformity. The lines“–” mean that D13S1492 have not been detected in these samples

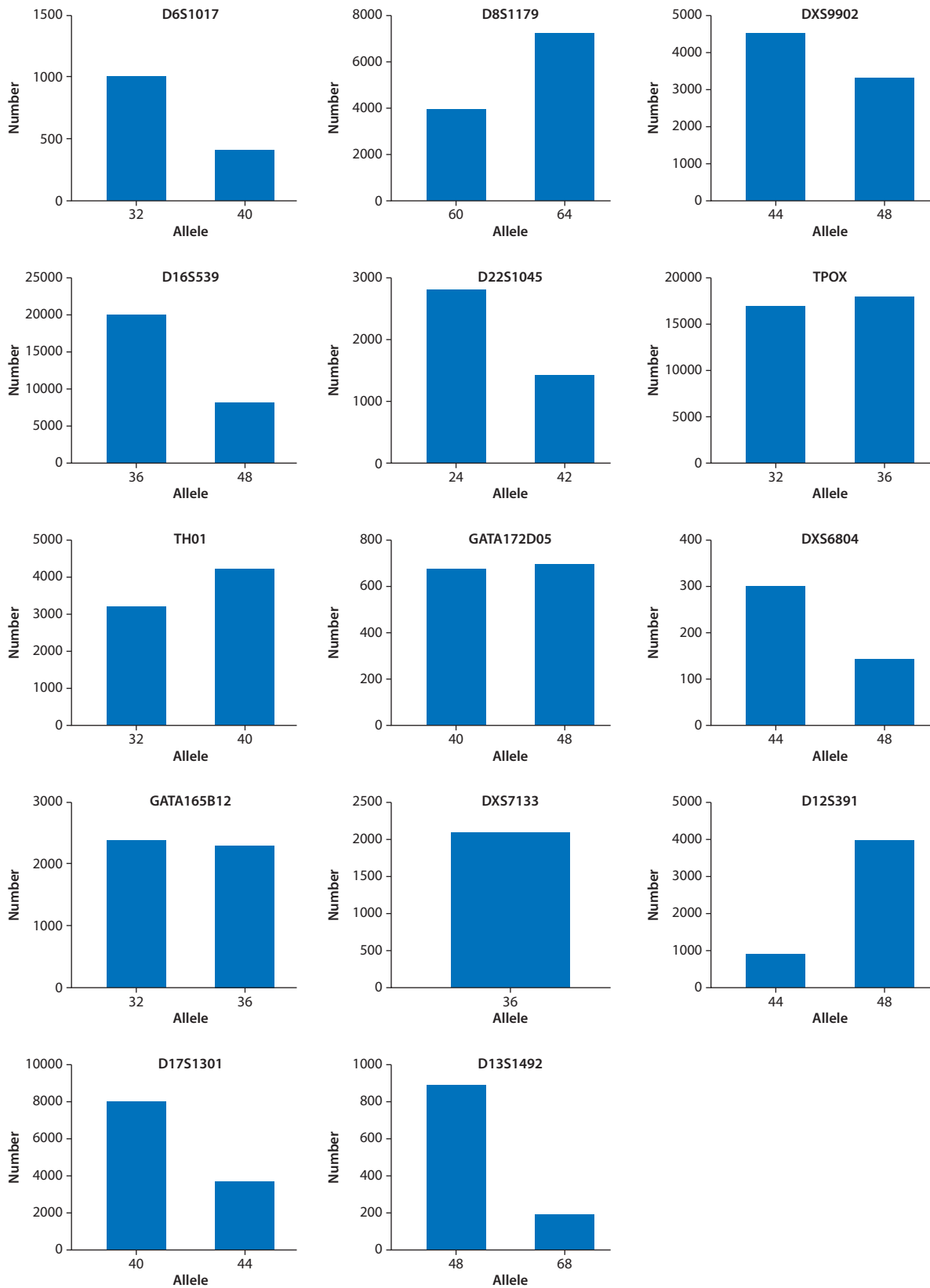


Figure 2. The sequencing map of 14 STR on Ion Proton System 14 STR peaks in NGS data. x-axis represents repeated length of different alleles, while the y-axis is the number of reads. The peak height represents the number for corresponding alleles. The figure shows the alleles with reads filter rate of 10%

and process. The deviation is an objective existence. Since the primer sequences were the same in two testing platforms, the STR length should in theory be the same. The STR length in NGS method (Tab. 1) is the actual length obtained by adding the detected sequencing length in repeat region and non-repeat region, while the length in capillary electrophoresis method shows the full length of STR product. Sometimes 3' A-tail was added to STR products by using the r-Taq polymerase, however, the addition of A-tail failed because of the amplification compositions. Therefore, taking into consideration the "A" tail and deviation in capillary electrophoresis platform, we assumed that the difference between two detection methods was objective and acceptable. Therefore, we analyzed the detecting consistency among NGS-STR and CE-STR. The aberration in alleles of one STR loci showed to be the same between NGS and CE, which supported the consistency among NGS-STR and CE-STR.

Standard of MCC interpretation

It is recommended that two to three informative microsatellite markers reflecting clearly definable, separate maternal and fetal genotypes from among a panel of approximately 7 to 10 markers be used to assess the presence of MCC7. While the maternal sample is absent, we can only get the information from fetal genotypes. One simple sample can provide only two different allele genotypes in one STR loci. If three alleles of the autosomal STR (or two X-chromosome STR alleles in sample of male fetus) appear in one fetal sample, one of the alleles must come from maternal DNA. Therefore, we regarded the STR loci as an informative microsatellite marker. Correspondingly, 3 main peaks appeared on the autosomal STR diagram, or 2 main peaks were displayed on the X-chromosome STR diagram. Generally, the contamination proportion was consistent with the rate of the low amount allele which was from maternal genotype. With maternal sample, MCC interpretation is much easier, since the fetal genotypes are compared with the maternal genotypes. If the fetal alleles are the same as maternal alleles in STR loci, the STR loci can be regarded as an informative microsatellite marker.

Standard of data analysis

The data were analyzed and evaluated according to the existing literature [8]. The STR analysis threshold was set based on the results detected from the accumulative samples. Sequencing error and PCR stutters exist objectively. Stutters are produced in PCR amplification when one repeat unit is shorter or longer than the parent allele. Stutters made up approximately 10% of the sequence reads, while the stutters rates differ in different STR [10]. Due to sequencing error and PCR stutters, these sequence reads can be filtered by analysis threshold so as to make accurate interpretation.

Table 2. Filter rate of STR

STR	Filter Rate
D6S1017	8.0%
D12S391	8.0%
D17S1301	8.0%
GATA165B12	9.0%
GATA172D05	5.0%
DXS6804	7.0%
DXS7133	6.0%
DXS9902	7.0%
D22S1045	8.0%
D8S1179	8.0%
TH01	6.5%
TPOX	5.0%
D16S539	7.0%
D13S1492	7.0%

The filter rate list is shown in Table 2. These analysis threshold values were determined in view of the expected allele reads for maternal minor component from the total reads at each of the mixture proportion in below 4 family samples, while in the previous studies threshold value was set to 10% or 5% following the same approach [11].

Sensitivity of NGS-STR

Four group mother-child samples were used to evaluate the detecting sensitivity. Mixtures were prepared in mixture ratios of 1:9, 1:4 and 3:7 (mother: child), with the proportion of maternal DNA 10%, 20% and 30%, respectively. The mother sample and child sample were tested so as to obtain the correct alleles in single sample. Consequently, the alleles in the mixtures were analyzed according to the results from a single sample. Thus, the detecting sensitivity was analyzed through the emerged maternal allele in corresponding proportion of mixtures. As shown in Table 3, we found that < 20% mixtures could be identified by 14 STR loci following this detection process. Unfortunately, certain 10% mixtures could not be identified, since the actual allele rate was similar to stutters rate in some STR loci.

Validation of positive and negative clinical samples

Three samples were not detected because of suspected MCC in chromosome karyotypes analysis. These three samples were also validated by capillary electrophoresis, which showed consistency with the NGS results. We tested the 3 samples with the following process and method. Sample 1 and Sample 3 were male fetuses, while Sample 2 was a female fetus. Sex of fetuses helped us to get more accurate

Table 3. The NGS-STR results from 4 samples groups

STR	A	B	A/B = 1:4	A/B = 3:7	C	D	C/D = 1:4	C/D = 3:7	E	F	E/F = 1:4	E/F = 3:7	G	H	G/H = 1:4	G/H = 3:7
D6S1017	40/48	48/52	40/48/52	40/48/52	40/52	40	40	40	32/40	32/40	32/40	32/40	40/48	32/48	32/40/48	32/40/48
D12S391	52/56	48/56	48/52/56	48/52/56	48/52	52/56	48/52/56	48/52/56	44/48	44/48	44/48	44/48	48/56	52/56	48/52/56	48/52/56
D17S1301	48/52	44/48	44/48	44/48/52	40/44	40/44	40/44	40/44	40/44	40/44	40/44	40/44	40/48	36/40	36/40	40/48/36
GATA165B12	32/36	36	32/36	32/36	36	36/40	36/40	36/40	32/40	32	32/40	32/40	32/40	32	32/40	32/40
GATA172D05	32/40	40	32/40	32/40	40/44	32/40	32/40/44	32/40/44	32/44	44	32/44	32/44	24/44	24/44	24/44	24/44
DXS6804	44/48	44/48	44/48	44/48	52/56	52/56	52/56	52/56	52/56	52/56	52/56	52/56	44	44/56	44/56	44/56
DXS7133	36	36	36	36	36/40	36/40	36/40	32/40	36	36	36	36	36/40	36	36/40	36/40
DXS9902	40/48	48	40/48	40/48	40/44	40	40	40/44	44/48	48	44/48	44/48	44/48	44/48	44/48	44/48
D22S1045	30/36	36	30/36	30/36	36	36/42	36/42	36/42	24/36	24/42	24/36/42	24/36/42	21/24	24/42	24/42	21/24/42
D8S1179	48/52	44/52	44/48/52	44/48/52	52/56	48/56	48/52/56	48/52/56	56/64	60/64	56/60/64	56/60/64	48/52	48/52	48/52	48/52
TH01	28/36	28/36	28/36	28/36	28	28/36	28/36	28/36	32/36	36	32/36	32/36	36	36	36	36
TPOX	32/44	32/44	32/44	32/44	32/44	32/44	32/44	32/44	32	32/36	32/36	32/36	32/44	32/44	32/44	32/44
D16S539	36/48	36/48	36/48	36/48	36/44	36/52	36/44/52	36/44/52	48/52	36/48	36/48	36/48	40/44	40/44	40/44	40/44
D13S1492	48/68	48/56	48/56/68	48/56/68	52/60	56/60	52/56/60	-	-	-	-	-	-	-	-	-

Samples A, C, E and G represent maternal DNA, while B, D, F and H represent child's DNA. The numbers shown in the table stand for the repeat region length of STR allele. The red fonts are the maternal allele which weren't detected in the mixtures. The lines "—" mean that D13S1492 were not detected in those samples. Data shown in the tables are the final alleles after the sequencing raw data were filtered. The detected results in 10% mixture were not shown in the table due to low detection rate of maternal STR allele

and convenient discrimination in laboratory experiments; no more than one allele was found in a single male fetus while the STR were located in chromosome X. STR of DXS6804 and GATA172D05 were in X chromosome, while other STR loci were autosomal STR. According to the standard criteria for MCC detection, more than 2 informative microsatellite markers need to be detected in 3 samples. The 3 abortive tissue samples revealed MCC, not only in capillary electrophoresis system but also using the next generation system. In Figure 3, we show both the CE result and the NGS result, while the CE figure implies the total length of STR products in this sample, and the NGS figure stands for the repeat length of STR in the sample. Although the results displayed the different form of length, the number of alleles and the difference in length between alleles showed high consistency in the same STR.

Twenty normal samples in chromosome karyotypes analysis, which were from different sample types of CVS, AF and blood, were collected to verify the detecting method. All of the samples suggested the absence of MCC under this test. Also, all of the twenty samples were tested by capillary electrophoresis, which revealed the same results as NGS, i.e. 20 examined samples were negative for MCC.

DISCUSSION

MCC in a prenatal diagnosis is an important factor, which is often overlooked by many laboratories. The British Association of Clinical Molecular Genetics (CMGS) developed practice guidelines for testing maternal cell contamination in prenatal samples for molecular studies. According to these, all prenatal diagnoses for single gene disorders should include MCC testing [12]. In 2010, American Society for Investigative Pathology and the Association for Molecular Pathology published the Laboratory Guidelines for Detection, Interpretation, and Reporting of Maternal Cell Contamination in Prenatal Analyses, suggesting that maternal samples should be obtained parallel with prenatal specimens so as to eliminate the potential impact of MCC [7]. These guidelines state that maternal and prenatal specimens should be tested and analyzed for MCC concurrently within the same analysis so as to allow for a direct comparison of results. The basic premise is the comparison of highly polymorphic short tandem repeat/microsatellite loci between the maternal and fetal DNA samples. It has been suggested that two to three informative microsatellite markers can clearly reflect the presence of MCC, while 7 to 10 markers are enough to assess the

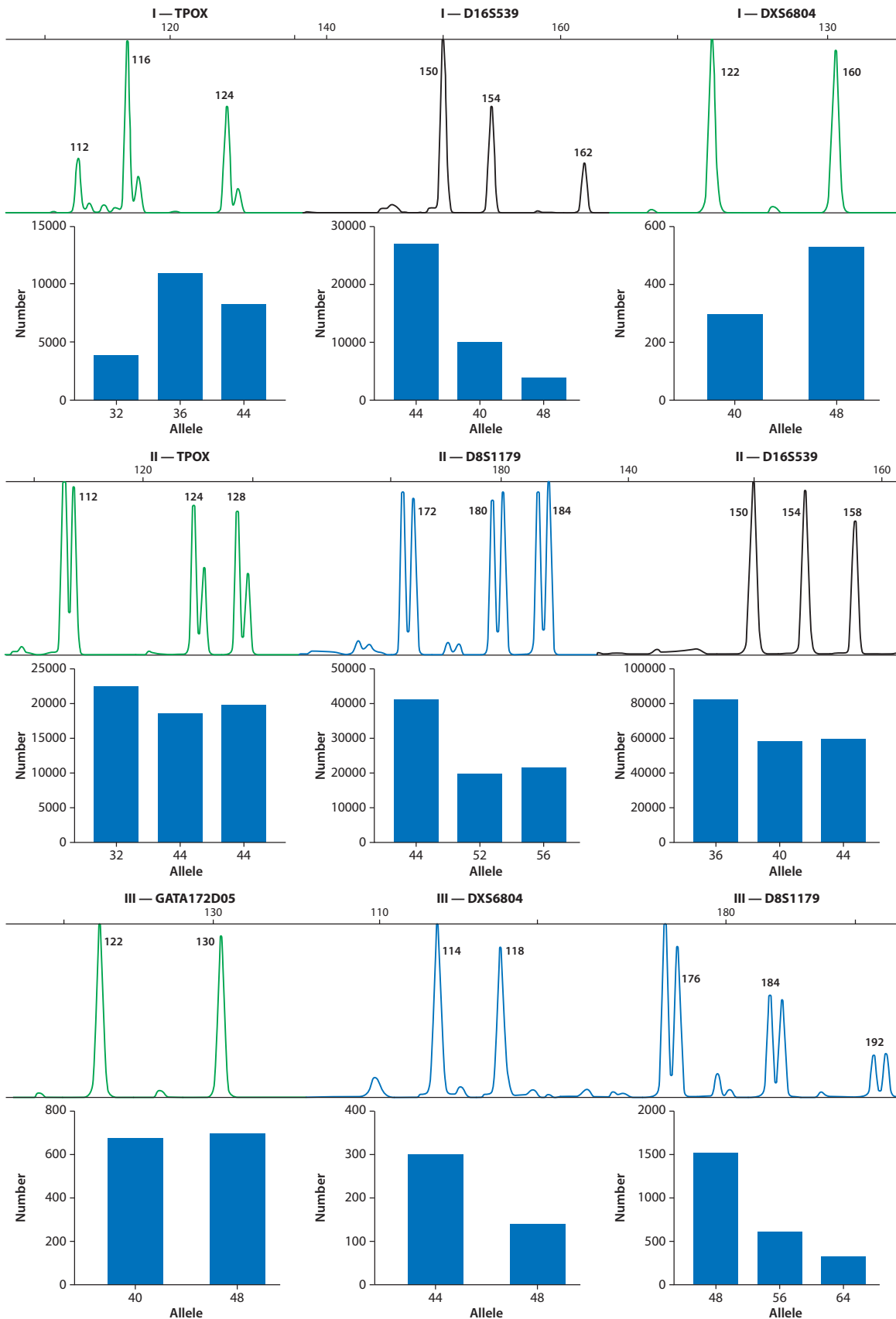


Figure 3. The detection results of 3 positive MCC samples by using NGS-STR and CE-STR
 The MCC detected results using NGS-STR from 3 uncertain MCC in chromosomes karyotypes analysis. Sample I, II and III represents different positive sample. The upper graphs are the informative STR results by capillary electrophoresis, while the below graph shows the same informative STR results by NGS. Other non-informative results were not displayed

presence of MCC. However, in order to save time and cost in detection of prenatal analyses, in most of the cases only the fetus analysis is performed. In the US, most of the laboratories perform MCC detection on prenatal diagnosis clinical sample [6]. The level of detection for MCC assays varies from 1 to 20%, while the number of markers ranges from 9 to 16 [13]. In addition, previous studies have suggested the use of STR biomarkers for detection of MCC [14, 15].

Different studies have revealed different incidence rates of maternal contamination in chorionic villus, miscarriage tissue or amniotic fluid samples in prenatal diagnosis [16, 17]. Some of the existing studies have shown that the proportion of MCC in amniotic fluid samples is higher compared to sample types, while some other studies have indicated that the chorionic villus has higher proportion [18, 19]. However, it is necessary to perform MCC detection in prenatal diagnosis regardless of these incidence variations among sample types [6, 20]. The existing methods used in clinical applications for the routine detection of MCC have many disadvantages such as the high cost, long application period and lack of automatic data analysis.

In the present study, we developed an effective method for detection of maternal cell contamination using STR biomarkers. Fourteen STRs with high polymorphism were verified in MCC detection using Ion Proton system. The validation with positive and negative samples supported the feasibility of the process suggested for the detection of MCC. The developed assay shows many advantages in MCC testing such as lower detection limit of 20%; simple process of detection in the absence of maternal samples; quick testing time (within one working day); automatic analysis in sequencing data; lower cost and extremely low output data. Also, the STR products can be prepared for library construction and can be mixed with any other fragmented product samples.

CONCLUSIONS

In the present study, we introduced a rapid and highly-sensitive method for MCC in prenatal diagnosis, which may be used as a reference method for MCC detection.

Conflict of interests

The authors declare no competing financial interests.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

REFERENCES

- Martín J, Cervero A, Mir P, et al. The impact of next-generation sequencing technology on preimplantation genetic diagnosis and screening. *Fertil Steril*. 2013; 99(4): 1054–61.e3, doi: [10.1016/j.fertnstert.2013.02.001](https://doi.org/10.1016/j.fertnstert.2013.02.001), indexed in Pubmed: [23499002](https://pubmed.ncbi.nlm.nih.gov/23499002/).
- Benn PA, Gilbert F, Hsu LY. Maternal cell contamination of amniotic fluid cell cultures from two consecutive pregnancies complicated by fibroids. *Prenat Diagn*. 1984; 4(2): 151–153, doi: [10.1002/pd.1970040211](https://doi.org/10.1002/pd.1970040211), indexed in Pubmed: [6739438](https://pubmed.ncbi.nlm.nih.gov/6739438/).
- Weida J, Patil AS, Schubert FP, et al. Prevalence of maternal cell contamination in amniotic fluid samples. *J Matern Fetal Neonatal Med*. 2017; 30(17): 2133–2137, doi: [10.1080/14767058.2016.1240162](https://doi.org/10.1080/14767058.2016.1240162), indexed in Pubmed: [27744775](https://pubmed.ncbi.nlm.nih.gov/27744775/).
- Ellegren H. Microsatellites: simple sequences with complex evolution. *Nat Rev Genet*. 2004; 5(6): 435–445, doi: [10.1038/nrg1348](https://doi.org/10.1038/nrg1348), indexed in Pubmed: [15153996](https://pubmed.ncbi.nlm.nih.gov/15153996/).
- Novokmet N, Marjanović D, Skaro V, et al. Forensic efficiency parameters for the 15 STR loci in the population of the island of Cres (Croatia). *Coll Antropol*. 2009; 33(4): 1319–1322, indexed in Pubmed: [20102087](https://pubmed.ncbi.nlm.nih.gov/20102087/).
- Schrijver I, Cherny SC, Zehnder JL. Testing for maternal cell contamination in prenatal samples: a comprehensive survey of current diagnostic practices in 35 molecular diagnostic laboratories. *J Mol Diagn*. 2007; 9(3): 394–400, doi: [10.2353/jmoldx.2007.070017](https://doi.org/10.2353/jmoldx.2007.070017), indexed in Pubmed: [17591939](https://pubmed.ncbi.nlm.nih.gov/17591939/).
- Nagan N, Faulkner NE, Curtis C, et al. MCC Guidelines Working Group of the Association for Molecular Pathology Clinical Practice Committee. Laboratory guidelines for detection, interpretation, and reporting of maternal cell contamination in prenatal analyses a report of the association for molecular pathology. *J Mol Diagn*. 2011; 13(1): 7–11, doi: [10.1016/j.jmoldx.2010.11.013](https://doi.org/10.1016/j.jmoldx.2010.11.013), indexed in Pubmed: [21227389](https://pubmed.ncbi.nlm.nih.gov/21227389/).
- Gymrek M, Golan D, Rosset S, et al. lobSTR: A short tandem repeat profiler for personal genomes. *Genome Res*. 2012; 22(6): 1154–1162, doi: [10.1101/gr.135780.111](https://doi.org/10.1101/gr.135780.111), indexed in Pubmed: [22522390](https://pubmed.ncbi.nlm.nih.gov/22522390/).
- Brookes C, Bright JA, Harbison S, et al. Characterising stutter in forensic STR multiplexes. *Forensic Sci Int Genet*. 2012; 6(1): 58–63, doi: [10.1016/j.fsigen.2011.02.001](https://doi.org/10.1016/j.fsigen.2011.02.001), indexed in Pubmed: [21388903](https://pubmed.ncbi.nlm.nih.gov/21388903/).
- Bornman DM, Hester ME, Schuetter JM, et al. Short-read, high-throughput sequencing technology for STR genotyping. *Biotech Rapid Dispat-ches*. 2012; 2012: 1–6, doi: [10.2144/000113857](https://doi.org/10.2144/000113857), indexed in Pubmed: [25621315](https://pubmed.ncbi.nlm.nih.gov/25621315/).
- Kim EH, Lee HY, Yang InS, et al. Massively parallel sequencing of 17 commonly used forensic autosomal STRs and amelogenin with small amplicons. *Forensic Sci Int Genet*. 2016; 22: 1–7, doi: [10.1016/j.fsigen.2016.01.001](https://doi.org/10.1016/j.fsigen.2016.01.001), indexed in Pubmed: [26799314](https://pubmed.ncbi.nlm.nih.gov/26799314/).
- Allen S, Mountford R, Butler A et al. Practice guidelines for the Testing for maternal cell contamination (MCC) in prenatal samples for molecular studies. 2007.
- Schrijver I, Cherny SC, Zehnder JL. Testing for Maternal Cell Contamination in Prenatal Samples. *British Journal of Pharmacology*. *Br J Pharmacol*. 2010; 161(3): 680–694.
- Smith GW, Graham CA, Nevin J, et al. Detection of maternal cell contamination in amniotic fluid cell cultures using fluorescent labelled microsatellites. *J Med Genet*. 1995; 32(1): 61–64, doi: [10.1136/jmg.32.1.61](https://doi.org/10.1136/jmg.32.1.61), indexed in Pubmed: [7897630](https://pubmed.ncbi.nlm.nih.gov/7897630/).
- Jarrett KL, Michaelis RC, Phelan MC, et al. Microsatellite analysis reveals a high incidence of maternal cell contamination in 46,XX products of conception consisting of villi or a combination of villi and membranous material. *Am J Obstet Gynecol*. 2001; 185(1): 198–203, doi: [10.1067/mob.2001.114692](https://doi.org/10.1067/mob.2001.114692), indexed in Pubmed: [11483928](https://pubmed.ncbi.nlm.nih.gov/11483928/).
- Jarrett KL, Michaelis RC, Phelan MC, et al. Microsatellite analysis reveals a high incidence of maternal cell contamination in 46,XX products of conception consisting of villi or a combination of villi and membranous material. *Am J Obstet Gynecol*. 2001; 185(1): 198–203, doi: [10.1067/mob.2001.114692](https://doi.org/10.1067/mob.2001.114692), indexed in Pubmed: [11483928](https://pubmed.ncbi.nlm.nih.gov/11483928/).
- Benn PA, Schonhaut AG, Hsu LY. A high incidence of maternal cell contamination of amniotic fluid cell cultures. *Am J Med Genet*. 1983; 14(2): 361–365, doi: [10.1002/ajmg.1320140216](https://doi.org/10.1002/ajmg.1320140216), indexed in Pubmed: [6837631](https://pubmed.ncbi.nlm.nih.gov/6837631/).
- Winsor EJT, Akoury H, Chitayat D, et al. The role of molecular microsatellite identity testing to detect sampling errors in prenatal diagnosis. *Prenat Diagn*. 2010; 30(8): 746–752, doi: [10.1002/pd.2530](https://doi.org/10.1002/pd.2530), indexed in Pubmed: [20661888](https://pubmed.ncbi.nlm.nih.gov/20661888/).
- Weida J, Patil AS, Schubert FP, et al. Prevalence of maternal cell contamination in amniotic fluid samples. *Am J Obstet Gynecol*. 2015; 212(1): S177–S177.
- Dória S, Lima V, Carvalho B, et al. Application of touch FISH in the study of mosaic tetraploidy and maternal cell contamination in pregnancy losses. *J Assist Reprod Genet*. 2010; 27(11): 657–662, doi: [10.1007/s10815-010-9460-1](https://doi.org/10.1007/s10815-010-9460-1), indexed in Pubmed: [20676750](https://pubmed.ncbi.nlm.nih.gov/20676750/).