# Polymerase chain reaction-based detection of chromosomal imbalances on embryos: the evolution of preimplantation genetic diagnosis for chromosomal translocations

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**Objective:** To develop and assess a polymerase chain reaction (PCR)-based preimplantation genetic diagnosis (PGD) approach for detection of chromosomal imbalances in embryos.

**Design:** A prospective study of embryos derived from chromosome translocation carriers that have undergone PGD using a novel molecular-based approach.

**Setting:** A reference molecular genetics laboratory specialized in the provision of transport PGD services and a private IVF clinic.

**Patient(s):** Twenty-seven couples carrying 12 different reciprocal translocations and 2 Robertsonian translocations.

**Intervention(s):** Preimplantation genetic diagnosis from chromosome translocation carriers on blastomeres biopsied from cleavage stage embryos.

**Main Outcome Measure(s):** Embryo diagnosis rate, pregnancy rate (PR), implantation rate, take-home-baby rate.

**Result(s):** Overall, 241/251 (96.0%) embryos were successfully diagnosed for chromosome rearrangements. Preimplantation genetic screening was included in the protocol of 12 couples, involving analysis of 90 embryos, 84 (93.3%) of which were successfully diagnosed and 53 (63.1%) showed aneuploidies. Embryos suitable for transfer were identified in 24 cycles. Eighteen couples achieved a clinical pregnancy (75.0% PR/embryo transfer), with a total of 31 embryos implanted (59.6% implantation rate). Ten patients (1 triplet, 1 twin, and 8 singleton pregnancies) have delivered 13 healthy babies, and the other patients (3 twins and 5 singletons) have currently ongoing pregnancies.

**Conclusion(s):** The PCR-based PGD protocol for translocations has the potential to overcome several inherent limitations of fluorescence in situ hybridization-based tests, providing potential improvements in terms of test performance, automation, turnaround time, sensitivity, and reliability. (Fertil Steril® 2010;■:■–■. ©2010 by American Society for Reproductive Medicine.)

**Key Words:** Preimplantation genetic diagnosis, preimplantation genetic screening, Robertsonian translocation, reciprocal translocation, short tandem repeats, pregnancy outcome, aneuploidy origin, uniparental disomy, blastocyst, chromosomal abnormalities

Individuals who carry a balanced chromosomal translocation (reciprocal or Robertsonian) typically suffer no outward manifestations of the rearrangement. The translocations, however, are associated with the production of large numbers of gametes with an unbalanced genetic complement. These unbalanced gametes lead to a greater chance of the patient being infertile or at high risk of conceiving chromosomally abnormal pregnancies that lead to recurrent spontaneous abortions or children with congenital anomalies and mental retardation (1).

Preimplantation genetic diagnosis (PGD) has been offered to carriers of balanced translocations as an alternative to prenatal diagnosis and termination of unbalanced pregnancies (2–5). Application of PGD to such couples can decrease the risk of these adverse outcomes by selecting for transfer only those embryos with a normal/balanced chromosomal complement (3, 6, 7).

Fluorescence in situ hybridization (FISH) is the method of choice for detecting unbalanced chromosome rearrangements in PGD. A commonly used FISH strategy for detection of the abnormal segregation in reciprocal translocations involves the simultaneous use of commercially available telomeric probes in combination with centromeric probes (1, 4). Analysis of Robertsonian translocations is simpler, involving the use of locus-specific enumerator probes enabling the detection of aneuploid embryos (2, 8).

Fluorescence in situ hybridization is known to have limitations, which primarily derive from errors inherent to the procedure and include signal overlap, signal splitting, and poor probe hybridization. As a result, interpretation errors due to the technical issues mentioned previously can lead to the loss of suitable (normal/balanced) embryos for transfer or the errant transfer of unbalanced embryos. Both of these mistakes are considered a misdiagnosis; however,

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the different types of misdiagnoses have far different outcomes. Loss of suitable normal/balanced embryos leads to less embryos available for transfer, which can impact pregnancy rates (PR), whereas errant transfer of unbalanced embryos can lead to pregnancy loss or the birth of children with congenital anomalies and mental retardation (9). Since FISH was first introduced in clinical diagnosis, improvements have been established to diminish the error rate of the technique (10–12). These improvements have increased PGD sensitivity, but certain shortcomings remain. The FISH error rates, including false negatives and false positives, have been estimated to be 7%–10% (13–15).

In this article, we present the development of a polymerase chain reaction (PCR)-based PGD approach for detection of chromosomal imbalances on embryos derived from both reciprocal and Robertsonian translocation carriers. The assay was clinically applied in 27 PGD cycles, resulting in the establishment of chromosomally balanced pregnancies in 18 couples.

## MATERIALS AND METHODS

### Clinical Cases

The details of the 27 couples are summarized in Table 1. Briefly, the couples in this study consisted of 15 Robertsonian translocation carriers and 12 reciprocal translocation carriers. The male partners of 20 couples presented sperm parameters requiring the use of intracytoplasmic sperm injection (ICSI). Eight couples had previously undergone infertility treatment using ICSI without PGD, whereas six other couples had already had an unsuccessful translocation PGD cycle in another center. Three of the six couples who had undergone PGD previously had also included preimplantation genetic screening (PGS) during the cycle and one couple had one IVF cycle that included PGS without PGD for translocation, using a sperm sample from a donor. Finally, one patient (couple 2) was advised of a very low chance of success due to her maternal age; however, the patient decided to proceed with the cycle seeing this as her last chance at pregnancy.

### Preclinical PGD Workup

Typically, the preclinical workup consisted of the following steps: confirmation of the karyotype of the partner carrying the balanced translocation by conventional cytogenetic banding and karyotyping, design and optimization of PCR primers for amplification of the short tandem repeat (STR) polymorphisms, identification of informative STR markers by testing both partners, and optimization of the multiplex PCR in single cells. The final stage of the validation of the PGD protocols involved amplification of a minimum of 50 single lymphocytes derived from both partners (25 cells from each partner), as previously described (16). This allowed evaluation of amplification efficiency and allele drop-out (ADO) rates in single cells, thus permitting a rough estimate of the efficiency of the protocol.

### STR Markers Selection and Primer Design

To detect chromosome imbalances in embryos derived from reciprocal translocation carriers, STR markers that flank each breakpoint were selected along each chromosome. For Robertsonian translocations, STR markers located at any point along the chromosomes involved allows for differentiation between aneuploid embryos and normal/balanced embryos by simply enumerating peak signals. In some cases, STR markers were also included to determine the copy number of chromosomes 13, 14, 15, 16, 18, 21, 22, X, Y. Only tetranucleotides markers were selected to achieve reduced stuttering artifacts that can confound analysis of dinucleotide and trinucleotide repeat STR markers (17). Primer sequences of these markers and their chromosomal location are shown in Tables 2 and 3 (Table 3 available online).

### Informativity Testing on Individual Couples

The STR genotyping for both partners of each couple was performed to identify the most informative STR markers to be used in the clinical PGD cycles. For each couple, only fully informative heterozygous markers presenting nonshared alleles (i.e., 4 different alleles, male partner a/b and female partner c/d; or 3 different alleles, translocation carrier a/b, other partner c/c) were selected so that segregation of each allele could be clearly determined. To avoid misdiagnosis due to possible ADO occurrences, at least three fully informative STR for each chromosome were included in the PGD protocol.

### IVF and Embryo Biopsy Procedure

Cleavage stage embryos were obtained using a standard IVF procedure, as previously described (18). At 62–64 hours after insemination, embryos with $\geq 6$ cells and $\leq 50\%$ fragmentation were placed into 20 $\mu$L of G-MOPS medium (Vitrolife, Goteborg, Sweden) under mineral oil and subjected to biopsy after zona ablation using a noncontact laser (ZILOS-tk; Hamilton Thorne Biosciences, Beverley, MA). One blastomere from each embryo was removed with macromanipulation and placed immediately into sterile 0.2-mL PCR tubes containing 5 $\mu$L of alkaline lysis buffer (200 mM KOH, 50 mM dithiothreitol [DTT]).

### Cell Lysis and Multiplex PCR

Before proceeding to multiplex PCR, blastomeres were lysed by incubation at 65°C for 10 minutes, followed by neutralization with 5 $\mu$L of neutralization buffer (900 mmol/L Tris-HCl, 300 mmol/L KCl, 200 mmol/L HCl). A heminested PCR protocol was used to coamplify all the selected informative STR markers. The first round multiplex PCR was performed with “outer” primers, followed by separate second round PCR reactions for each locus with one of the same outer primers combined with a unique “inner” primer that was labeled with a fluorescent tag (Tables 2 and 3) (Table 3 available online). The PCR reactions were performed as previously described (16). Fluorescent PCR products were then analyzed by 30 minutes of capillary electrophoresis on an automatic DNA sequencer, ABI Prism 3100 (Applied Biosystems, Rome, Italy).

### Classification of the Results

Embryos were diagnosed as “normal/balanced” if PCR results clearly indicated two signals (peaks) for each chromosome tested. Embryos were diagnosed as “unbalanced” if the PCR results showed a clear and consistent deviation from the “normal/balanced” signal pattern, such as (partial or full) trisomies (three peaks), (partial or full) monosomies (one peak), and nullisomies (no PCR signals) (Fig. 1). The presence of one signal for each chromosome tested was classified as haploid. An “inconclusive” diagnosis was assigned for those embryos where the signal pattern was not a clear-cut normal result.

### Confirmation of PGD Results

After successful embryo transfer, unbalanced or morphologically incompetent embryos were collected in individual tubes and reanalyzed to confirm the PGD results. In cases in which pregnancies were achieved, patients were advised to undergo conventional prenatal diagnosis to confirm the karyotype of the fetus.

### Clinical Data and Definitions

The number of fertilized (two pronuclei [2PN]) oocytes and the number of biopsied embryos were calculated on the basis of the total number of mature injected oocytes. For the outcome of the pregnancies (19), according to The International Committee Monitoring Assisted Reproductive Technologies, a clinical pregnancy is “evidence of pregnancy by clinical or ultrasound parameters (ultrasound visualization of a gestational sac—thus with and without fetal heart beat).” The implantation rate was calculated as the number of embryos (with and without fetal heart beat) implanted over the total number of embryos transferred.

## RESULTS

### Preclinical Workup

In total, 27 couples were included in the preclinical workup; in each case an informative set of STR markers was available, therefore all couples were considered suitable for the PGD procedure. The

**TABLE 1**

**Patient characteristics.**

| Couple | Translocation | Maternal age (y) | Sperm abnormality | No. of years infertile | Previous ICSI cycles w/o PGD | Previous PGD cycles | Previous PGS |
|---|---|---|---|---|---|---|---|
| 1 | 45,XY, t(13;14)(q10;q10) | 36.3 | OAT | 8 | 0 | 0 | 0 |
| 2 | 45,XX, t(13;14)(q10;q10) | 45.0 | OAT | 6 | 3 | 0 | 0 |
| 3 | 45, XY, t(13;14)(q10;q10) | 39.0 | AT | 3 | 0 | 0 | 0 |
| 4 | 45,XY, t(13;14)(q10;q10) | 41.4 | OA | 2 | 1 | 0[a] | 1 |
| 5 | 45,XY, t(13;14)(q10;q10) | 41.9 | OAT | 6 | 0 | 2 | 0 |
| 6 | 45,XX, t(13;14)(q10;q10) | 39.1 | OA | 5 | 0 | 2 | 0 |
| 7 | 45,XY, t(13;14)(q10;q10) | 33.4 | OA | 2 | 0 | 0 | 0 |
| 8 | 45,XY, t(13;14)(q10;q10) | 41.2 | OAT | 4 | 2 | 0 | 0 |
| 9 | 45, XX, t (13;14)(q10;q10) | 32.4 | None | 3 | 0 | 0 | 0 |
| 10 | 45, XX, t(13;14)(q10;q10) | 33.0 | OAT | 4 | 0 | 0 | 0 |
| 11 | 45,XY, t(13;14)(q10;q10) | 36.9 | OAT | 5 | 2 | 0 | 0 |
| 12 | 45,XY; t(14/21)(q10;q10) | 41.9 | OAT | 8 | 0 | 3 | 3 |
| 13 | 45, XY, t(14;21)(q10;q10) | 29.7 | OAT | 1 | 0 | 0 | 0 |
| 14 | 45 XX, t(14;21)(q10;q10) | 33.0 | None | 2 | 0 | 0 | 0 |
| 15 | 45, XY, t(14;21)(q10;q10) | 30.8 | AT | 3 | 1 | 0 | 0 |
| 16 | 46, XY, t(5;19)(q31.1;q13.1) | 36.2 | AS | 3 | 2 | 0 | 0 |
| 17 | 46 XY, t(1;12)(p36.1;q13.1) | 32.9 | None | 5 | 0 | 0 | 0 |
| 18 | 46 XX,t(5;7)(q35;q32) | 32.6 | OAT | 3 | 0 | 1 | 1 |
| 19 | 46,XY t(10;13)(p13;q32) | 35.2 | OAT | 3 | 2 | 0 | 0 |
| 20 | 46, XX, t(2;6)(p23;q22) | 36.5 | None | 3 | 0 | 0 | 0 |
| 21 | 46,XY t(7;11)(q21.1; p13) | 40.6 | OA | 11 | 0 | 1 | 0 |
| 22 | 46,XX t(3;18)(q25;q23) | 34.5 | None | 3 | 0 | 0 | 0 |
| 23 | 46, XY, t(9;15)(q34;q13) | 37.4 | OAT | 2 | 0 | 0 | 0 |
| 24 | 46, XX, t(5;9)(q33;q21.3) | 35.9 | OA | 5 | 0 | 0 | 0 |
| 25 | 46, XX, t(8;22)(p11;pter) | 29.0 | None | 3 | 0 | 0 | 0 |
| 26 | 46 XX t(1;8)(p34;p12) | 31.3 | OAT | 3 | 2 | 1 | 0 |
| 27 | 46,XX, t(7;18)(p15;p11.1) | 32.4 | None | 4 | 0 | 0 | 0 |

*Note:* OAT = oligoasthenoteratozoospermia; AT = asthenoteratozoospermia; AS = asthenozoospermia; OA = oligoasthenozoospermia; ICSI = intracytoplasmic sperm injection; PGD = preimplantation genetic diagnosis; PGS = preimplantation genetic screening.
[a] No PGD for translocation was performed because a sperm sample from a donor was used for ICSI.

*Fiorentino. A PCR-based PGD protocol for chromosomal translocation. Fertil Steril 2010.*

number of markers included in the PGD protocol varied from 8 (in PGD cases for Robertsonian translocation) to 30 (in PGD cases for reciprocal translocation with PGS STR markers used for aneuploidy detection), 15.3 ± 6.0 on average. A total of 434 single lymphocytes were individually tested. A positive amplification signal was obtained in 409/434 (94.2%) cells. Amplification rates were generally high for all loci tested, ranging from 88.9%–100 %. The ADO rates varied among the different loci investigated, ranging from 0–6.8%, with an average ADO rate of 2.4%. None of the 540 blank controls used for each locus-specific trial showed amplification.

## Clinical PGD Cycles and Follow-up Analysis

Twenty-seven cycles of PGD were carried out for 27 couples carrying 12 different reciprocal translocations and 2 different Robertsonian translocations (Table 4). Eight cycles (for couples 3, 4, 6, 15, 19, 23, 26, and 27) involved freezing of embryos at the 2PN stage for collection due to low response to ovarian stimulation.

A total of 451 oocytes were collected (range 7–35 oocytes), 351 (77.8%) of them were mature metaphase II stage, 284 (80.9%) fertilized normally (range 5–23) resulting in 251 embryos (mean number 9.3 ± 3.6 per cycle; range 4–19), which were biopsied on day 3. The PCR was successful in 263 of 276 blastomeres (95.3%). Amplification failed for all the markers tested in 13 blastomeres. The efficiency of amplification of the individual STR markers on blastomeres ranged from 84.6%–100%, with an overall amplification rate of 95.0%. The ADO rates varied between the different markers investigated, ranging from 0–8.3%, with an average ADO rate of 2.6%. No contamination was detected in 251 blank controls collected during the biopsy procedure or in the blanks from the PCR reagents.

A reliable genotype (i.e., a profile with PCR results obtained from at least 2 STR markers on either side of the translocation breakpoints, for reciprocal translocation, or at least 2 STR markers for each chromosome, for Robertsonian translocation) was achieved in 263/263 (100%) of the blastomeres with positive PCR results. Overall, 241 (96.0%) embryos were successfully diagnosed. No diagnosis was obtained for 10 embryos, because of a total amplification failure of all markers tested.

Preimplantation genetic screening was included in the PGD protocol of 12 couples; for eight of them (couples 2–6, 8, 12, 21) PGS was indicated because of advanced maternal age (average 40.9 ±2.2 years; range 37.4–45 years). For couple 18, despite the patient's low reproductive age (32 years), PGS was performed because a previous PGD+PGS cycle resulted in a diagnosis of aneuploid embryos; for couples 11 and 23, instead, PGS was included because of their reproductive history. For couple 16, analysis of chromosome 21 only was included to reduce the risk of a pregnancy with Down syndrome. In total, 90 embryos were tested for aneuploidy along with the

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**TABLE 2**

**Oligonucleotide primer sequences for amplification of STR markers used for detection of chromosomal imbalances on embryos derived from Robertsonian translocation carriers.**

| Chromosome | STR markers | Chromosomal band | Outer primers (5′ to 3′) | Inner primers (5′ to 3′) | PCR product size | Dye label |
|---|---|---|---|---|---|---|
| 13 | D13S634 | q21.33 | F: 5′-CTTCAGATAGGCAGATTCAATAGGA-3′<br>R: 5′-CAGTTTGCAGACTATTGTGAGAGTT-3′ | [a]R: 5′-CCAATTCCCCTATTTAGTCATCTGT-3′ | 248 | TAMRA |
| | D13S258 | q21.33 | F: 5′-TTTTACCAGGAGGAGAGGGACTA-3′<br>R: 5′-AATGGGATGAGAGAGGAAGACAG-3′ | F: 5′-GGGACTACCTATGCACACAAAGT-3′[a] | 172 | FAM |
| | D13S256 | q14.3 | F: 5′-TGGTGAAACCTGTGAGGCAG-3′<br>R: 5′-GGCCACAGAGGAAGCACATA-3′ | F: 5′-CTGGGCAACAAGAGCAAAACT-3′[a] | 265 | TAMRA |
| | D13S240 | q13.3 | F: 5′-GTCCATTCTTTAACATGTACGCA-3′<br>R: 5′-AACAGAGCAAGACTCCATCTCA-3′ | F: 5′-CCATTCCCCATCTTTATTGACT-3′[a] | 244 | FAM |
| | D13S217 | q12.3 | F: 5′-GGATGTGGAGGAGAGTTCATTT-3′<br>R: 5′-AGCCCAAAAAGACAGACAGAAA-3′ | F: 5′-TCTGACCCATCTAACGCCTATC-3′[a] | 128 | FAM |
| | D13S243 | q12.2 | F: 5′-GTAATGCCTCAACCATGAATTC-3′<br>R: 5′-CAGTTAAATCCAGGAGGTGGAG-3′ | [a]R: 5′-GATTGTGCCACTGTACTTCTGC-3′ | 160 | HEX |
| | D13S252 | q12.2 | F: 5′-TTTCTGCCCCCTAGGTGAGTAT-3′<br>R: 5′-GCTTCTCAGCTTGCAGATGGTA-3′ | [a]R: 5′-GGACCTTGTGATCGTGTGAGTT-3′ | 206 | HEX |
| | D13S251 | q31.1 | F: 5′-AGCAGGAACAATGTTTGTTGGT-3′<br>R: 5′-TGCCATAATTGCATATTGCAGT-3′ | F: 5′-GATGTTCCAGCTAATGCCATTAG-3′[a] | 231 | FAM |
| | D13S631 | q32.1 | F: 5′-CAAGATCACACCATTCCACTCC-3′<br>R: 5′-GCAGTTTCTTAGCCCTCACCAT-3′ | F: 5′-GGCAACAAGAGCAAAACTCTGT-3′[a] | 218 | HEX |
| | D13S1823 | q32.1 | F: 5′-GCCTTGTGATCGTGTGAGTTAA-3′<br>R: 5′-TCTCCCCTGAATGCTACAAATC-3′ | [a]R: 5′-ACCCAGAATTGGAAAGAACTGT-3′ | 186 | HEX |
| | D13S892 | q32.1 | F: 5′-TCAACTCAACCATTCCATTTCTAG-3′<br>R: 5′-CAAGTCATGTGTTTGTAATCTTGG-3′ | F: 5′-AATACTTGTGCATTTGAACAGAGG-3′[a] | 202 | FAM |
| | D13S797 | q33.2 | F: 5′-CTGGCATCTGTATTAGGGTTCTC-3′<br>R: 5′-CAGTCTCTATAATCACGAGCCAAT-3′ | F: 5′-TCCAGACAGATAGAACCAATAGGA-3′[a] | 154 | HEX |
| | D13S763 | q33.2 | F: 5′-CAATGAGCCGTGATCATGCTAC-3′<br>R: 5′-ATCCATGTCAAAACCTCTTGGC-3′ | F: 5′-GCATGGGTGACAGAGTGAGATT-3′[a] | 206 | FAM |
| | G15773 | q33.2 | F: 5′-GGTAGACATTTGCCACTTGGTT-3′<br>R: 5′-CACTACTCCATGCCAGGATCTC-3′ | [a]R: 5′-CACGAGCCAATTCTCCTAACAA-3′ | 192 | HEX |
| | D13S248 | q33.3 | F: 5′-GCTATTGACAATAGCCAAATAT-3′<br>R: 5′-TACTTACCATAATGTCCTCAAG-3′ | F: 5′-AACTTAAATGTCCATCAATAAA-3′[a] | 201 | TAMRA |
| | D13S796 | q33.3 | F: 5′-TGAATCTCATCTCCCTGTTTGGT-3′<br>R: 5′-ATTTGAGGTTGCTTGAATCCATG-3′ | [a]R: 5′-TCACAGATATGGAGGGATGACTG-3′ | 155 | HEX |
| | D13S783 | q33.3 | F: 5′-CAGGTATCTAATGTGCTCTTTAAA-3′<br>R: 5′-AAATTCATTCTCTCTCTGTCTCC-3′ | F: 5′-CACCCAATTTTTTGTATGTTTAC-3′[a] | 175 | FAM |
| 14 | D14S122 | q11.2 | F: 5′-CCGAATAAATTGGAAAGTTGCG-3′<br>R: 5′-CCTGGGTGAGACTCCATCTCA-3′ | F: 5′-GTTGCGTTCATGTACCACTGC-3′[a] | 215 | FAM |
| | D14S608 | q12 | F: 5′-CTTTCGTGGTTTTTGTCTTTCAAG-3′<br>R: 5′-GGATCTCCTTCTTTTTATGGATGA-3′ | F: 5′-ACGTGGTACAGGTAGATAAATGGA-3′[a] | 175 | FAM |
| | D14S121 | q13.1 | F: 5′-GGATTGAATGTCTTTCCTGGT-3′<br>R: 5′-TGGTATGTTCCTAGCATTTGC-3′ | F: 5′-CAAGATCCCCTTTCCCATATA-3′[a] | 162 | HEX |
| | D14S551 | q13.1 | F: 5′-TCAGCCATGATTGTGCCACT-3′<br>R: 5′-CATGTGGTCCCAGCTCACAT-3′ | F: 5′-CTGGGCAACAGAGCAAGATG-3′[a] | 198 | HEX |
| | D14S125 | q23.3 | F: 5′-AGACATACAATTCGTGAGGAACAGA-3′<br>R: 5′-GCGTGTTCCACTCTTTAATTACAGT-3′ | F: 5′-GCTCTTAACCTCTCATCATATCACA-3′[a] | 180 | TAMRA |

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**TABLE 2**

**Continued.**

| Chromosome | STR markers | Chromosomal band | Outer primers (5′ to 3′) | Inner primers (5′ to 3′) | PCR product size | Dye label |
|---|---|---|---|---|---|---|
| | D14S549 | q24.1 | F: 5′-AGGCACCCCATATAAGCACTATTTA-3′<br>R: 5′-GGTTTGAACATGTAATCACACTTGG-3′ | F: 5′-GCACTCAGAGAATCAGAAAAACTGA-3′[a] | 121 | TAMRA |
| | D14S120 | q24.3 | F: 5′-TCACTACAAACTCCACCTCCCAG-3′<br>R: 5′-GCTATTCCTTCTGCCATCAAATC-3′ | F: 5′-ACTATATTGGCATGGCTGGTCTC-3′[a] | 275 | HEX |
| | D14S127 | q31.2 | F: 5′-GATGCAAAATACGTGGCTTCTT-3′<br>R: 5′-CATCCACATGGTCTTGGTTCTAG-3′ | F: 5′-GAGGTGAAAGAAGAACCAACAAGT-3′[a] | 396 | FAM |
| | D14S126 | q31.3 | F: 5′-GTTTCCTTGGACTATTTCCCTGT-3′<br>R: 5′-CATGGAAAACCTCCTCATATAGC-3′ | F: 5′-GGTTGGTTGTTACACAGCAAAG-3′[a] | 151 | HEX |
| | D14S128 | q31.3 | F: 5′-GTTGAGGTTATGCATGTGTGAGTG-3′<br>R: 5′-CCCCAAAACTCTCAGAAGATACTTT-3′ | F: 5′-GGCTTTTATGGGAAATCTCTGTACT-3′[a] | 331 | HEX |
| | D14S617 | q32.12 | F: 5′-AAGTTGTTAGTAATCTCCGCCTCC-3′<br>R: 5′-CAGTTTAGGCAACAGAACAAGATCT-3′ | F: 5′-GGAGAAATTAAGTTTTAGGTGGCC-3′[a] | 152 | FAM |
| | D14S553 | q32.13 | F: 5′-ACCACTGCCTGCTATTTACAAA-3′<br>R: 5′-GCCTGTGTGACAGAGTAAGACC-3′ | F: 5′-GGGAGTGAAAAGGCTTTTCTAG-3′[a] | 248 | HEX |
| | D14S1434 | q32.13 | F: 5′-CCACCACTGGGTTCTATAGTTCTC-3′<br>R: 5′-CATGGATTCCACATTAAGAGCTCT-3′ | F: 5′-TCAGATTCAGACTGAATCACACCA-3′[a] | 252 | FAM |
| 21 | D21S11 | q21.1 | F: 5′-GAGTCAATTCCCCAAGTGAATT-3′<br>R: 5′-GAAGGGAGAAACACTGTAAGGTT-3′ | [a]R: 5′-TGTTGTATTAGTCAATGTTCTCCAG-3′ | 240 | HEX |
| | D21S1414 | q21.1 | F: 5′-AGAAGGGAGAAACACTGTAAGGT-3′<br>R: 5′-CCAAGTGAATTGCCTTCTATCTA-3′ | F: 5′-TCCAGAGACAGACTAATAGGAGGT-3′[a] | 190 | FAM |
| | D21S1437 | q21.1 | F: 5′-ACTGATGGACATTTAGGTTGATTC-3′<br>R: 5′-TTCTCTACATATTTACTGCCAACAC-3′ | F: 5′-GAATAGTGCTGCAATGAACATACAT-3′[a] | 150 | HEX |
| | D21S1244 | q21.2 | F: 5′-TGTCAAAGGAGTATGTCCCCAT-3′<br>R: 5′-TAGTGAGGAAGAATAGGGATTATCC-3′ | F: 5′-CTAGTACCACAGAATTCAGTCCAAA-3′[a] | 170 | FAM |
| | D21S1409 | q21.2 | F: 5′-ATACAAGCGAAGGATTTGGATC-3′<br>R: 5′-CATATGCGTGTATTTTTGCCTC-3′ | [a]R: 5′-GGAACATACGCTCTCTCCCTTA-3′ | 172 | HEX |
| | D21S1250 | q21.2 | F: 5′-TGGGTAAAGAAAATGTGCTCTC-3′<br>R: 5′-GGAATCATGCAGTGCTTGTAGT-3′ | [a]R: 5′-GTTCAATGGTGTCACAAAGGAT-3′ | 103 | FAM |
| | D21S1914 | q21.2 | F: 5′-AGATTACATTGGGCCTTCTGTC-3′<br>R: 5′-ATCTGAACCAGGGCATGTAAAC-3′ | [a]R: 5′-GGAGCCTTACAAAAGATTTGGA-3′ | 178 | TAMRA |
| | D21S1413 | q22.11 | F: 5′-CTCTTAAATTGGAAGCATGCAG-3′<br>R: 5′-CCCGGAAGTTTTATACCAAAAG-3′ | F: 5′-ATACATAAAGCTGCCAGCGTTG-3′[a] | 184 | TAMRA |
| | D21S1444 | q22.13 | F: 5′-ACAACACCCTTATCAACCTGC-3′<br>R: 5′-GGCTTTGGATCACTCGTAACT-3′ | F: 5′-TTAGAGCTTCCTTTGCCATCT-3′[a] | 242 | FAM |
| | D21S1245 | q22.2 | F: 5′-TGAAAAACAGAGAAGGAGGGAA-3′<br>R: 5′-TTGTTGAGGATTTTTGCATCAG-3′ | F: 5′-ACCAAAACCAGAAAATGACACA-3′[a] | 274 | HEX |
| | D21S1412 | q22.2 | F: 5′-GGAAGGAATTCACTCTACCACTACA-3′<br>R: 5′-AGTGAGTTGAGATCGCACCATT-3′ | F: 5′-AGTGAGTTGAGATCGCACCATT-3′[a] | 283 | FAM |
| | D21S2055 | q22.2 | F: 5′-ATTCTGGTCCTTTGAGGTAACTG-3′<br>R: 5′-CATCCTCCATAATAGCATGAGCT-3′ | F: 5′-CAGAGAAACAGAACCAATAGGCT-3′[a] | 165 | FAM |
| | D21S1411 | q22.3 | F: 5′-ATATGATGAATGCATAGATGGA-3′<br>R: 5′-CCCAGAAAACAACTCAGTTAATA-3′ | [a]R: 5′-TTGTATTAATGTGTGTCCTTCC-3′ | 240 | TAMRA |

*Note:* F = Forward; R = Reverse; STR = short tandem repeat; PCR = polymerase chain reaction.
[a] A heminested approach was used.

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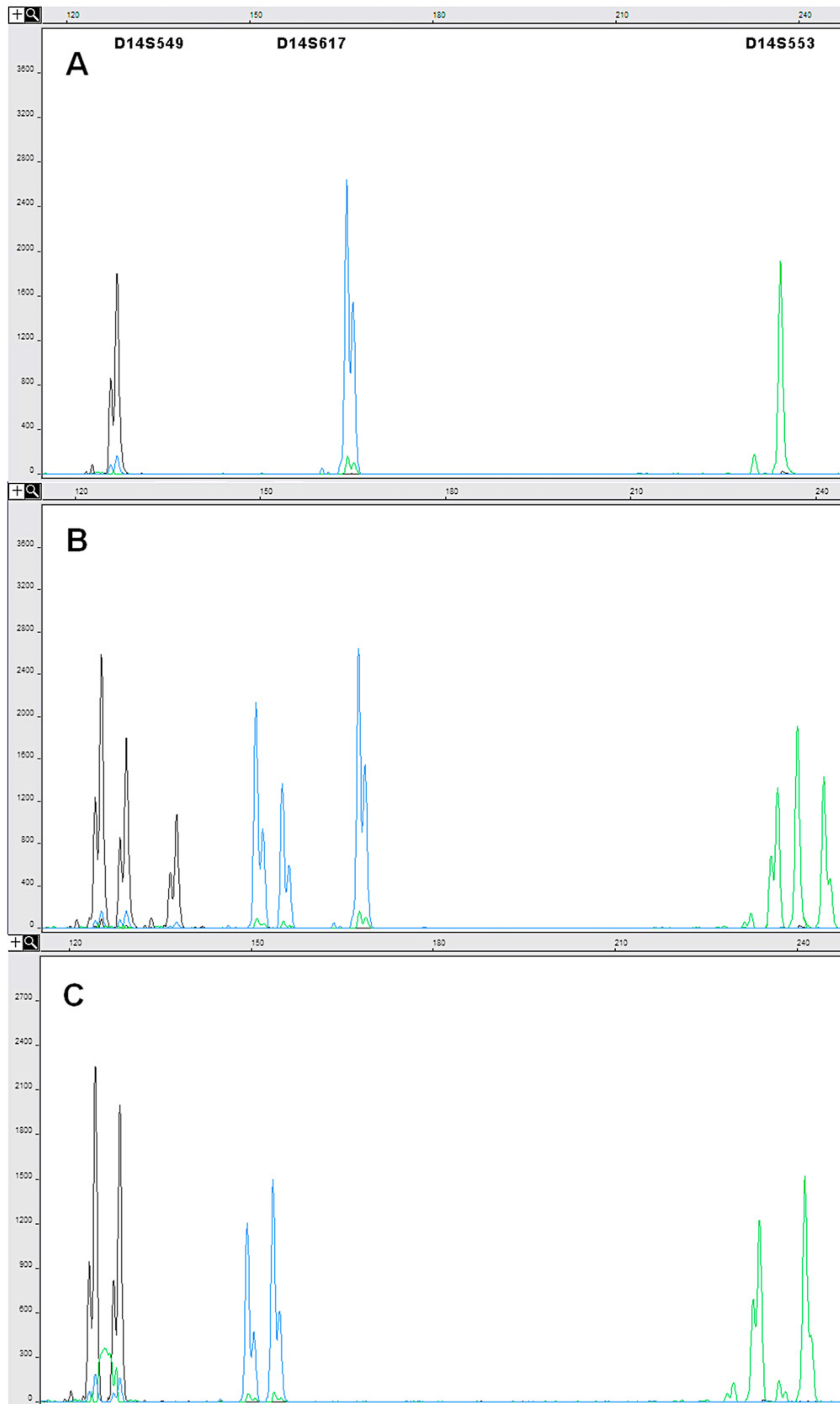
FIGURE 1

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## FIGURE 1 Continued

Capillary electrophoresis of fluorescent polymerase chain reaction (PCR) products obtained from a preimplantation genetic diagnosis case for Robertsonian translocation (13;14), after multiplex amplification of a set of polymorphic short tandem repeat (STR) markers located along chromosome 14. The x-axis shows the length of PCR products in base pair and the y-axis shows the fluorescence intensity in relative fluorescence units. On top of the electropherogram the marker name is located above the corresponding alleles (peaks). A normal diploid embryo (**C**) has the normal complement of each parental chromosome, thus two alleles of a chromosome-specific STR are determined as two peaks. Embryos with a normal copy number for a given chromosome will show a heterozygous pattern for all the STRs used. The observation of an extra STR allele as a three peak pattern is diagnostic of the presence of an additional sequence, which represents an additional chromosome, as in the case of a trisomy. Trisomic embryos will produce trisomic patterns for all markers on the same chromosome (**B**). The observation of only one STR allele as a one peak pattern is diagnostic of the missing of the sequence from one chromosome, as in the case of a monosomy. Monosomic embryos will show a homozygote pattern for all the STRs used for a given chromosome (**A**).

chromosome rearrangement, 84 (93.3%) of which were successfully diagnosed. Amplification failed for all the markers tested in six embryos. A total of 53 (63.1%) embryos were found to be aneuploid; in 54 (69.6%) embryos aneuploidies were of maternal origin and 24 (30.4%) were of paternal origin. Five embryos (9.4%) had a combination of aneuploidies from both maternal and paternal origin.

Embryos suitable for transfer at the blastocyst stage were identified in 24 of the 27 cycles (88.9%). In three PGD cycles (couples 10, 14, and 16), embryo transfer was canceled because the morphology of the embryos was not sufficient for transfer. After transfer of 52 embryos (mean 1.7 ± 0.9, range 1–3), 18 women (mean age 35.1 ± 4.3 years, range 29.0–41.9 years) had positive hCG levels (75.0% PR per embryo transfer). All pregnancies continued on to confirm at least one fetal sac and heart beat. A total of 31 embryos implanted and led to the presence of a fetal sac (59.6% implantation rate), resulting in 29 fetuses with a heart beat detected (55.8 % fetal heart beat/embryo transferred). Prenatal diagnosis was performed in two pregnancies and both were confirmed to be karyotypically balanced. All pregnancies (1 triplet, 4 twins, and 13 singleton) have completed at least 12 weeks of gestation. Ten patients (1 triplet, 1 twin, and 8 singleton pregnancies) have delivered 13 healthy babies, and the other patients have currently ongoing pregnancies.

After the clinical cases, 33 nontransferred embryos were reanalyzed using the same PCR conditions and procedure as in the clinical

## FIGURE 2

Uniparental disomy (UPD) detection on embryos from a preimplantation genetic diagnosis case for Robertsonian translocation (13;14), by using the polymerase chain reaction (PCR)-based preimplantation genetic diagnosis protocol for detection of chromosomal imbalances. The x-axis shows the length of PCR products in base pair and the y-axis shows the fluorescence intensity in relative fluorescence units. On top of the electropherogram the marker name is located above the corresponding alleles (peaks). For the chromosome 14 STR markers, the embryo (**A**) inherited alleles only from one parent (**B**) and failed to inherit an allele from the other (**C**), consistent with UPD14. Highlighted in blue are maternal alleles.

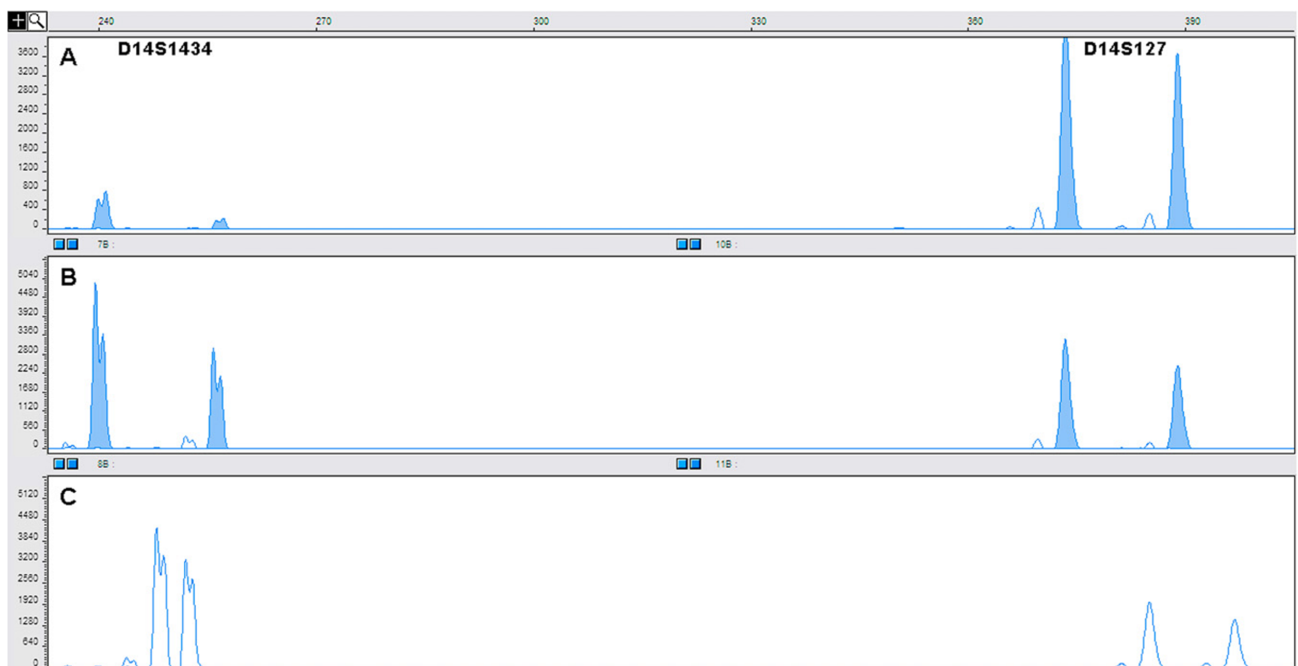


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**TABLE 4**

**Clinical results from 27 PGD cycles for reciprocal and Robertsonian translocations.**

| | Couples | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Robertsonian translocation | | | | | | | | | | | | | | | | Reciprocal translocation | | | | | | | | | | | | | |
| **Clinical data** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **Total** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** | **25** | **26** | **27** | **Total** | **Total** |
| No. of couples treated | | | | | | | | | | | | | | | | 15 | | | | | | | | | | | | | 12 | 27 |
| Maternal age (average; y) | | | | | | | | | | | | | | | | 37.6 ± 4.8 | | | | | | | | | | | | | 34.4 ± 3.2 | 36.1 ± 4.4 |
| No. of cycles performed | | | | | | | | | | | | | | | | 15 | | | | | | | | | | | | | 12 | 27 |
| PGS (Y/N) | N | Y | Y | Y | Y | Y | N | Y | N | N | Y | Y | N | N | N | 8 | Y[c] | N | Y | N | N | Y | N | Y | N | N | N | N | 4 | 12[c] |
| No. of oocytes retrieved | 15 | 8 | 5 | 7 | 21 | 15 | 12 | 14 | 16 | 10 | 19 | 19 | 29 | 15 | 26 | 231 | 10 | 16 | 31 | 14 | 35 | 11 | 19 | 10 | 26 | 18 | 17 | 13 | 220 | 451 |
| No. of mature oocytes injected (%)[a] | 12 | 8 | 5 | 7 | 18 | 10 | 9 | 11 | 16 | 9 | 14 | 17 | 25 | 11 | 20 | 192 (83.1) | 9 | 12 | 21 | 8 | 23 | 9 | 15 | 8 | 16 | 16 | 12 | 10 | 159 (72.3) | 351 (77.8) |
| No. of oocytes fertilized (%)[b] | 7 | 8 | 5 | 5 | 14 | 7 | 7 | 9 | 12 | 8 | 10 | 14 | 23 | 11 | 19 | 159 (82.8) | 8 | 10 | 10 | 5 | 20 | 8 | 13 | 6 | 13 | 15 | 9 | 8 | 125 (78.6) | 284 (80.9) |
| No. of embryos thawed | 0 | 0 | 3 | 2 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 17 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 3 | 0 | 0 | 8 | 7 | 20 | 37 |
| No. of embryos survived after thawing | 0 | 0 | 3 | 1 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 13 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 3 | 0 | 0 | 7 | 6 | 18 | 31 |
| No. of embryos biopsied | 5 | 8 | 5 | 4 | 10 | 6 | 5 | 8 | 10 | 8 | 8 | 13 | 19 | 8 | 13 | 130 | 7 | 10 | 8 | 7 | 16 | 5 | 12 | 8 | 11 | 12 | 13 | 12 | 121 | 251 |
| No. of blastomeres analyzed | 5 | 8 | 5 | 4 | 10 | 6 | 10 | 16 | 10 | 8 | 8 | 13 | 19 | 8 | 13 | 143 | 7 | 10 | 8 | 7 | 16 | 5 | 12 | 8 | 11 | 24 | 13 | 12 | 133 | 276 |
| No. of blastomeres with total PCR failure | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 2 | 0 | 0 | 0 | 2 | 1 | 1 | 0 | 8 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 5 | 13 |
| No. of embryos diagnosed (%) | 5 | 8 | 4 | 4 | 10 | 5 | 5 | 8 | 10 | 8 | 8 | 11 | 18 | 7 | 13 | 124 (95.4) | 7 | 10 | 6 | 7 | 15 | 5 | 12 | 8 | 11 | 12 | 12 | 12 | 117 (96.7) | 241 (96.0) |
| Balanced (%) | 3 | 3 | 4 | 3 | 7 | 4 | 5 | 5 | 6 | 6 | 6 | 6 | 14 | 3 | 7 | 82 (66.1) | 3 | 3 | 3 | 2 | 5 | 3 | 5 | 4 | 3 | 3 | 4 | 7 | 45 (38.5) | 127 (52.7) |
| Unbalanced (%) | 1 | 5 | 0 | 0 | 3 | 1 | 0 | 2 | 4 | 2 | 2 | 5 | 4 | 4 | 6 | 39 (31.5) | 3 | 7 | 3 | 4 | 8 | 2 | 7 | 4 | 8 | 9 | 8 | 5 | 68 (58.1) | 107 (44.4) |
| Aneuploid (%) | 2 | 6 | 1 | 2 | 8 | 3 | 0 | 6 | 0 | 2 | 4 | 6 | 0 | 0 | 0 | 40 (69.0) | 3 | 0 | 1 | 1 | 2 | 2 | 0 | 2 | 0 | 2 | 0 | 0 | 13 (50.0) | 53 (63.1) |
| Balanced + aneuploid (%) | 0 | 2 | 0 | 1 | 6 | 2 | 0 | 3 | 0 | 2 | 2 | 4 | 0 | 0 | 0 | 22 (37.9) | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 5 (19.2) | 27 (32.1) |
| No. of embryos tested for PGS | 0 | 8 | 5 | 4 | 10 | 6 | 0 | 8 | 0 | 0 | 8 | 13 | 0 | 0 | 0 | 62 | 7 | 0 | 8 | 0 | 0 | 5 | 0 | 8 | 0 | 0 | 0 | 0 | 28 | 90 |
| No. of embryos diagnosed for PGS (%) | 0 | 8 | 4 | 4 | 10 | 5 | 0 | 8 | 0 | 0 | 8 | 11 | 0 | 0 | 0 | 58 (93.5) | 7 | 0 | 6 | 0 | 0 | 5 | 0 | 8 | 0 | 0 | 0 | 0 | 26 (92.9) | 84 (93.3) |
| No. of aneuploid chromosomes | 2 | 10 | 1 | 3 | 11 | 8 | 0 | 16 | 0 | 2 | 4 | 8 | 0 | 0 | 0 | 65 | 3 | 0 | 1 | 1 | 2 | 2 | 0 | 2 | 0 | 3 | 0 | 0 | 14 | 79 |
| Trisomies (%) | 0 | 5 | 0 | 2 | 3 | 1 | 0 | 1 | 0 | 0 | 1 | 5 | 0 | 0 | 0 | 18 (27.7) | 2 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 4 (6.2) | 22 (27.8) |
| Monosomies (%) | 1 | 5 | 0 | 0 | 8 | 6 | 0 | 12 | 0 | 2 | 2 | 3 | 0 | 0 | 0 | 39 (60.0) | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 2 | 0 | 0 | 5 (35.7) | 44 (55.7) |
| Haploidy (%) | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 (6.2) | 1 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 (28.6) | 8 (10.1) |
| Triploidy (%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 (1.5) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 (0) | 1 (1.3) |
| UPD (%) | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 (3.1) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 (7.1) | 3 (3.8) |
| XXY | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (1.5) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 (0) | 1 (1.2) |
| Aneuploidies of maternal origin (%) | 1 | 10 | 1 | 1 | 11 | 4 | 0 | 7 | 0 | 0 | 3 | 7 | 0 | 0 | 0 | 45 (69.2) | 3 | 0 | 0 | 1 | 1 | 2 | 0 | 2 | 0 | 1 | 0 | 0 | 10 (71.4) | 55 (69.6) |
| Aneuploidies of paternal origin (%) | 1 | 0 | 0 | 2 | 0 | 4 | 0 | 9 | 0 | 2 | 1 | 1 | 0 | 0 | 0 | 20 (30.8) | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 4 (28.6) | 24 (30.4) |
| No. of embryos transferable | 3 | 1 | 2 | 2 | 1 | 2 | 5 | 2 | 6 | 0 | 3 | 2 | 11 | 3 | 3 | 46 | 1 | 3 | 3 | 2 | 5 | 1 | 5 | 3 | 2 | 2 | 2 | 5 | 34 | 80 |
| Embryo transfer (Y/N) | Y | Y | Y | Y | Y | Y | Y | Y | Y | N | Y | Y | Y | N | Y | 13 | N | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | 11 | 24 |
| No. of embryos transferred | 3 | 1 | 2 | 2 | 1 | 2 | 2 | 1 | 2 | 0 | 3 | 2 | 2 | 0 | 3 | 26 | 0 | 3 | 3 | 2 | 3 | 1 | 2 | 3 | 2 | 2 | 2 | 3 | 26 | 52 |
| Day of transfer | 5 | 6 | 6 | 6 | 6 | 6 | 5 | 5 | 6 | - | 6 | 6 | 6 | - | 6 | | - | 5 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 5 | 6 | 6 | | |
| No. of clinical pregnancies | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | - | 0 | 1 | 1 | - | 1 | 9 | - | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 9 | 18 |
| No. of embryos implanted (gestational sacs) | 3 | 0 | 2 | - | 1 | - | 2 | 1 | 2 | - | 0 | 1 | 1 | - | 2 | 15 | - | 3 | 1 | - | 2 | 1 | 2 | 0 | 2 | 1 | 2 | 2 | 16 | 31 |
| No. of fetal heart beats | 3 | 0 | 2 | - | 1 | - | 2 | 1 | 2 | - | 0 | 1 | 1 | - | 2 | 14 | - | 3 | 1 | - | 1 | 1 | 2 | 0 | 2 | 1 | 2 | 2 | 15 | 29 |
| No. fetuses after 12 weeks of gestation | 3 | 0 | 1 | - | 1 | - | 2 | 1 | 1 | - | 0 | 1 | 1 | - | 1 | 12 | - | 1 | 1 | - | 1 | 1 | 2 | 0 | 1 | 1 | 2 | 2 | 12 | 24 |
| Clinical pregnancy rate per OR | | | | | | | | | | | | | | | | 60.0% | | | | | | | | | | | | | 75.0% | 66.7% |

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**TABLE 4**

**Continued.**

| Clinical data | Couples | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Robertsonian translocation | | | | | | | | | | | | | | | | Reciprocal translocation | | | | | | | | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | Total | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | Total | Total |
| Clinical pregnancy rate per embryo transfer | | | | | | | | | | | | | | | | 69.2% | | | | | | | | | | | | | 81.8% | 75.0% |
| Implantation rate | | | | | | | | | | | | | | | | 57.7% | | | | | | | | | | | | | 61.5% | 59.6% |
| No. of pregnancies still ongoing | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | - | 0 | 0 | 0 | - | 1 | 7 | - | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 7 | 14 |
| No. of pregnancies went to term | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | - | 0 | 1 | 1 | - | 0 | 5 | - | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 5 | 10 |
| No. of babies born | 3 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | - | 0 | 1 | 1 | - | 0 | 7 | - | 1 | 1 | 0 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 6 | 13 |

NPGD = preimplantation genetic diagnosis; PGS = preimplantation genetic screening; PCR = polymerase chain reaction; OR = ; UPD = uniparental disomy; Y/N = yes/no.
[a] Calculated on no. of oocytes retrieved.
[b] calculated on no. of mature oocytes injected.
[c] only for chromosome 21.

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PGD samples. The follow-up PCR was successful in all embryos, confirming the diagnosis in all nontransferred embryos reanalyzed.

## DISCUSSION

Currently, the diagnosis of chromosomal imbalances on embryos obtained from both reciprocal and Robertsonian translocation carriers is performed using a FISH-based method (1, 2, 4). Although FISH has been applied extensively to identify structural and numerical chromosome abnormalities in embryos, several technical limitations have been described, all of which can confound diagnosis and may lead to incorrect interpretation of the results and a potentially adverse outcome (9).

A major limitation of the FISH procedure is that it is dependent on fixation of a single cell onto a microscope slide, a critical step that requires skill and experience. Suboptimal fixation (condensed nuclei, cytoplasm covering the nucleus, expanded nuclei) can produce a host of issues that are difficult to overcome. The techniques used to spread/fix nuclei on slides (20, 21) and the ability of each technologist to deliver consistent results on each embryo drives the success of the test.

Beyond the technical skill necessary to place and keep the nucleus on the slide, FISH-based tests suffer from other inherent issues that must be overcome, or at least minimized. These issues include hybridization failure (lack of FISH signals), overlapping FISH signals (two FISH signals of the same color lying in close proximity that cannot be discerned as one or two signals, or two FISH signals of different colors lying in close proximity where one signal obscures the second), and split signals (one FISH signal appearing as two due to the separation of sister chromatids).

Each of the limitations listed can lead to misdiagnosis of embryos both in eliminating embryos that could be transferred, or worse, including abnormal embryos in the transfer cohort errantly.

In the present study we have described a new approach for the detection of chromosomal imbalance in embryos derived from translocation carriers. The approach consists of a fluorescent multiplex PCR using STR markers located along the chromosomes involved in the rearrangement. Using STR markers simultaneously for each arm involved in the translocation, one can detect all abnormal segregations for any translocation.

The coamplification of at least three fully informative STR markers lying on either side of the translocation breakpoints increases the accuracy of the test. Allele dropout can lead to misdiagnosis if all markers tested are affected simultaneously. However, the use of a strategy involving at least three informative markers on each side of the breakpoints reduces this risk substantially as ADO in any one marker is not likely to be repeated in the others (Fig. 1). This series of checks and double checks increases the accuracy of the test and would only fail if ADO occurred in all markers at the same time, estimated at about a 0.002% chance per test. The multiplex STR marker system also provides an additional control for contamination with exogenous DNA, as other alleles, differing in size from those of the parents, would be detected (22). However, the approach does not discriminate between noncarrier embryos and those that carry the balanced form of the translocation.

In addition to the ability to test for virtually any chromosome translocation, additional markers can be added to the multiplex to assess common aneuploidies for patients of advanced maternal age or those who wish additional information during genetic testing.

Beyond the technical advantages of the described molecular-based technique lies another, perhaps more important, advantage. The system described not only diagnoses unbalanced inheritance

TABLE 5

**Pregnancy outcome in translocation carriers after PGD treatment.**

| Reference | Cycles/couples | Maternal age (mean ±SD) | No. of clinical pregnancies | Clinical pregnancy rate/embryo transfer | Clinical pregnancy rate/OR | Implantation rate |
|---|---|---|---|---|---|---|
| Robertsonian translocation | | | | | | |
| Goossens et al. (26) | 1,009 / NA | 33.5 | 213 | 29.0 | 21.1 | 16.0% |
| Verpoest et al. (27) | 94 / 54 | 32.2 ± 5.0 | 24 | 38.1% | 25.5% | NA |
| Munné et al. (28) | 133 / 88 | 34.0 | 30 | 42.7% | 37.6 | NA |
| Gianaroli et al. (29) | 35 / 22 | 35.5 ± 3.7 | 13 | 59.1% | 37.1% | 44.4% |
| Present study | 15 / 15 | 37.6 ± 4.8 | 9 | 69.2% | 60.0% | 57.7% |
| Reciprocal translocation | | | | | | |
| Goossens et al. (26) | 1,973 / NA | 33.0 | 264 | 22.9% | 13.4% | 13.1% |
| Verpoest et al. (27) | 190 / 90 | 33.0 ± 4.5 | 22 | 23.2% | 11.6% | NA |
| Lim et al. (30) | 51 / 34 | 31.3 ± 3.1 | 14 | 38.6% | 33.3% | 24% |
| Otani et al. (7) | 36 / 29 | 32.7 ± 2.9 | 17 | NA | 47.2% | NA |
| Munné et al. (28) | 338 / 239 | 36.1 | 79 | 34.1% | 23.4% | NA |
| Gianaroli et al. (29) | 29/ 24 | 34.0 ± 5.3 | 3 | 27.3% | 10.3% | 20.0% |
| Present study | 12 / 12 | 34.4 ± 3.2 | 9 | 81.8% | 75.0% | 61.5% |
| Cumulative translocations | | | | | | |
| Goossens et al. (26) | 2,982 / NA | 33.2 | 477 | 25.3% | 16.0% | 14.2% |
| Verlinsky et al. (31) | 469 / NA | NA | 123 | 34.6% | NA | NA |
| McArthur et al. (32)[a] | 21 / NA | NA | 7 | 50% | NA | 50% |
| Verlinsky et al. (6) | 183 / 130 | 33.2 | 45 | 35.7% | 24.6% | 24.7% |
| Present study | 27 / 27 | 36.1 ± 4.4 | 18 | 75.0% | 66.7% | 59.6% |

*Note:* NA = not available; PGD = preimplantation genetic diagnosis; OR = Oocyte retieval.
[a] Embryo transfer at blastocyst stage.

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of chromosomes in translocation carriers, it allows for tracking of inheritance of each chromosome. This tracking of parental origin allows for the diagnosis of uniparental disomy (UPD) where both chromosomes are inherited from one parent and no chromosomes are inherited from the other (Figure 2) (23, 24). The FISH-based techniques cannot discern the origin of the chromosome that can lead to chromosomally balanced embryos for transfer that suffer from UPD. Although UPD is usually a rare event, UPD14 was detected in three (3.8%) embryos that, otherwise, would have been considered for transfer, potentially leading to phenotypic consequences.

The ability of the PCR-based approach to distinguish the parental origin of inherited chromosomes offers some additional unique data for each test. For instance, in this series of cases, 11 embryos that showed an unbalanced profile for the translocated chromosome were, in the end, scored as aneuploid because the missing/extra alleles actually originated from the partners not carrying the translocation. With the FISH-based protocol, the aneuploidy would be simply scored as an unbalanced inheritance of the translocation. More interesting, the inherent ability to determine parental inheritance of either chromosome allows for the identification of the parental origin of aneuploidy. In the embryos that were tested for both the chromosome rearrangement and aneuploidy due to advanced reproductive age, 30.4% of aneuploidies were of paternal origin, and 5 embryos were carrying both paternal and maternal aneuploidies. These findings deserve further assessment and might represent an important parameter to take into consideration when choosing the kind of cells (polar bodies or blastomeres) to be tested for PGS.

The molecular-based approach carried out for this series of patients has been shown to be efficient at the single cell level. Each multiplex PCR was successfully adapted to single lymphocytes as well as to blastomeres, showing amplification and ADO rates within the expected range for single cell PCR (25). An accurate genotyping was achieved in 263/263 (100%) of the blastomeres with positive PCR and a successful diagnosis in 241 (96.0%) of the embryos analyzed.

Furthermore, the PCR-based protocol is fast (approximately 12-hour turnaround times) and fairly inexpensive to run (~80 € for testing of 10 embryos + controls), compared with purchasing commercial FISH probes for each translocation. In fact, a typical FISH test costs approximately 1000 € to cover probes for parental workup and 1 or 2 clinical PGD cycles of approximately 10 embryos each.

Finally, the molecular approach is also amenable to automation and allows for easy data interpretation. It may also make transport PGD easier, because placing a cell in a tube is far easier to train and monitor than teaching any of the current spreading methods.

Although these results indicate that the molecular-based procedure is reliable and suitable for routine clinical application, some limitations must be considered. Allele dropout occurrences affecting multiple markers simultaneously can potentially lead to misdiagnosis, although the estimated risk is very low (~0.002%). In addition, the occurrence of a contamination event with exogenous DNA, or amplification failure of all markers analyzed, may reduce the number of embryos with a conclusive diagnosis using this technique. In the current study, however, a high percentage (96.0%) of embryos was successfully diagnosed, and no contamination events were detected in blank controls. These limitations do not affect the traditional FISH-based approach and represent the main drawbacks of the described procedure.

Clinical outcomes from PGD cycles performed with the PCR-based approach were very encouraging. Eighteen of 24 patients

(75.0% clinical PR/embryo transfer) with embryo transfer achieved an ongoing pregnancy (mean maternal age 36.1 ± 4.4 years). The embryo implantation rate was 59.6%. The most recent European Society of Human Reproduction and Embryology (ESHRE) PGD Consortium data collection (26) reported an ongoing PR per embryo transfer of 25.3% (mean maternal age 33.2 years) and an implantation rate of 14.2%. In addition, other studies (27–32) (Table 5) using FISH-based testing of blastomeres biopsied from cleavage-stage embryos reported an ongoing PR ranging from 22.9%–59.1% and an implantation rate ranging from 13.1%–44.4%. These values are significantly lower compared with those obtained in this study.

Although the implantation rate and PR in our series of cycles are encouraging, they cannot be attributed only to the technique being used to analyze the embryos. However, a technique that has a lower error rate should lead to more normal/balanced embryos available for transfer, which should positively impact both PR and implantation rates. Despite these considerations, this initial study of a PCR-based approach to chromosome rearrangement PGD has shown that the use of multiple STR markers along each chromosome may lead to more embryos with a correct diagnosis and less embryos with no diagnosis, which will certainly have a positive effect on PR and implantation rates over time.

In conclusion, these results demonstrate the reliability and feasibility of our PCR-based PGD protocol for detection of chromosomal imbalances. Considering the encouraging clinical outcome obtained for the first cases, this approach has the potential to represent a valuable alternative to the FISH-based PGD protocols.

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**TABLE 3**

**Oligonucleotide primer sequences for amplification of STR markers used for detection of chromosomal imbalances on embryos derived from reciprocal translocation carriers.**

| Chromosome | STR markers | Chromosomal band | Outer primers (5′ to 3′) | Inner primers (5′ to 3′) | PCR product size | Dye label |
|---|---|---|---|---|---|---|
| 1 | D1S1612 | p36.23 | F: 5′-ATAGAGGATGGCTCTCTGCCAC-3′<br>R: 5′-GGCAACAAGAGCAAAAATCTGT-3′ | F: 5′- GTCCCATGCCAAAATTCTTAGG-3′[a] | 176 | HEX |
| | D1S1160 | p36.23 | F: 5′-ACCAGTCAATAGCAAGCTGAGG-3′<br>R: 5′-TGGCAAGTTCGTAAATGCTAGT-3′ | [a]R: 5′- CCTTTATTGCCCACCTTACAGA-3′ | 164 | TAMRA |
| | D1S1151 | p36.22 | F: 5′-TTCCAAGCAGTGCTGTTTCATT-3′<br>R: 5′-GGGTGACAGAGCAAGACTCTGT-3′ | F: 5'- GATTCCTGCTCTGATAATCCCC-3'[a] | 252 | TAMRA |
| | D1S1598 | p34.2 | F: 5′-ACCAAGGGACCTAGAAACTCATTA-3′<br>R: 5′-CAAAGGCAGTCTTTTAGAAGGACT-3′ | [a]R: 5′- GGACTGAGACTTACAAGAGGCTAAA-3′ | 132 | HEX |
| | D1S394 | q22 | F: 5′-GTAAATTGCCCAGTTTCACATG-3′<br>R: 5′-CCTTACCTCTAACACTGGAAATCA-3′ | F: 5′- ACTTATAAAGAGACCCTGCCTGA-3′[a] | 189 | FAM |
| | D1S1609 | q41 | F: 5′-CTGACTTGGTACAAACCCACAA-3′<br>R: 5′-TGATTCTAGGTTGGTCACTCGA-3′ | [a]R: 5′- GGGAGAAATTCTATGTGCCACT-3′ | 206 | FAM |
| 2 | D2S1275 | p25.1 | F: 5′-TCCTTGTAAATCTCAGATCCCC-3′<br>R: 5′-TTGCTGCTGGCCTAATTGTTAT-3′ | [a]R: 5′- CATCCTCGACTTCTCTGCTTTT-3′ | 201 | HEX |
| | D2S262 | p25.1 | F: 5′-CCAGAGACCTTTGTTCACTTGTT-3′<br>R: 5′-GCTAGGATGCAGAGATGTGGTA-3′ | F: 5′- CCCTCCACTGTTGTCCTATGAC-3′[a] | 201 | FAM |
| | D2S272 | p24.2 | F: 5′-GGCCTCTCCAAGCACTAGATC-3′<br>R: 5′-GAAAACAATAGTCTGGCTTGGG-3′ | F: 5′- AGGTTGAAGAACAGGGTTTTGA-3′[a] | 216 | TAMRA |
| | D2S1360 | p24.2 | F: 5′-GCCTTTGGACTAGGACTGAATTAT-3′<br>R: 5′-GGCATATACAAAACAGAAACAGAAC-3′ | F: 5′- TATAACCTTGTGAGCCAATTCCTAT-3′[a] | 157 | FAM |
| | D2S150 | q22.1 | F: 5′-ATCACCTCATTCTTCCTCTCTC-3′<br>R: 5′-GTAGGATTTGTGTAAGAAGGCA-3′ | [a]R: 5′- GGGCTCAAAGATCTAAAGTCTT-3′ | 179 | FAM |
| | D2S434 | q35 | F: 5′-CCATCTGTACTGTTCCCAGAAA-3′<br>R: 5′-TATGGATGTGGAAATCATAGCC-3′ | F: 5′- GTGGGATACCTGGAGAAGACTC-3′[a] | 132 | FAM |
| | D2S1338 | q35 | F: 5′-AGGTGGCCCATAATCATGAGTT-3′<br>R: 5′-ATTCCTACTGGCCCATAATCCA-3′ | [a]R: 5′- CCAGTGGATTTGGAAACAGAAA-3′ | 174 | FAM |
| | D2S1363 | q36.3 | F: 5′-TCTGCTTTCTCTGACTGTATCATG-3′<br>R: 5′-CTTTTATTCTTTGTCTCCCCAGTT-3′ | [a]R: 5′- GCTACTTCACTCCATCATTTGCT-3′ | 169 | HEX |
| 3 | D3S1767 | p21.31 | F: 5′-GGGTGACAGAGAATCCATCTCT-3′<br>R: 5′-CCTTTGAGATAATGGCAAAGTG-3′ | F: 5′- GGCTTGAGTGATGGGAATTTAT-3′[a] | 199 | FAM |
| | D3S2456 | p21.31 | F: 5′-AGTCCAGGAGTTCAAGACCAG-3′<br>R: 5′-GATATCCAGTTTTCCCAGCTCT-3′ | F: 5′- ATACACAAAATTGGCTGGTGTG-3′[a] | 209 | HEX |
| | D3S1581 | p21.31 | F: 5′-ATGTGGTGGCCAGTTCTCAAAG-3′<br>R: 5′-TATGTGCTCCAGGCTGGGTAAC-3′ | F: 5′- GTCCTGACAGAACTGCCAAACC-3′[a] | 114 | FAM |
| | D3S2329 | p14.1 | F: 5′-AAGAGTATTCCATAGCGTGTGT-3′<br>R: 5′-ACCATTTGCTAGACAAGGTCTA-3′ | [a]R: 5′- ATGTGCTCAAATACTGTGTGTG-3′ | 244 | FAM |
| | D3S3041 | q26.32 | F: 5′-ATCTCTATCAGGCAGGGCTCTC-3′<br>R: 5′-AGCCACCCCTGTCAGATTTTAG-3′ | [a]R: 5′- TCACCAGACTTCCACAATCACA-3′ | 168 | FAM |
| | D3S2427 | q26.32 | F: 5′-ATTCGTCTCTCTTGCTCCACTG-3′<br>R: 5′-GTGACCTGCACTCAGCAGATCT-3′ | [a]R: 5′- CTGCCTCATCTCTTCAGGATGT-3′ | 186 | HEX |

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## TABLE 3

**Continued.**

| Chromosome | STR markers | Chromosomal band | Outer primers (5′ to 3′) | Inner primers (5′ to 3′) | PCR product size | Dye label |
|---|---|---|---|---|---|---|
| | D3S1754 | q26.32 | F: 5′-GCTTAAAGTGTTTTTTTGAGACA-3′<br>R: 5′-CGCTATTTGGTCTTTAACTGTTA-3′ | F: 5′- ACTTTTTAATACGCTTTTAAGGG-3′[a] | 182 | TAMRA |
| | D3S2398 | q28 | F: 5′-ATTGTAGTGAGCCAAGATCGTG-3′<br>R: 5′-CCCTAGAAAATAGCATCAGGGA-3′ | F: 5′- CTTGGGTGACAGAGTAAGACCC-3′[a] | 102 | HEX |
| 5 | D5S2505 | p15.32 | F: 5′-CTTCTCAGCCTCTGTAATTGCA-3′<br>R: 5′-ACACATGCTGTGTCTCTCAACA-3′ | F: 5′- TGAGCCAATTCCCTTAGTAAGC-3′[a] | 252 | TAMRA |
| | D5S580 | p15.32 | F: 5′-TTGTGCCACTGCATTCTAGC-3′<br>R: 5′-CATTTGCCTGCTGATTTAAAAG-3′ | F: 5′- ACAGAGCCAGACCCTGTCAA-3′[a] | 254 | HEX |
| | D5S807 | p15.2 | F: 5′-CAGGAAACAGAGAAAATATACTGG-3′<br>R: 5′-AAGTTCTTTTCCTGAGGTTTCTAC-3′ | F: 5′- TGAGTAAATTCACACAGCCAGTA-3′[a] | 236 | FAM |
| | D5S1486 | p15.2 | F: 5′-GGCTTAAGGTCAGGAGTTGGAG-3′<br>R: 5′-TGCTCACACTTCAACAAAGCAA-3′ | F: 5′- TAGCCTGGGCAACATAGTGAGA-3′[a] | 170 | HEX |
| | D5S1465 | q34 | F: 5′-GGTTCTTCAAATGACCCAGTTT-3′<br>R: 5′-ATCCTTCTCTCCTACTCCCCAC-3′ | F: 5′- AATGACTAAATTTGGACCCTGC-3′[a] | 204 | HEX |
| | D5S1349 | q35.1 | F: 5′-TATCGTGTGTTATTGCTGCTTG-3′<br>R: 5′-GCATCTACATTTTTGGCAACTC-3′ | F: 5′- CTGACACATAGTTGGCACTCAA-3′[a] | 283 | FAM |
| | D5S1456 | q35.1 | F: 5′-CCATGTGAACCAATTCTTGAAAG-3′<br>R: 5′-ATTGTAACCCCGTTGTAGGTCA-3′ | F: 5′- TCTCTGGAAAACCCTAATTCTCC-3′[a] | 190 | FAM |
| | D5S614 | q35.2 | F: 5′-CACTGGCATGGCTTTGGTTAAT-3′<br>R: 5′-ATCACACAGCTGCCAAGAGACA-3′ | [a]R: 5′- ATCTGTGTGGCTTCTGAATTGC-3′ | 156 | TAMRA |
| 6 | D6S477 | p25.1 | F: 5′-ATAGATGGCTTCCTAGGCTCAA-3′<br>R: 5′-GGACTTCAAAGACAAAAGAGGG-3′ | F: 5′- TTTACTTCTGTCACAGGGCTGA-3′[a] | 219 | HEX |
| | D6S399 | p24.1 | F: 5′-GTGAGACATGATTGCACCACTG-3′<br>R: 5′-GAGCTTGCTGTGAGCATTTACC-3′ | [a]R: 5′- GTAACAAATGGTGACCTGCAGC-3′ | 288 | HEX |
| | D6S1279 | p24.1 | F: 5′-TTTAAGGAGCTACAGTGGCAGG-3′<br>R: 5′-TCCCCAGGTAAAATGATTTGAC-3′ | [a]R: 5′- TCTGACAAATTGCTCCCACTTC-3′ | 164 | TAMRA |
| | G15833 | q24.3 | F: 5′-CTCTCGGATCATCAGTTTGTGA-3′<br>R: 5′-CTTGAATTTTTGACTGCACAGG-3′ | F: 5′- TGTTCAAGGGTCAACTGTGAAT-3′[a] | 209 | FAM |
| | D6S1009 | q23.3 | F: 5′-GCTGTTCTCAGAGCCCTAAAAA-3′<br>R: 5′-AGCTATGATCACAATGCTGCAC-3′ | [a]R: 5′- GCAACAGAGTGAAAGACCCTGT-3′ | 206 | HEX |
| | D6S960 | q25.1 | F: 5′-GAAGATGCTGGAGCTCTAAGCC-3′<br>R: 5′-TTAGTGTGCCTTTGACAAGCCT-3′ | F: 5′- TTTTCTCCAGGTGTTTCCTGTG-3′[a] | 180 | HEX |
| 7 | D7S620 | p21.3 | F: 5′-TGTATTTGTCAGGGTTCTCCAG-3′<br>R: 5′-CCAATTCTCCTCATAAGTCTCCTC-3′ | F: 5′- CAGAGAGACACAGCCAATTAAATG-3′[a] | 164 | HEX |
| | D7S2210 | p15.3 | F: 5′-AGCCTCTCAAGGACTGAGAGAG-3′<br>R: 5′-CAAATAGAAGCTCATCTGGAAGC-3′ | F: 5′- AGTGAGGTGGACAAGCAGGTAG-3′[a] | 218 | FAM |
| | D7S460 | p14.3 | F: 5′-CTTCAGTTCCCTGTCACATTTGT-3′<br>R: 5′-CCTAGAAAGATGGGAAACAGCTG-3′ | F: 5′- GCCACAGGGTTTATGAAACTGAT-3′[a] | 205 | TAMRA |
| | D7S2847 | q31.2 | F: 5′-CACCTTCAGAAAGTATTGCCTA-3′<br>R: 5′-CATAGTGAGGTGTTTCTCCAAG-3′ | [a]R: 5′- CAAGCTCTGTTCCTCATTAATG -3′ | 182 | TAMRA |

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**TABLE 3**

**Continued.**

| Chromosome | STR markers | Chromosomal band | Outer primers (5′ to 3′) | Inner primers (5′ to 3′) | PCR product size | Dye label |
|---|---|---|---|---|---|---|
| | D7S1805 | q35 | F: 5′-AAAGTGCTGGGATTACAGACAT-3′<br>R: 5′-CCCACTTCTCTGCTATTACATATAT-3′ | F: 5′- CTGTATCTCTCAGTTAATTCCCAG-3′[a] | 182 | FAM |
| | D7S2208 | q36.1 | F: 5′-ATGCCTTTACCCACCCCTCTA-3′<br>R: 5′-GGGAAAGAAGATACAGGTGGAAT-3′ | F: 5′- AATCAGTATGCCTGTATCCACCA-3′[a] | 248 | TAMRA |
| | D7S1823 | q36.1 | F: 5′-AGTGGCTTCTATTTCTCTCCAAGT-3′<br>R: 5′-AGATCCTCGTATGGGAGTGACC-3′ | F: 5′- TCCTACAGTGGACTCAGAAGCC-3′[a] | 200 | HEX |
| 8 | D8S492 | p22 | F: 5′-CAGACAGTTGCTGCTAACCC-3′<br>R: 5′-GCAAGTAGGCCTGTTTCATG-3′ | [a]R: 5′- TTGGCTGGTTAGTGACACAGA-3′ | 162 | FAM |
| | D8S1477 | p12 | F: 5′-AGAGGGTTTCGCCAAAAGATT-3′<br>R: 5′-CTGAGCCTTCCTGTCCTCTAAT-3′ | F: 5′- ATGAGCAAACTTCATCCACTTG-3′[a] | 180 | HEX |
| | D8S532 | p11.21 | F: 5′-GCTCAAAGCCTCCAATGACTGT-3′<br>R: 5′-GCAACCAGAATGGACTAGGACA-3′ | F: 5′- GTAATTATGCAAGGCCCACATG-3′[a] | 165 | TAMRA |
| | D8S2317 | p11.21 | F: 5′-TGATGGGAATGAGAGAATCTCA-3′<br>R: 5′-CCCCAGGAAGTATACCATTCAA-3′ | F: 5′- CAGAACTGGCTCTTCAATGACA-3′[a] | 208 | HEX |
| | D8S1104 | p11.21 | F: 5′-CAGCTATGAGAAAAGTTGAATGGT-3′<br>R: 5′-GGAGGTATATTTTGACCCTTGTTT-3′ | [a]R: 5′- TGTACGGTATGCATCAGAGGTTT-3′ | 115 | HEX |
| | D8S592 | q24.11 | F: 5′-TTGCCTTAGGTGGACTGAATATA-3′<br>R: 5′-TCAGTGTTTCCACAGGATAGATG-3′ | F: 5′- GTTGGCTAATGTTCCTGTCTTCT-3′[a] | 173 | HEX |
| | D8S586 | q24.12 | F: 5′-GAAGGAGGGCTAAAATAAACCC-3′<br>R: 5′-GGGTACTAGCTGTGCCTATTGC-3′ | [a]R: 5′- TGCTTCTGGAGTGTCATACCAT-3′ | 265 | FAM |
| 9 | D9S254 | p23 | F: 5′-CTGCTTCTGCTTCACTCAAAGAT-3′<br>R: 5′-GGATAAACCTGCTTCACTCAAAG-3′ | F: 5′- GTAATAACTGCCGGAGAGATGG-3′[a] | 112 | TAMRA |
| | D9S746 | p21.2 | F: 5′-TTCACTACAACAACTGCTGTCACTC-3′<br>R: 5′-ACAGCGAGACTCCATCTCAAAAAT-3′ | F: 5′- CTCACTGATTCACAAAAAATTGGGT-3′[a] | 215 | FAM |
| | D9S251 | p21.1 | F: 5′-GTAGGCAAAACTAACCCTTTTGTAG-3′<br>R: 5′-TCCAGCTTGCTCTATTTCAGACT-3′ | F: 5′- GAAATTTTGACTGGAAAGTGCC-3′[a] | 255 | HEX |
| | D9S752 | q34.11 | F: 5′-GAGGCAGGAGAATCACTTGAAT-3′<br>R: 5′-TCAGGCCATTATACTCATTGGA-3′ | F: 5′- GTTGCAGTGAGCTAAGATCACG-3′[a] | 169 | FAM |
| | D9S766 | q34.11 | F: 5′-TGCTTGACCTCAGGAAGTGG-3′<br>R: 5′-CCACACCGCCTCTATTCATC-3′ | F: 5′- GAGCCAAGATTGCACCACTG-3′[a] | 208 | HEX |
| | D9S1830 | q34.13 | F: 5′-CCATTCCTAACCTCCAGAGTGG-3′<br>R: 5′-GACTGCCTTCCTCATGCCTG-3′ | F: 5′- TGGGACTCAATCCCAAGTGG-3′[a] | 94 | FAM |
| 10 | D10S2483 | p15.3 | F: 5′-TCTCCACCCACATAACACAGAC-3′<br>R: 5′-GTAATTGCAGGTTTTGCAATTG-3′ | [a]R: 5′- TGGCAGAAACCACAATTACTTT-3′ | 122 | TAMRA |
| | D10S1435 | p15.3 | F: 5′-GCCCTCGAAGAGGTTCTAGAGT-3′<br>R: 5′-GCCAGACCCTGTCTCAAAAATA-3′ | F: 5′- CTTGTGTGGTGCTGTGTTTGTT-3′[a] | 133 | FAM |
| | D10S526 | p15.3 | F: 5′-CACTAGCCAGGGTTCTCCAGAG-3′<br>R: 5′-TGGACGCTGAACTGATGAGTGT-3′ | [a]R: 5′- ACCCAAATCCCAAGTGAACATC-3′ | 281 | HEX |
| | D10S1237 | q25.3 | F: 5′-ACTCTCTTAGGTTTCTAGCTTGC-3′<br>R: 5′-TCTGTACTAGTCAGGTTCTCCAG-3′ | F: 5′- GCCTCTATAATTGTGTGAGTCAT-3′[a] | 222 | TAMRA |
| | D10S1213 | q26.13 | F: 5′-ATGAAGAGACCAGGCCAGTGTC-3′<br>R: 5′-CTGTAGTGAGCGGAGATCATGC-3′ | [a]R: 5′- GCCTGGGCAATAAGAGTGAAAC-3′ | 252 | FAM |

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**TABLE 3**

**Continued.**

| Chromosome | STR markers | Chromosomal band | Outer primers (5′ to 3′) | Inner primers (5′ to 3′) | PCR product size | Dye label |
|---|---|---|---|---|---|---|
| | D10S1248 | q26.3 | F: 5′-AATAAGTGCAGTGCTTGGCAAA-3′<br>R: 5′-AAAGCAAACCTGAGCATTAGCC-3′ | F: 5′- CTCTGTATCCCACCCCTGGATA-3′[a] | 238 | HEX |
| 11 | D11S1984 | p15.5 | F: 5′-GGGTGACAGAGCAAAATTCTGT-3′<br>R: 5′-GCCTACACCTGGATCTTGGACT-3′ | [a]R: 5′- GCCTCCAGACCTTGTAGAGACA-3′ | 160 | HEX |
| | D11S1997 | p15.4 | F: 5′-TTCCTAAGAAAGATAAAGCACCAG-3′<br>R: 5′-GGACAAAATAAAGACCAGCTTTAC-3′ | [a]R: 5′- CAATTGACAGTGGATTTTTGAC-3′ | 143 | FAM |
| | D11S1999 | p15.4 | F: 5′-GACTCACAGTTCTACATGGCTGG-3′<br>R: 5′-GGAACTGGAGTAAACAAGATTGC-3′ | F: 5′- AGTCATGTCTTACATGGCAGCAG-3′[a] | 133 | TAMRA |
| | D11S1981 | p15.1 | F: 5′-ACCTCGGCCTCTCAAAGTACTG-3′<br>R: 5′-CTTTCCCAGACAGAGCTCAGGA-3′ | F: 5′- ACTGCACCCGGTCACTAAAAAA-3′[a] | 176 | TAMRA |
| | D11S1978 | p11.2 | F: 5′-GACAGATGAATGGATAAAGGCA-3′<br>R: 5′-ATCTGCACTCCACAAATACACAC-3′ | [a]R: 5′- GCCTTCCAGGTTCATTCATATT-3′ | 209 | HEX |
| | D11S2016 | p11.2 | F: 5′-TGTTGGATGAAGTAATACTGGTGA-3′<br>R: 5′-CCATATGAACTGCAGCATTATTC-3′ | [a]F: 5′- GAATGAATAGAGGCAATGTGACAT-3′ | 185 | FAM |
| | D11S2179 | q22.3 | F: 5′-CTGAAGTGGGAGGATTGTTTGA-3′<br>R: 5′-GCACTGGAATACGATTCTAGCAC-3′ | F: 5′- GCCTAGGCAATACAGCAAGACC-3′[a] | 147 | HEX |
| | D11S1304 | q25 | F: 5′-TCCTTCACAGGGTTCATTTTTC-3′<br>R: 5′-CTGCTAAAAGACTGTGGGAACA-3′ | F: 5′- GGCATTTGGCTTTTTCAGATTA-3′[a] | 175 | HEX |
| 12 | D12S372 | p13.32 | F: 5′-GTAGAATAAATCCCTGCATGGC-3′<br>R: 5′-ACTCTCCAATGGAAAGAAATGG-3′ | F: 5′- GTGGACCACAGGGTATCATCTA-3′[a] | 193 | HEX |
| | D12S391 | p13.2 | F: 5′-GAATCAACAGGATCAATGGATG-3′<br>R: 5′-TGCAGATGGACTGTCATGAGAT-3′ | [a]R: 5′-TTCAGCCTCCATATCACTTGAG-3′ | 156 | FAM |
| | D12S373 | p12.3 | F: 5′-ACCAAGTTGCAGAGCTACTCTAGG-3′<br>R: 5′-CACTAGATGTTTTCAAGGCTCACA-3′ | F: 5′- TAGAGAAAGGCAGACAGATAGGTG-3′[a] | 196 | FAM |
| | D12S1066 | p12.1 | F: 5′-TGACGTTTATTGGCCACTTGTA-3′<br>R: 5′-TGTGGGACCTTGTGATTGTGTA-3′ | F: 5′- AAATGTCTGCTCAGGACCTGAG-3′[a] | 176 | TAMRA |
| | D12S395 | q24.23 | F: 5′-CCATTTCCGTCAGTGAACATCT-3′<br>R: 5′-CCAACAATGGGCAATAACTTCT-3′ | F: 5′- TACCTCGATGTAATGCACGTGT-3′[a] | 129 | TAMRA |
| | D12S378 | q24.31 | F: 5′-CTTGGCCCTGATTGATTTTTTG-3′<br>R: 5′-AGGACACCAGCCATACTGGATC-3′ | [a]R: 5′- GGACCCACCTCATGACCTTATC-3′ | 259 | FAM |
| | D12S2078 | q24.32 | F: 5′-CACAATTTCACGTACTTGGCAA-3′<br>R: 5′- CCCCATCCTTCTTTTTATGACA-3′ | F: 5′- GCTGAGAACTGGAACCATCAAT-3′[a] | 173 | HEX |
| 15 | D15S817 | q11.2 | F: 5′-AGACTATGGTACCCAACAAGCA-3′<br>R: 5′-TAGTCAGGGTTATCGAGAGCAA-3′ | F: 5′- CATGTGATTCAGTTCCCCTAAA-3′[a] | 119 | FAM |
| | D15S1365 | q12 | F: 5′-ACAAAAATTAGCCTGACGTGGT-3′<br>R: 5′-CATTTATTGTCTCTATGGGCAGC-3′ | F: 5′- CAGAGTGAGACACTCTTGGGAAA-3′[a] | 256 | HEX |
| | D15S822 | q12 | F: 5′- AACTGTATCCAGCATGAATCTCTG-3′<br>R: 5′- AATGAAGGAAAGTCAACAGTCTCA-3′ | F: 5′- TTCTCTTTCTCACCTCTCCTTCTG-3′[a] | 185 | FAM |
| | D15S192 | q24.1 | F: 5′-AATTGCAGTGAGCCAAGAT-3′<br>R: 5′-TTCTGCACATAGTCTGCATC-3′ | F: 5′- AACAAGAGCAAGACTCCGAG-3′[a] | 253 | FAM |
| | D15S526 | q26.1 | F: 5′-AGCCACTGAACTTTTGGCTAGG-3′<br>R: 5′-CATGAGACTGAGGCAGGAGGAT-3′ | [a]R: 5′- TTATCATGGCACTGCACTCCAG-3′ | 244 | TAMRA |

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## TABLE 3

**Continued.**

| Chromosome | STR markers | Chromosomal band | Outer primers (5′ to 3′) | Inner primers (5′ to 3′) | PCR product size | Dye label |
|---|---|---|---|---|---|---|
| | D15S230 | q26.2 | F: 5′-CTGTCTAGAGTTTTTAGCCTGTCG-3′ R: 5′-CCAGAAAATACAGAACCAGTAAGG-3′ | F: 5′- TTTAAGATTTACCTAGCCAAACCC-3′[a] | 188 | TAMRA |
| | D15S642 | q26.3 | F: 5′-AGGATCACTTGAGGTCAGGAGTTT-3′ R: 5′-ACTAATGCTTTCAGGAATGTGAGG-3′ | F: 5′- TAATCTCAGTTACCCAGGAAGCTG-3′[a] | 189 | FAM |
| 18 | D18S391 | p11.31 | F: 5′-TCATGAGGTGGACTTACCACAG-3′ R: 5′-CCCATCTGAGTCACTCAGCTAA-3′ | F: 5′- GCAATGTGACTTGAGGAAGAGA-3′[a] | 133 | FAM |
| | D18S976 | p11.31 | F: 5′-TCCTTAGCAAGAAACTCCCTGA-3′ R: 5′-GGACTTCTCTGCTGCCATAATC-3′ | [a]R: 5′- ACCAATTCCCCTAATAAATGCC-3′ | 166 | HEX |
| | D18S542 | p11.21 | F: 5′-TCTGTTTCCAGTGGAAACCAAA-3′ R: 5′-GGAGGGTCTTGGAACAAATTCT-3′ | [a]R: 5′- CCAGCAACAACAAGAGACAGCT-3′ | 199 | FAM |
| | D18S1371 | q23 | F: 5′-TCTCTCTTCATCCACCATTGGT-3′ R: 5′- AGCAGGTACAGACGTGAAAAGG-3′ | [a]R: 5′- GCTGTCAGAGACCTGTGTTGTG-3′ | 145 | TAMRA |
| | D18S821 | q23 | F: 5′- CTCTAGAGGATCCGAATTATTATA-3′ R: 5′- TGTAAAGTGCCTCATCATTTAT-3′ | [a]R: 5′- TAGATTTCTGCTTGTCATTTTC-3′ | 165 | FAM |
| | MBP | q23 | F: 5′-CCCACTAGAATGTAGGTTTC-3′ R: 5′-ATTTACCTACCTGTTCATCC-3′ | F: 5′- GGACCTCGTGAATTACAATC-3′[a] | 141 | HEX |
| | D18S70 | q23 | F: 5′-TACTGGTGCCCCATAGAGAGAC-3′ R: 5′-CCTCTCTCCCAGAAAGATCTCC-3′ | F: 5′- CACACACAATGTTTTGGGAATG-3′[a] | 109 | FAM |
| 19 | D19S403 | p13.2 | F: 5′-CGGGAAGTCTAGGCTACAGAGA-3′ R: 5′-ATAGCCATCCTTCCCAGTAGCT-3′ | F: 5′- TAGTGCCATTGGATTTCAACCT-3′[a] | 223 | HEX |
| | D19S581 | p13.2 | F: 5′-CCAGACAGAAGTGTTTACTATTATGG-3′ R: 5′-CTAGGAGGTCGAGACTACAGTGAG-3′ | F: 5′- TTACTATTATGGGTGTCTTCCTGAC-3′[a] | 271 | HEX |
| | D19S564 | p13.13 | F: 5′-GATCTTGCCATTGCACTTCAGTC-3′ R: 5′- CACCAACACACCTGGCTAATTT-3′ | [a]R: 5′- GCCCAGGCTTCTATCTGCTTTTA-3′ | 212 | FAM |
| | D19S556 | p13.12 | F: 5′- TTGCAGTGAGTCAATATCATGCC-3′ R: 5′-GGGAGACTTTTTGGATAGAAGGG-3′ | F: 5′- TTGCACTCTAGTTTGGGTGACAG-3′[a] | 300 | TAMRA |
| | D19S543 | q13.32 | F: 5′- TCTTTCCTTCTGAGGGTCCCTT-3′ R: 5′-ACACTAAGCCAGGGAGGTTGAG-3′ | F: 5′- AGATGTCCACTTCCACTGGTCC-3′[a] | 245 | FAM |
| | D19S562 | q13.32 | F: 5′- GAGTTTGCAGTGAGCCAAGAT-3′ R: 5′-TCCACAGGGCAACCTATTATAA-3′ | F: 5′- CTGGGTGACAGAGTGATATTCTG-3′[a] | 234 | TAMRA |
| | D19S553 | q13.33 | F: 5′- AACCTGGACAAATGCCAGAAAG-3′ R: 5′-CCACTGCACTCCAGATTAGGCT-3′ | F: 5′- CTTTTGACAAAATCCCCCTTGA-3′[a] | 339 | HEX |
| | D19S402 | q13.33 | F: 5′- CAGAATTTCAAAAGCAGCCTG-3′ R: 5′-TGGGTTTCTTCTCTTGGCTAAG-3′ | F: 5′- AGGGAGACCCTGTCTCTACACA-3′[a] | 295 | FAM |
| 22 | D22S689 | q12.3 | F: 5′- CAGGAAGTCAAGGCTGTAGGG-3′ R: 5′- ACCTGCAACTTGCTTTTCTGC-3′ | F: 5′- AGACCCCATCTCAATCTGCCT-3′[a] | 233 | FAM |
| | D22S691 | q12.3 | F: 5′- GAGCCTCTTCTGCAAAATGAGA-3′ R: 5′- TGTGATTCAATTAACCAGGCCT-3′ | F: 5′- CATCAACCACACAGGGATGTTAT-3′[a] | 169 | FAM |
| | D22S685 | q12.3 | F: 5′- TCTCTTACAGGGCAGTTGTTTA-3′ R: 5′- GGGAAAGAAAGGAAATAGGTTC-3′ | F: 5′- AGGGATCCTAGTTATCACCTACA-3′[a] | 213 | HEX |

*Fiorentino. A PCR-based PGD protocol for chromosomal translocation. Fertil Steril 2010.*

## TABLE 3

**Continued.**

| Chromosome | STR markers | Chromosomal band | Outer primers (5′ to 3′) | Inner primers (5′ to 3′) | PCR product size | Dye label |
|---|---|---|---|---|---|---|
| | D22S534 | q13.1 | F: 5′- AGAGATGGAGTTTGCCGTAAGC-3′<br>R: 5′- CAAACAGAGACATCACCCCCTT-3′ | F: 5′- TGACAGGGCAAGACTCCATAATT-3′[a] | 109 | TAMRA |
| | D22S417 | q13.2 | F: 5′- GAGCCTGGGAAGTTAAGACTGC-3′<br>R: 5′- CCCCACCCTCCTATCTTGATTA-3′ | F: 5′- CACTTCAGCCTGTATGACACGG-3′[a] | 223 | HEX |
| | D22S526 | q13.33 | F: 5′- AGATCACACCACTGTACTCCAGC-3′<br>R: 5′- TCCATTAAACCAACCTACCTACCT-3′ | F: 5′- AGAGCAAGACTCTGTCTCAACAAA-3′[a] | 219 | FAM |

F = Forward; R = Reverse; STR = short tandem repeat; PCR = polymerase chain reaction.
[a] A heminested approach was used.

*Fiorentino. A PCR-based PGD protocol for chromosomal translocation. Fertil Steril 2010.*



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