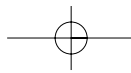




METHODS EXPRESS

# Whole Genome Amplification

edited by S. Hughes and R. Lasken



# CHAPTER 11

## Pre-implantation genetic diagnosis using whole genome amplification

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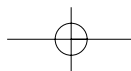
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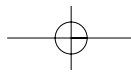
### 1. INTRODUCTION

Pre-implantation genetic diagnosis (PGD) following assisted conception is now well established clinically as an alternative to conventional pre-natal diagnosis in couples at risk of having children with an inherited disease (1). Controlled ovarian stimulation, egg collection by ultrasound-guided transvaginal needle aspiration and insemination with the partner's washed sperm provide access to fertilized pre-implantation-stage embryos *in vitro*. Single cells, typically the first and second polar bodies and/or one or two blastomeres, are then removed by micromanipulation from each fertilized zygote or cleavage-stage embryo, respectively, for genetic analysis. This typically involves fluorescent *in situ* hybridization (FISH) and other molecular cytogenetic techniques for detection of chromosomal abnormalities in interphase nuclei, or for detection of single gene defects, PCR-based strategies for DNA amplification and mutation detection. Finally, unaffected embryos are selected for transfer to the uterus, avoiding the possibility of terminating an affected pregnancy diagnosed at later stages.

The range of genetic defects that can be diagnosed has expanded dramatically since the first births were reported in couples at risk of X-linked conditions and cystic fibrosis (2, 3), and now includes numerical and structural chromosomal abnormalities and most of the common single gene defects (4). The scope of PGD has also been extended to screening for chromosomal aneuploidy in infertile couples (5–7) and for human leukocyte antigen (HLA) typing with or without single gene defect diagnosis with the aim of recovering compatible stem cells from cord blood at birth for transplantation to an existing sick child (8–10). Although precise data are not available, it is now estimated that approaching 1500 babies have been born worldwide following PGD (11).

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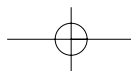
Diagnosis of single gene defects requires sequence information from both parental chromosomes in the single cell removed from each embryo. This was made possible initially by amplifying a short DNA fragment encompassing the mutation with two rounds of PCR, generally with a nested pair of oligonucleotide primers in the second round (see *Fig. 1A*) (3, 12, 13). With the advent of automated sequencers offering highly sensitive detection and analysis of fluorescent PCR products, strategies were developed for multiplex amplification of several fragments, for example, to combine mutation detection with chromosome-specific sequences to identify the sex of embryos and contamination with exogenous DNA (see *Fig. 1B*) (14, 15). Most recently, this has evolved further to combine multiplex amplification of several short fragments, followed by rapid sequencing or mini-sequencing for sequence/mutation analysis (see *Fig. 1C*) (16, 17).

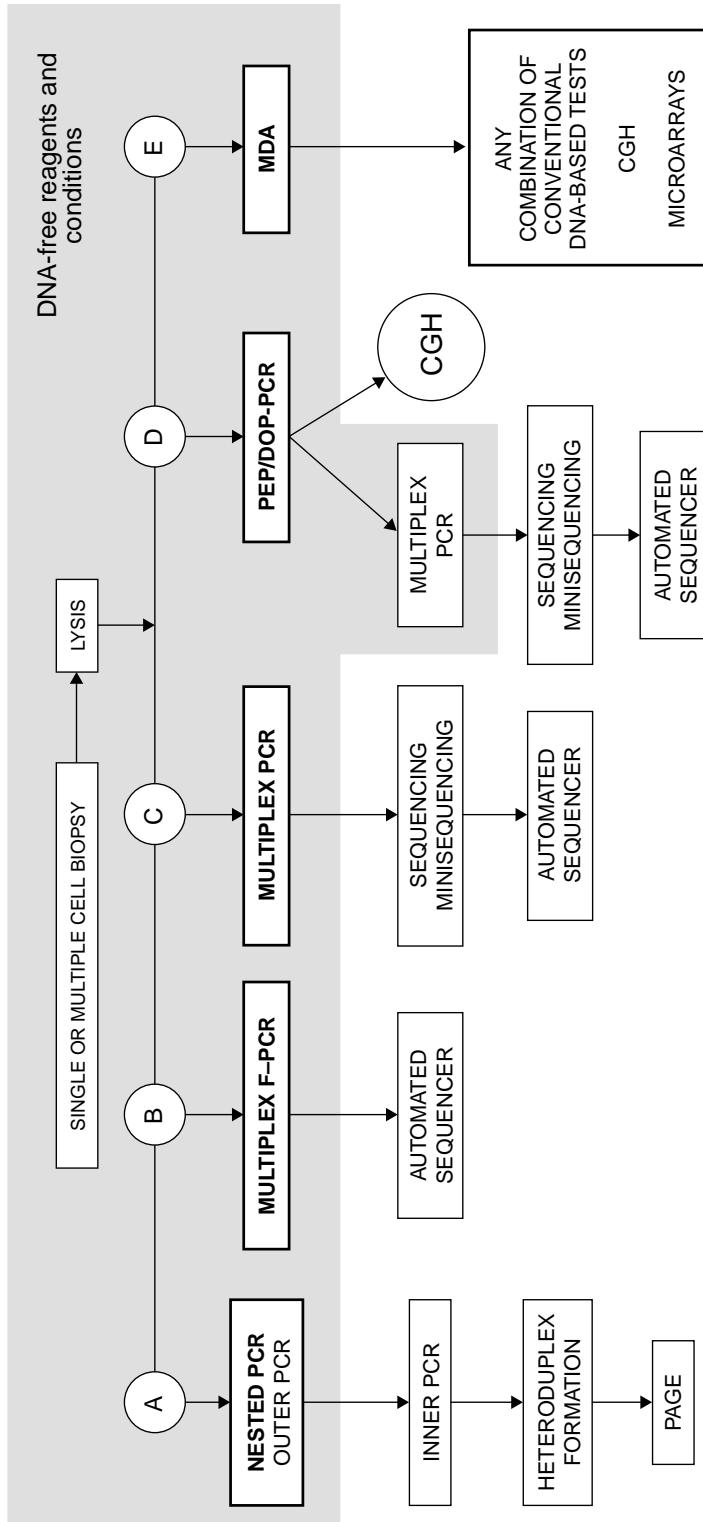
Highly sensitive amplification strategies, which are capable of detecting as few as one or two target dsDNA molecules in a single cell, are equally highly susceptible to errors through contamination with foreign or previously amplified DNA. One of the advantages of multiplex PCR strategies including chromosome-specific sequences, therefore, is to detect amplification from contaminating exogenous target DNA by detecting markers, often highly polymorphic repeats, not present in the parental chromosomes (see *Table 1*). Another problem when amplifying from single cells is that occasionally one parental allele fails to amplify at random, resulting in allele dropout (ADO). This can also occur by chromosome malsegregation during the early cleavage divisions of the human embryo when only one of the two parental chromosomes has segregated into the cell removed from the embryo for analysis (18). In these situations, multiplex strategies with chromosome-specific markers can identify when ADO occurs and, if the marker is closely linked or intragenic to the gene defect being diagnosed, has the additional advantage of providing a second linkage-based verification of mutation status (19).

## 2. METHODS AND APPROACHES

### 2.1. PGD using WGA

The idea of using WGA as a universal first step to enable secondary analysis of a range of sequences without the need to optimize primers and reaction conditions for multiplexing (*Fig. 1D*) (20), followed the development of the PCR-based primer-extension pre-amplification (PEP) method for haplotyping single sperm (21). PEP has been used for analysis of sex-linked sequences, deletions of the dystrophin gene for PGD of Duchenne muscular dystrophy (22) and to detect a mutation causing familial adenomatous polyposis coli, an autosomal dominant cancer-predisposing syndrome (23). However, a number of disadvantages including the limited amplification achieved and consequent inaccuracies in the amplification of highly polymorphic microsatellite repeat sequences, particularly the common dinucleotide repeats, which are valuable as linked markers, have





**Figure 1. Strategies for amplifying target sequences from single and small numbers of cells for genetic analysis of mutations and other sequences.** PAGE, polyacrylamide gel electrophoresis; F-PCR, fluorescent PCR; PEP, primer-extension pre-amplification; DOP, degenerate-oligonucleotide priming; MDA, multiple-displacement amplification; CGH, comparative genomic hybridization (see Table 1 for a more detailed explanation).

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**Table 1. Comparison of different strategies for amplifying target sequences from single and small numbers of cells for genetic analysis of mutations and other sequences**

Method	Feature	Time required (h)*	Advantages	Disadvantages
<b>Amplification of specific target sequences</b>				
A Nested PCR	Outer PCR can be multiplexed	6	<ul style="list-style-type: none"> <li>• Increased specificity</li> <li>• Decreased carry-over contamination</li> <li>• Sufficient DNA amplified for conventional analysis</li> </ul>	<ul style="list-style-type: none"> <li>• Repetitive sequences not amplified accurately</li> <li>• Not quantitative</li> </ul>
B Single multiplex fluorescent PCR	Up to 8 target fragments	3	<ul style="list-style-type: none"> <li>• Fast and quantitative</li> <li>• Fingerprinting detects contamination</li> <li>• Screening for major aneuploidies (if informative) can be combined with mutation detection</li> </ul>	<ul style="list-style-type: none"> <li>• Requires carefully optimised set of primers and reaction conditions</li> </ul>
C Multiplex PCR (plus sequencing/mini-sequencing)	Up to 15 target fragments	8	<ul style="list-style-type: none"> <li>• Moderately fast</li> <li>• Quantitation variable</li> <li>• Screening for major aneuploidies (if informative) can be combined with mutation detection and linked STR markers</li> <li>• Sequencing/mini-sequencing can be applied to any mutation</li> <li>• Reduced ADO</li> </ul>	<ul style="list-style-type: none"> <li>• Limits to multiplexing</li> </ul>
<b>WGA</b>				
D PEP	Linear amplification 80–100 fragments ~800 bp	12	<ul style="list-style-type: none"> <li>• Multiple fragment analysis without the need for optimization</li> </ul>	<ul style="list-style-type: none"> <li>• Amplification of specific target sequences requires sensitive methods</li> <li>• Repetitive DNA not amplified accurately</li> </ul>
DOP-PCR	Greater quantitative yield Less coverage			
E MDA	~50 ?g DNA product Average 10 kb	10–18	<ul style="list-style-type: none"> <li>• Universal initial amplification</li> <li>• Sufficient DNA amplified for extensive conventional genetic analysis not requiring specialist facilities</li> </ul>	<ul style="list-style-type: none"> <li>• Variable proportion of human sequence in amplified product</li> <li>• Extensive preferential amplification</li> </ul>

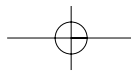
\*Time required to amplify target sequences prior to analysis by a range of different methods.

discouraged widespread application of this approach (see *Table 1*) (24). Another PCR-based method for WGA, degenerate-oligonucleotide priming (DOP), which provides greater amplification but less uniform genome coverage, has been used for comparative genomic hybridization (CGH) and identification of aneuploidy and unbalanced translocations in single cells (6, 7, 24).

The development of WGA using the bacteriophage  $\phi$ 29 DNA polymerase for isothermal multiple-displacement amplification (MDA) has several advantages (25, 26).  $\phi$ 29 DNA polymerase has high processivity generating amplified fragments of >10 kb by strand displacement and has proofreading activity resulting in lower misincorporation rates compared with *Taq* DNA polymerase. The random hexamer primers must be thiophosphate modified to protect them from degradation by the 3'→5' exonuclease proofreading activity of  $\phi$ 29 DNA polymerase (27). Isothermal WGA directly from clinical samples such as blood and buccal swabs has allowed high-throughput genotyping without the need for time-consuming DNA purification steps (28). Sequence representation in the amplified DNA, assessed by multiple single nucleotide polymorphism (SNP) analysis, is equivalent to genomic DNA when amplifying from as little as 0.3 ng target DNA and amplification bias is superior to PCR-based methods (29).

The principal advantage of MDA for PGD is that sufficient amplified DNA is produced to allow extensive parallel genetic testing and accurate mutation detection by conventional relatively low-sensitivity methods (*Fig. 1D*) (30, 31). Even from single lymphocytes, the yield of amplified DNA is in the microgram range, allowing, for example, analysis of 20 different loci (including the  $\Delta$ F508 deletion in exon 10 and two intragenic microsatellite markers in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, and nine short tandem repeats used in DNA fingerprinting) by standard, relatively low-sensitivity PCR methods (30). This equals or exceeds the maximum number of loci that have been amplified directly from single cells by multiplex fluorescent PCR, without any need to optimize the conditions for efficient co-amplification (9, 14) and only using a small fraction of the amplified DNA. Furthermore, unlike PCR-based methods (24), the size of all of the polymorphic repeat alleles, including dinucleotide and short tandem repeats, was accurately identified. However, preferential amplification and ADO at heterozygous loci is not eliminated by MDA, and subsequent analysis needs to be carefully optimized and compensated for by increasing the number of loci analysed (see *Table 1*). Alternatively, increasing the number of cells sampled to between 2 and 20 rapidly reduces these problems (30).

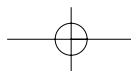
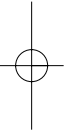
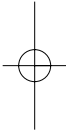
With PCR-based strategies (with or without WGA), separate equipment, isolated clean room facilities and stringent precautions are essential for the initial stages of amplification to prevent contamination (see *Fig. 1A–D*). This effectively excludes the use of most laboratories where amplification and handling of PCR products on the laboratory bench are commonplace. As a consequence, PGD is a costly, highly specialized procedure only available in a handful of centers with the necessary resources and expertise. In contrast, MDA is easily carried out following embryo biopsy in the DNA-free conditions of clinical embryology laboratories and the products analysed elsewhere by conventional relatively low-sensitivity approaches (*Fig. 1E*). By eliminating a significant part of the preliminary work



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involved in test development by PCR methods alone, this should make it possible to offer PGD for any known genetic defect based on established protocols at a significantly reduced cost. With microgram amounts of MDA product, it may also be feasible to use DNA microarrays (i) to identify karyotype abnormalities by CGH (31); (ii) to identify haplotypes by large-scale SNP analysis; and (iii) to screen for a broad range of single gene defects. A disadvantage of WGA, by either PCR-based or MDA methods, is the significantly increased time involved (see *Table 1*). However, with improvements in embryo culture media, embryo transfer to the uterus is now routinely delayed by 24–30 h following embryo biopsy early on day 3 post-insemination and the successful application of this approach for PGD of  $\beta$ -thalassemia has been reported (32).

To illustrate the power of using MDA for PGD from single or small numbers of cells removed from human embryos, we present here our current methods for cell lysis and MDA, and a combination of protocols that combine testing for (i) mutations causing  $\beta$ -thalassemia by mini-sequencing; (ii) closely linked short tandem repeat (STR) markers for independent linkage-based verification of mutation status; (iii) HLA matching using multiple STR markers across the HLA region of chromosome 6; and (4) chromosome-specific markers for molecular genetic detection of common aneuploidies.



### 3. RECOMMENDED PROTOCOLS

#### Protocol 1

#### Preparation and lysis of single cells

##### Equipment and Reagents

- Histopaque-1077 (Sigma-Aldrich)
- Phosphate-buffered saline (sterile and calcium/magnesium-free) (PBS)
- PBS with 15 mg/ml polyvinylpyrrolidone (PVP) (high molecular weight; Sigma-Aldrich)
- Alkaline lysis buffer (0.4 M KOH; 10 mM EDTA; 100 mM dithiothreitol)
- Neutralizing buffer (REPLI-g Kit; Qiagen)
- 0.2 ml PCR tubes (DNase, RNase and DNA free)
- Mouth pipette with 0.22  $\mu\text{m}$  filter (Millipore) and capillary tube adapter (Sigma-Aldrich)
- Finely pulled Pasteur pipettes or thick-walled micromanipulator capillary tubing (1 mm outer diameter) (Research Instruments), heat sterilized at 200°C for 2 h
- Mineral oil (embryo culture grade; Sigma-Aldrich)
- 60 mm tissue culture Petri dishes
- Stereo microscope (Leica MZ12 or equivalent)

##### Method

1. Separate lymphocytes (and mononuclear cells) from 3 ml of whole blood by centrifugation over Histopaque-1077, according to the manufacturer's instructions<sup>a,b</sup>.
2. Carefully remove the buffy coat on the surface of the Histopaque-1077 layer.
3. Wash three times by resuspending cells in 1 ml of PBS (with PVP) and centrifuging at 500 *g*.
4. Resuspend cells in 1 ml of PBS (with PVP).
5. Drop 10  $\mu\text{l}$  of lymphocyte suspension and a series of 5  $\mu\text{l}$  drops of PBS (with PVP) on to a Petri dish and cover drops with 5–7.5 ml mineral oil (sufficient to cover the drops).
6. Transfer a small number of lymphocytes from the lymphocyte suspension drop into the top of one of the PBS (with PVP) drops using a mouth pipette connected to a finely pulled Pasteur pipette or capillary tube.
7. Pick and transfer single cells using a fresh Pasteur pipette or capillary tube, while the lymphocytes remain floating, into 3.5  $\mu\text{l}$  of PBS in PCR tubes, under a stereomicroscope to confirm transfer of the cell.
8. Add 3.5  $\mu\text{l}$  of freshly prepared alkaline lysis buffer to each sample and place the tubes on ice for 10 min to lyse the cells.
9. Stop lysis by adding 3.5  $\mu\text{l}$  of neutralizing buffer and, if not used immediately, store at –20°C.

##### Notes

<sup>a</sup>Lymphocytes should be prepared in a dedicated lab with positive-pressure high-efficiency particulate air (HEPA) filters taking precautions to avoid contamination.

<sup>b</sup>All sample tubes should be kept in cool racks at approximately ice temperature throughout the procedure.

## Protocol 2

### WGA using MDA

#### Equipment and Reagents

- REPLI-g Kit (4× REPLI-g buffer containing exonuclease-resistant phosphorothioate-modified random hexamer oligonucleotide primers; REPLI-g DNA polymerase (φ29 DNA polymerase); nuclease-free water; Qiagen)

#### Methods

1. Prepare a master mix of 26.5 μl of nuclease-free water, 12.5 μl of 4× REPLI-g buffer and 0.5 μl of REPLI-g DNA polymerase<sup>a</sup>, for each reaction.
2. Combine the 10.5 μl of solution from *Protocol 1* with 39.5 μl of master mix (final reaction volume 50 μl) and mix well by pipetting.
3. Incubate at 30°C on a thermocycler for 16 h or overnight.
4. Terminate the reaction by raising the temperature to 65°C for 3 min to inactivate the enzyme.
5. Store amplified DNA at 4°C if it is to be used immediately or at -20°C for long-term storage<sup>b,c,d</sup>.

#### Notes

<sup>a</sup>REPLI-g DNA polymerase should be thawed on ice. However, all other components can be thawed at room temperature.

<sup>b</sup>Following amplification, the yield of dsDNA can be measured using PicoGreen reagent (Molecular Probes), following the manufacturer's instructions.

<sup>c</sup>In control reactions without target DNA, amplification still occurs from the primers and gives similar yields.

<sup>d</sup>Due to the presence of amplification in the negative control, if necessary, the proportion of human sequence can be determined using real-time PCR for a chosen target sequence and compared with an unamplified genomic DNA control.

## Protocol 3

### Multiplex PCR amplification following WGA

#### Equipment and Reagents

- GeneAmp PCR System 9700 (Applied Biosystems) (or comparable real-time PCR detection instrument)
- 10× PCR Buffer II (500 mmol/l KCl; 100 mmol/l Tris-HCl (pH 8.3)) (Applied Biosystems)
- 10× MgCl<sub>2</sub> (15 mmol/l)
- dNTPs (25 mM) (Roche Diagnostic)
- AmpliTaq Gold Polymerase (5 units/μl) (Applied Biosystems)
- Nuclease-free water (Sigma)

#### Methods

1. Prepare the PCR according to the test being performed. The reaction constituents and cycling conditions for the specific tests are detailed in *Table 2*.

#### Additional Protocols

The PCR products generated using the profiles described in *Protocol 3* were analysed by either STR genotyping<sup>a</sup> or by mini-sequencing<sup>b</sup>. Both applications were performed using an ABI PRISM 3100 DNA sequencer (Applied Biosystems), according to the manufacturer's protocol.

#### Notes

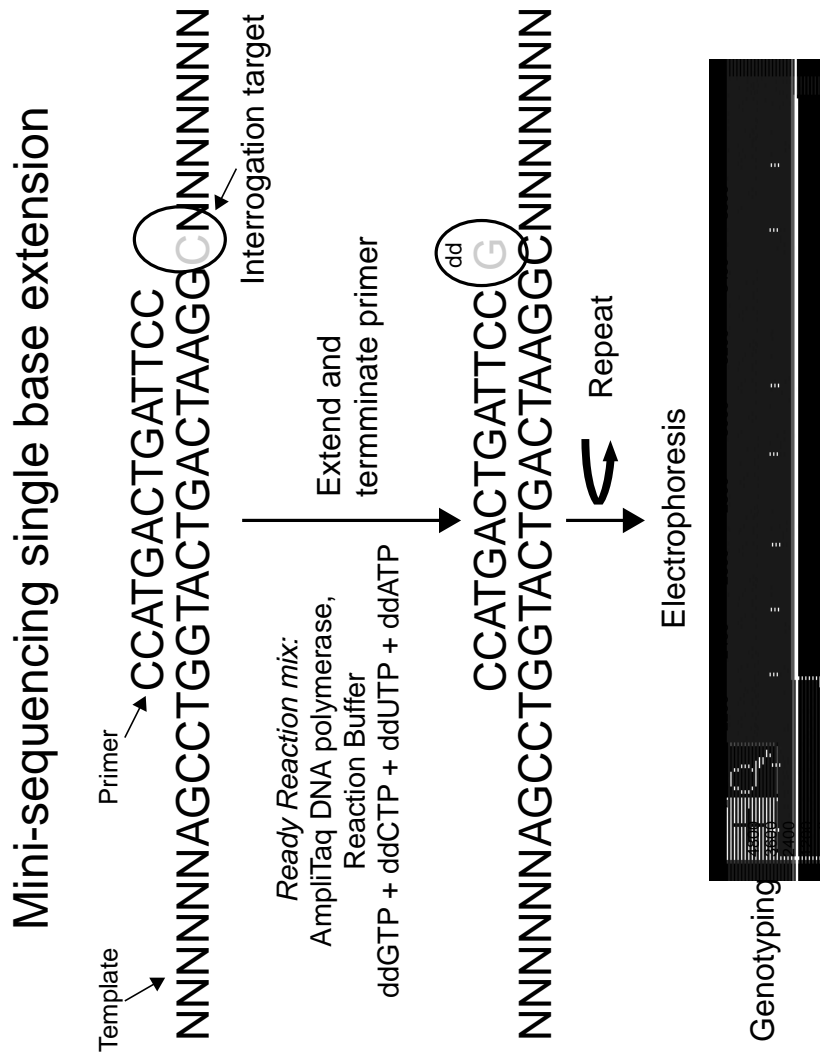
<sup>a</sup>Combine 1 μl of each dye-labeled PCR with 0.5 μl of GS500 TAMRA (Applied Biosystems) and 15 μl of Hi-Di formamide (Applied Biosystems) and denature for 4 min at 94°C. Resolve samples by capillary electrophoresis for 30 min on an ABI PRISM 3100 DNA sequencer (Applied Biosystems) and analyze the results using GeneScan Analysis software (Applied Biosystems).

<sup>b</sup>To avoid participation in the mini-sequencing primer-extension reaction, remove primers and unincorporated dNTPs using a Microcon 100 filter (Millipore), according to the manufacturer's protocol. Combine 10 ng of purified PCR product, 5 μl of Ready Reaction Pre-mix (ABI PRISM SnaPshot Multiplex Kit; Applied Biosystems) and 5 pmol of each mini-sequencing primer. Resolve samples by capillary electrophoresis for 15 min on an ABI PRISM 3100 DNA sequencer (Applied Biosystems) using POP-4 polymer (Applied Biosystems).

## 3.1. Downstream applications

### 3.1.1. Single gene defects

Many different approaches have been used for mutation detection following DNA amplification from single cells (4). However, with the new generation of automated sequencers using capillary electrophoresis, mini-sequencing is being used increasingly because it is universally applicable to mutation detection and can be applied to short amplified fragments, which minimizes ADO (16, 17). Mini-sequencing chemistry is based on the single dideoxynucleotide extension of unlabeled oligonucleotide primers annealing to purified amplified target DNA. Specific mini-sequencing primers, which are exactly one base short of the mutation sites, are used for each mutation under investigation. Primers bind to



**Figure 2. Mini-sequencing single-base extension technique.** Primers bind to their complementary templates and *Taq* DNA polymerase then adds a single fluorescent-labeled dideoxynucleoside triphosphate (ddNTP) to the 3' end of each primer. Since the reaction contains only template, primer, and dye-labeled ddNTPs, and not deoxynucleoside phosphates as in a full sequencing protocol, interruption of the reaction occurs after incorporation of only one of the dideoxy terminators. This process is repeated in successive rounds of extension and termination. The resulting products, varying in color, can then be analyzed by electrophoresis. The mutation site can thus be reliably differentiated from the homozygous wild type, mutant or heterozygote.

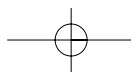
**Table 2. PCR constituents for PGD screening**

	<b>Informativity testing on individual couples</b>	<b>HLA matching</b>	<b>Aneuploidy screening</b>	<b>Thalassemia and linked STR markers</b>
Genomic DNA	50 ng	1 µl of MDA product (~1 µg)	1 µl of MDA product (~1 µg)	1 µl of MDA product (~1 µg)
10× PCR Buffer II	5 µl (1×)	5 µl (1×)	5 µl (1×)	5 µl (1×)
Concentration of each pair of primers	10 pmol (see <i>Table 3</i> )	5 pmol (see <i>Table 3</i> )	5 pmol (see <i>Table 4</i> )	10 pmol (see <i>Table 5</i> )
MgCl <sub>2</sub> (mmol)	1.5 mmol/l	1.5 mmol/l	1.5 mmol/l	1.5 mmol/l
dNTPs (mmol)	200 mmol/l	200 mmol/l	200 mmol/l	200 mmol/l
AmpliQa Gold polymerase	2.5 units	2.5 units	2.5 units	2.5 units
Ultra-pure water	Up to 50 µl	Up to 50 µl	Up to 50 µl	Up to 50 µl
Cycling conditions	<ul style="list-style-type: none"> <li>• Initial denaturation of 95°C/10 min</li> <li>• 32 cycles of 95°C/30 s; 60°C/30 s; 72°C/30 s</li> <li>• Final extension of 65°C for 60 min</li> </ul>	<ul style="list-style-type: none"> <li>• Initial denaturation of 95°C/10 min</li> <li>• 32 cycles of 95°C/30 s; 55°C/30 s; 72°C/30 s</li> <li>• Final extension of 65°C for 60 min</li> </ul>	<ul style="list-style-type: none"> <li>• Initial denaturation of 95°C/10 min</li> <li>• 32 cycles of 95°C/30 s; 55°C/30 s; 72°C/30 s</li> <li>• Final extension of 65°C for 60 min</li> </ul>	<ul style="list-style-type: none"> <li>• Initial denaturation of 95°C/10 min</li> <li>• 32 cycles of 95°C/30 s; 55°C/30 s; 72°C/30 s</li> <li>• Final extension of 65°C for 60 min</li> </ul>

their complementary templates and *Taq* DNA polymerase then adds a complementary single fluorescent-labeled dideoxynucleoside triphosphate (ddNTP) at the 3' end of each primer, according to the sequence. Since the reaction contains only template, primer, and dye-labeled ddNTPs, not a mixture with deoxynucleoside phosphates as in a full sequencing protocol, interruption of the reaction occurs after only one incorporation of the dideoxy terminator. This process is repeated in successive rounds of extension and termination. The resulting products, varying in color, can then be analyzed by electrophoresis (see *Fig. 2*). The colors of the final peaks are determined by the specific genotype at the locus under investigation, making it possible to identify the base variation. The mutation site can thus be reliably differentiated between homozygous wild type and mutant (one peak of a specific color; A/green, C/black, G/blue, T/red) or heterozygote. In the latter case, two different-colored peaks occur in the electrophoretogram, one derived from the normal base and the other from the mutated base (see *Fig. 3* in colour section).

### 3.1.2. Aneuploidy screening

Pregnancy and live birth rates following *in vitro* fertilization decline rapidly with advancing maternal age. One of the main factors causing this decline is a decrease



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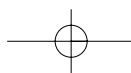
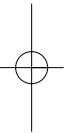
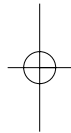
in egg quality, associated with an increase in errors of female meiosis, particularly meiosis I. As a consequence, a high incidence of aneuploid oocytes and embryos occurs, which are either not viable or develop abnormally and have a high risk of miscarriage. Chromosomal abnormalities also arise during the early cleavage divisions of the fertilized egg as a result of chromosome malsegregation. To screen for these abnormalities and avoid the transfer of aneuploid embryos, embryo biopsy and single-cell analysis by sequential FISH, typically with five to nine chromosome-specific probes, is used for interphase molecular cytogenetic analysis (11, 33, 34). Alternatively, molecular genetic approaches have been proposed that identify parental chromosomes using STR markers (see *Table 3*) and other markers, both qualitatively and quantitatively (6). In addition, CGH can be used to extend the analysis to the whole karyotype (6, 7, 24). Here, we present a protocol that enables identification of aneuploidy for chromosomes 21, 18, 13, X and Y using STR markers. Aneuploidy for 21, 18 and 13 are commonly associated with miscarriage or result in an abnormal pregnancy, and sex chromosome aneuploidy is quite common at pre-implantation stages of development. An example showing trisomy 21 is given in *Fig. 4* (in colour section).

### 3.1.3. HLA matching

For couples who have a child affected by a genetic condition that is treatable by transplantation of HLA matched hemopoietic stem cells, PGD offers the possibility of combining mutation testing, if the condition is inherited, to avoid the birth of another affected child, with HLA matching. Cord blood stem cells can then be recovered at birth for transfer to the affected child, as stem cells from an HLA-matched sibling donor have the best chance of success. Our approach to HLA matching involves using a number of STR markers across the HLA region of chromosome 6 to ensure that the embryo is HLA compatible and that there has been no recombination (9, 10).

STR haplotyping for family members (father, mother and affected child) is performed prior to pre-implantation HLA typing, in order to identify the most informative STR markers of the HLA complex to be used in the following clinical PGD cycles. A panel of 50 different STR markers (see *Fig. 5* and *Table 4*) are studied during the set-up phase, to ensure sufficient informativity in all families. For each family, only heterozygous markers presenting alleles not shared by the parents are selected, so that segregation of each allele and discrimination of the four parental HLA haplotypes can be clearly determined. Informativity is also evaluated for STR markers linked to the gene regions involved by mutation, and is thus used to avoid possible misdiagnosis due to the well-known ADO phenomena.

By selecting a consistent number of STR markers evenly spaced throughout the HLA complex, an accurate mapping of the whole region can be achieved. Because genes in the HLA complex are tightly linked and usually inherited in a block, profiles obtained from such markers in father, mother and affected child allow the determination of specific haplotypes. Thus, the HLA region can be indirectly typed by segregation analysis of the STR alleles and the HLA identity of the embryos matching the affected sibling can be ascertained by evaluating the inheritance of



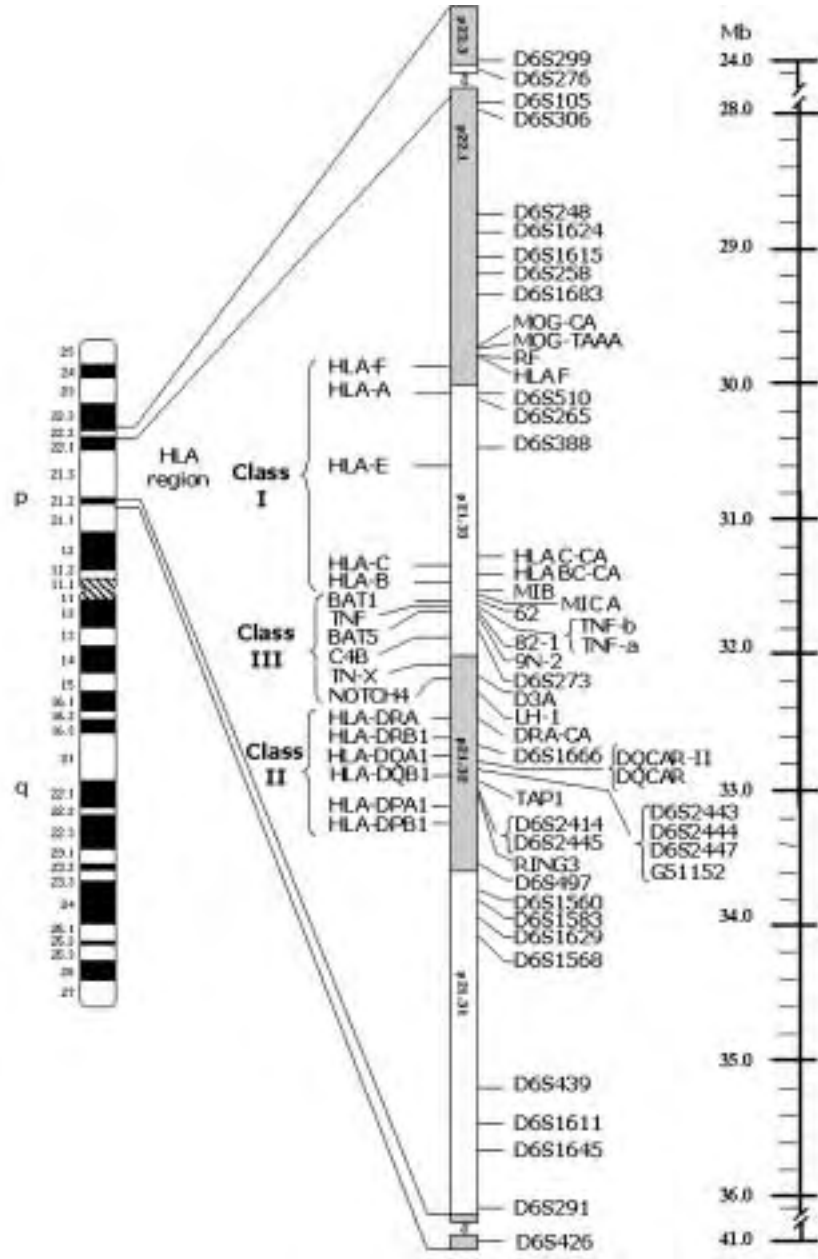
**Table 3. Primers used for screening aneuploidies for chromosomes 21, 18, 13, X and Y**

STR marker	Chromosome	Primer sequences (5'→3')	Size (bp)	Fluorescent label
D21S11	21	R TGTGTATTAGTCAATGTTCTCCAG F TCCAGAGACAGACTAATAGGAGGT	200–240	HEX
D21S1414	21	R CCAAGTGAATTGCCTTCTATCTA F GAATAGTGCTGCAATGAACATACAT	190–220	6-FAM
D21S1437	21	R TTCTCTACATATTTACTGCCAACAC F ATATGATGAATGCATAGATGGA	150	HEX
D21S1411	21	R TTGTATTAATGTGTGCTCTCC F GGAGGCTGAGTCAGGAGAATCA	240–300	TAMRA
D18S386	18	R GCAGGTAGAATCTACGCACCCT F GTACAAACAGCAAACCTTTACAGGG	352–370	6-FAM
D18S1002	18	R TGAAGTAGCGGAAGGCTGTAATAT F TCATGTGACAAAAGCCACAC	302–340	TAMRA
D18S535	18	R AGACAGAAATATAGATGAGAATGCA F AGCTGGAGAGGGATAGCATT	150–170	TAMRA
D18S858	18	R TGCATTGCATGAAAGTAGGA F CTGGGCAACAAGAGCAAACT	200–234	6-FAM
D13S256	13	R GGCCACAGAGGAAGCACATA F GGGACTACCTATGCACACAAAGT	265–290	TAMRA
D13S258	13	R AATGGGATGAGAGAGGAAGACAG F CTGGGCAACAAGAGCAAACT	172–190	6-FAM
D13S256	13	R GGCCACAGAGGAAGCACATA F TCCATGGATGCAGAATTCACAG	265–280	TAMRA
D13S796	13	R TCTCATCTCCCTGTTTGGTAGC F AAATGCTGGGATCACAGG	180–200	TAMRA
D13S217	13	R CCTGGTGGACTTTTGCTG F GAAGGGAAAATGATGAATAAACT	200–240	HEX
5'DYS-7	X	R GTCAGAACCTTTGTACCTGTC F GGGCAGTAGCTTTCAGCTTAAAC	156–180	6-FAM
HPRT	X	R CCCTGTCTATGGTCTCGAATCA F CCCTGGGCTCTGTAAAGAATAGTG	150–175	HEX
Amelogenin	X/Y	R ATCAGAGCTTAACTGGGAAGCTG F GGTGTCTGTGTACAGGTACCTCAG	103–109	6-FAM
DXS6941	X	R GGACCTCCAGATTACACATGC F GTGTTACTGGACTCCAGCCTGG	117–135	TAMRA
DXS722	X	R CCTGATCCTGTCCACTGGG F ACTGGCAACAGAACGAGACTCT	111–138	HEX
DXS1240	X	R AGATCTAGGCAAGGGCAATTAA F TACAACAAGCCAGGTCCTCACT	163–186	6-FAM
DXS1470	X	R GTGTAGTAACTCATATCAAGAGCCG	208–235	HEX

HPRT, hypoxanthine phosphoribosyltransferase; F, forward primer; R, reverse primer; HEX, hexamethylfluorescein; 6-FAM, 6-carboxyfluorescein; TAMRA, 6-carboxytetramethylrhodamine.

the matching haplotypes. Because segregation of the STR alleles fully corresponds to the direct HLA genotyping, STR haplotyping can be used as a reliable diagnostic tool for indirect HLA matching evaluation. The use of microsatellite markers for this purpose is very useful, since they may provide information on identity over a greater distance within the HLA region compared with classical HLA genes alone,

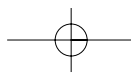
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**Figure 5. Polymorphic STR markers located throughout the HLA region, on chromosome 6, used in the pre-implantation HLA matching procedure.**  
 The STR markers are ordered from the telomere (top) to the centromere (bottom) and their position is compared with genes of the HLA complex. D6S299, D6S276 and D6S426 markers are located outside the HLA region. All STR markers are dinucleotide repeats, except for RF, which is a trinucleotide repeat, and MOG-TAAA, D6S2414, D6S2415 and D6S497, which are tetranucleotide repeats. Mb, megabases.

**Table 4. Oligonucleotide primer sequences of selected STR markers located in the HLA complex used for pre-implantation HLA matching**

STR locus	Primer sequences (5'→3')	Size range (bp)	Fluorescent label
D6S299	F CCATGCAGTAACTCAGATCTAGGA R ATGTCTCTTCTCTCCCTCCC	160-176	HEX
D6S306	F AAGGTTTGTCAAACATCCCATC R GGTTTGAGAGTTTCAGTGAGCC	155-160	6-FAM
D6S1615	F AATCTCTGCTCTCTGGGATT R AGCCTGGGTAACAGAGCAAGA	98-122	6-FAM
D6S258	F GCAAATCAAGAATGTAATTCCTC R GCCTTGTAGTTCTTTTGTGGA	121-131	HEX
D6S1683	F ACATGTATCCGAGAATTAAAGT R AAGTAGAGACAGGATTTCTGT	170-178	6-FAM
MOG-TAAA	F GTGGGCACCTATAATACCAGCTAC R GGGTTAGAAGTGTGCTTATGAA	215-227	TAMRA
HLA-F	F TATGCTCAGGTACAACITTTCCAG R TGAACCTGCTGAGAATGAAGG	260-275	6-FAM
D6S2971	F CTGCTTATTTTCATATGCTCAGGTA R TTGCTGAGAAATGAAGGTCTAGA	230-263	6-FAM
D6S388	F GCTGATGGAGAATGAAATATGG R GGTTAGACGTAGCTAAGAGAGAAT	150-155	TAMRA
D6S1666	F GTTGGGCAGCATTGTAGATTTC R ACCCAGCATTGGAGTTGTGT	112-142	HEX
D6S2443	F CCATACCAAAGTAAAACCCAGTG R CATTGATACTGAGGATGAAGGG	180-188	6-FAM
D6S2444	F GGGAGCATTGTGATTTCTGTATG R AATGATTCATGAGCCAAGAACC	137-145	HEX
D6S2414	F AACTGGGCTGAGATGTACCACT R GACTCAAGGAGAGGAATGTGTG	155-165	TAMRA
D6S2415	F CAGCCCTAACAGCTTTATTGG R ATGAACCTGACTGTGGTATGA	152-157	HEX
D6S497	F CCTGGGCAACAAGAGTGAAC R TTGGCTGTTGAATTGTGAGAGT	129-140	6-FAM
D6S1560	F TCCTGGTGGTAGTGTCTTCAA R TGAGTCAAGTGAGAAACAGAGAG	130-146	6-FAM
D6S1583	F CCCTAACCTGCTTCTACTGATCA R CTCAGGGACAGACAACCTCTG	130-138	TAMRA
D6S1629	F CACAGTGACTTGTACTGAAAGCTCA R GGCTCCCAATTATCTCTGC	155-165	HEX
D6S1568	F AGATATCCCACCAAGGCAG R AGCTAGGCCAGGCCGTGT	127-152	6-FAM
D6S1611	F GGATTTCTTGCAAAACAACCC R AAGGGCTGAGTTTCTTCTGGG	180-185	HEX
D6S1645	F ACAGAGTGAGACTCTGTCGCAAAC R CCCACTTAGCAGACAGAGATAGA	160-167	6-FAM
D6S276	F TCAATCAAATCATCCCGAGAAG R GGGTGCAACTGTTCCTCTCT	190-220	HEX
D6S291	F GTCTAAAATATCCATCCGGCAT R TTAATTGTGGTGATGGTTTAC	156-166	6-FAM
D6S426	F ACTCCCCAAAAATGTAGTCAT R AAAATGCACGTACCTAGTCCTC	112-130	NED
D6S273	F TGAGTATTTCTGCAACTTTTCTGTC R AAACCAAACCTCAAATTTTCGG	135-146	HEX
D6S265	F TCGTACCCATTAACCTACCTCTCT R TCGAGGTAACAGCAGAAAGATAG	110-125	6-FAM

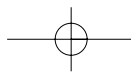


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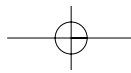
Table 4. Contd.

STR locus	Primer sequences (5'→3')	Size range (bp)	Fluorescent label
MICA	F GAAAGTGCTGGTGCCTCAGAGTC R CTACCATCTCCAGAACTGCC	170–180	NED
TNF- $\alpha$	F GCCTCTAGATTTCATCCAGCCAC R CCTCTCTCCCCTGCAACACA	97–121	6-FAM
TAP1CA	F TCATACATCTGCTTTGATCTCCC R GGACAATATTTGCTCCTGAGG	195–215	NED
TNF- $\beta$	F TGTGTTGCAGGGGAGAGAGG R GGCCACAGAGCAAGACACCA	100–118	HEX
D6S2447	F CTGCATTTCTTCTTATCACTTC R TTTGAGAGGTGTGCATGTTACC	180–202	6-FAM
D6S510	F TTTGCTTTCCCAATGTAACAC R GCTACTACTTCACACCAATTAGGA	140–155	HEX
D3A	F CATCCATGACAGAAAGCAGAGC R CCTGCCTTCTGTAAGCCTCAG	181–215	NED
62	F GATTCATCCAGCCACAGGA R TCCAATCACCTCTGCTCACC	140–170	HEX
82-1	F GAGCCAGGATGGAGACCAAA R CCTGGATAACAGAACGAGACCC	100–122	6-FAM
G51152	F GGAAAAGAGCTCACGCACAT R CCTGCCATCATGACTCAAG	145–158	NED
LH-1	F GCATCTGTGCCAAGAACTC R ACCTTACTGGGCACAAATTCAC	126–160	6-FAM
Ring3CA	F GCCGCAGTTAACTGTTCTT R GAAATGTTAGGTGAGAACCACAGA	124–130	HEX
MOG-CA	F AGATCACCTCGAGTGAAGTCTT R TTGACCATGGGTAAGTGAAGC	205–235	6-FAM
DRA-CA	F ACTTTCCTAATTCTCCTCCTC R GCATGAGTAAACTATGGAATCTC	122–140	HEX
D6S439	F CCCCTATTCTCCACCCACTAGA R CAGCCTCAGGGAAGACACATT	116–130	NED
DQCAR	F CTGCATTTCTTCTTATCACTTC R TGGCCAATCAGAATCTTCTCTA	150–175	6-FAM
9N-2	F TGGGTAACAGAGCAAGACTCTGT R TGGGATTGCAGATGTGTTACAC	100–110	HEX
MIB	F CGTTTTAGCCTGCTAGCTTAT R CCACAGTCTCTATCAGTCCAGATTC	155–186	HEX
D6S105	F AACCAAGAGCAAACTCCGTCTC R TCACCTTGATATCTTATTACCCTGG	141–155	NED
DQCARI	F TTGGGCAGCATTGTAGATTTC R GCAAGAATCCAGCATTTTGG	118–138	6-FAM
HLABC-CA	F GTCAAGCATATCTGCCATTTGG R ACTTGGGCAATGAGTCTATGA	113–144	HEX
HLAC-CA	F CGGCAAGAGACTCTGATGAGAA R GTAGCTGGGATTACAGGTGCCT	156–173	NED
D6S248	F CGAGATCAAGCCACTGCACT R CAGGAATGGTGAGAAGGGAAA	151–165	HEX
D6S1624	F TATAACCCAGGTGTTTGTGG R GGAAGTCTTCAGTGGAGAGAGTG	200–220	6-FAM

MOG-TAAA, myelin oligodendrocyte glycoprotein (TAAA)<sub>n</sub> repeat; HLA-F, human leukocyte antigen F; MICA, MHC class I polypeptide-related sequence A; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; TAP1CA, transporter associated with antigen processing 1CA; TNF- $\beta$ , tumor necrosis factor- $\beta$ ; Ring3CA, bromodomain-containing protein 2 (CA)<sub>n</sub> repeat; MOG-CA; myelin oligodendrocyte glycoprotein (CA)<sub>n</sub> repeat; DRA-CA, HLA class II histocompatibility antigen, DR- $\alpha$  chain precursor (HLA-DRA) (CA)<sub>n</sub> repeat; MIB, D6S2810, 24.9 kb centromeric of HLA-B; F, forward primer; R, reverse primer; HEX, hexamethylfluorescein; 6-FAM, 6-carboxyfluorescein; TAMRA, 6-carboxytetramethylrhodamine.

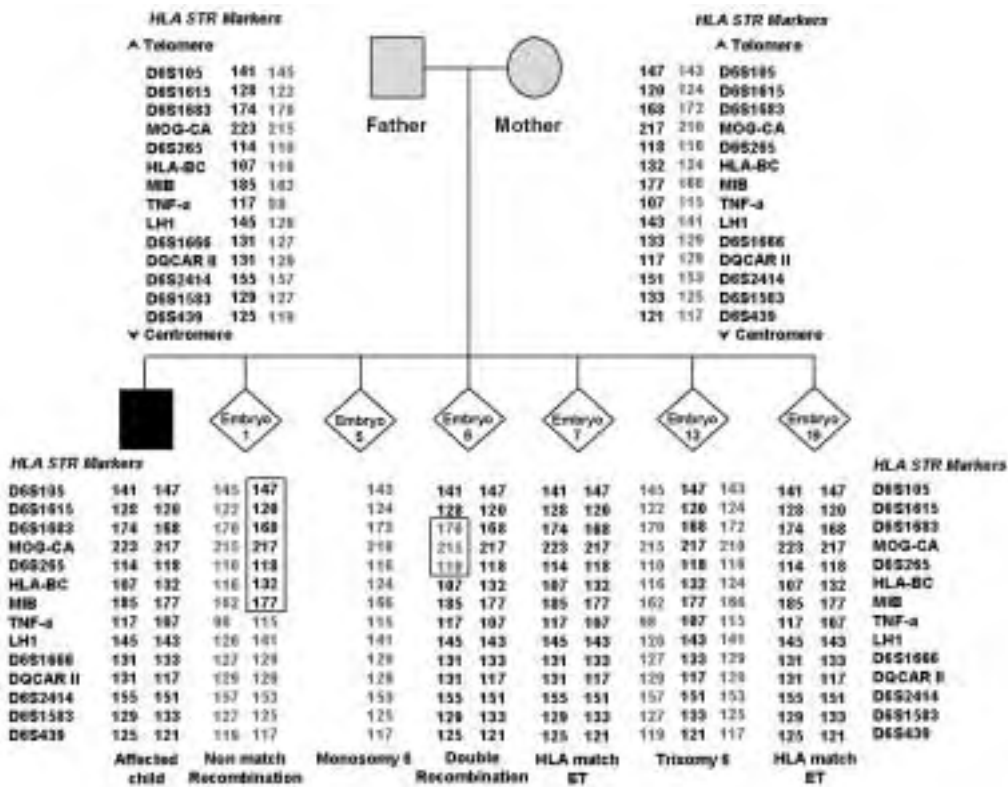






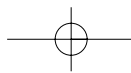
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An example of the pre-implantation HLA matching procedure using STR haplotyping, in combination with PGD for  $\beta$ -thalassaemia, is shown in Fig. 6. The strategy presented here enables the selection of HLA-matched embryos and can be performed for any genotype combination, without the need to develop a specific diagnostic experimental design for each couple, as the selected panel of STR markers has already been worked out and can be used for other patients. As a consequence, a substantial shortening of the preliminary phase can be achieved. Recombination, if not detected, could strongly affect the accuracy of the HLA



**Figure 7. Avoidance of pre-implantation HLA matching misdiagnosis due to recombination and aneuploidies.**

*Upper panel.* Determination of the different haplotypes from father, mother and affected child (lower panel, left side, black square) by segregation analysis of the alleles obtained after STR genotyping of the HLA region. Informative STR markers used are ordered from the telomere (top) to the centromere (bottom). Paternally and maternally derived HLA haplotypes, matched to the affected child, are shown in bold. Examples of different results of HLA haplotyping from biopsied blastomeres are shown in the lower panel. Embryo 5 has no paternal chromosome present (monosomy 6); embryo 13 shows an extra maternal chromosome (trisomy 6); embryo 1 shows a single recombination occurrence in maternal haplotypes between the alleles of the markers D6S105 and MIB (boxed); in embryo 6, initially appearing to be HLA matched with the affected sibling, a double recombination event was observed between the markers D6S1683 and D6S265 (boxed). Embryos 7 and 19 were diagnosed as HLA matched and were transferred. ET, embryo transfer; HBB, hemoglobin- $\beta$ ; MIB, D6S2810, 24.9 kb centromeric of HLA-B; MOG, myelin oligodendrocyte glycoprotein; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ .

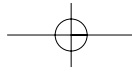


matching procedure. The importance of detecting recombination within the HLA region is demonstrated in *Fig. 7*. Recombination between flanking markers of the paternal or maternal haplotype is detected in two embryos (embryos 1 and 6). In one of them (embryo 1), a single recombination has occurred in the maternal haplotype, between the alleles of the markers D6S105 and MIB. In the other embryo (embryo 6), initially appearing to be HLA matched with the affected sibling, a double recombination event is evident, between markers D6S1683 and D6S265. This occurrence, which was only detected by using a consistent number of STR markers able to determine a fine mapping of the whole HLA region, if missed, could lead to an HLA-genotyping misdiagnosis, and the embryo would be erroneously diagnosed as HLA identical. Hence, the reliability of the procedure is strongly correlated with the number of STR markers used for HLA haplotyping. Furthermore, the combined use of a multiplex HLA STR marker system has allowed the detection of aneuploidies of chromosome 6. The relevance of aneuploidy testing for chromosome 6 is seen in *Fig. 7*. One of the embryos tested in this case

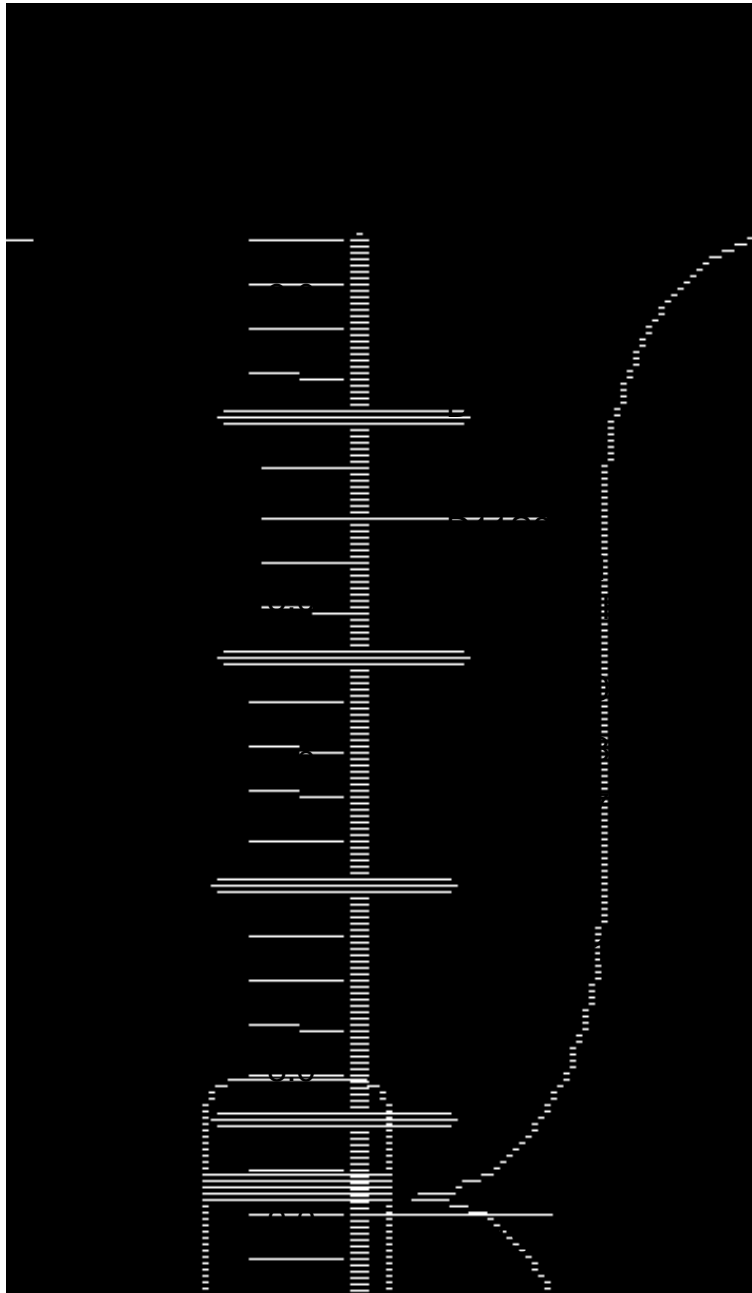
**Table 5. Primers used for amplification of the hemoglobin- $\beta$  (HBB) gene and linked STR markers for ADO detection**

Gene region/marker	Primer sequences (5'→3')	Size (bp)	Fluorescent label
HBB gene exon 1	F CATCACTAGACCTCACCCCTGT R TCTCCTAAACCTGTCTTGTAAACC	303	
HBB gene exon 2	F TGGGTTTCTCATAGGCACTGA R AAAGAAAACATCAAGGGTCCC	398	
HBB gene exon 3	F TATCATGCCTCTTGCACCAAT R CAGTTTAGTAGTTGGACTTAGGGAA	449	
HBB gene IVSII	F CCTAATCTCTTCTTTCAGGGCAAT R GGTATGAACATGATTAGCAAAAGGG	270	
TH01	F GTGGGCTGAAAAGCTCCCGATTAT R GTGATTCCCATTTGGCCTGTTCCCTC	156–178	6-FAM
D11S4146	F GGTAAAGCAGAGTTAATAGGC R CTACCAAACATGATTCCTAGGA	163–180	HEX
D11S988	F CACAGAAAATAGTTCAGACCACCAT R TGGGACAAGAGAAAGTTGAACATAC	127–148	HEX
D11S4181	F CTGGGCAACAAGAGTAAGTCTCT R CCTAAGAACTGAGACCAAGAACA	117–135	6-FAM
B-STR	F AGACTGGAGTAAAGGAAATGG R GATGCCACAGCAGGTG	100–118	6-FAM
D11S1760	F ATCTCAAGTGTTTCCCAACAAC R CTGCATCATGACTTGA AAAACG	106–125	6-FAM
D11S1338	F CCACACAGATTCACTTAAAGCAA R GCTACTTATTGGAGTGTGAATTC	135–148	HEX
D11S1997	F TTCCTAAGAAAGATAAAGCACCAG R CAATTGACAGTGGATTTTGAC	143–150	6-FAM
D11S1331	F GATGTTTAGATGCACAAGACACAGA R CTCCTTCGTCTTCTCACTTTTAC	156–178	HEX
D11S4149	F GGCTAAAAAGGCAACAGATAACATC R CCATATATAGAATCACACTGGCCAA	161–180	6-FAM

F, forward primer; R, reverse primer; HEX, hexamethylfluorescein; 6-FAM, 6-carboxyfluorescein.



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**Figure 8. Polymorphic STR markers linked to the hemoglobin- $\beta$  (HBB) gene on chromosome 11.**

STR markers are ordered from the telomere (top) to the centromere (bottom). Mb, mega bases.

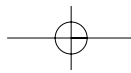
has only one maternal chromosome 6 (embryo 5), and one (embryo 13) has an extra maternal chromosome, consistent with a diagnosis of monosomy 6 and trisomy 6, respectively, making them unacceptable for transfer. The oligonucleotide primer sequences of selected STR markers located in the HLA complex that are used for pre-implantation HLA matching are shown in *Table 4*.

### 3.1.4. PGD of $\beta$ -thalassemia combined with HLA matching

STR markers were selected to cover the extended HLA complex (see *Fig. 8* and primer list in *Table 5*). One of the primers for each microsatellite was labeled with a fluorescent dye (e.g. 6-Fam, Hex, Ned) so that it could be visualized on an automatic DNA Sequencer (ABI Prism 3100; Applied Biosystems). STR markers with overlapping size ranges were labeled with different fluorochromes in order to analyze them in the same capillary electrophoresis run.

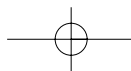
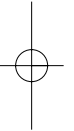
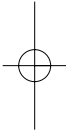
## 4. REFERENCES

1. Sermon K, van Steirteghem A & Liebaers I (2004) *Lancet*, **363**, 1633–1641.
2. Handyside AH, Kontogianni EH, Hardy K & Winston RM (1990) *Nature*, **344**, 768–770.
3. Handyside AH, Lesko JG, Tarin JJ, Winston RM & Hughes MR (1992) *N. Engl. J. Med.* **327**, 905–909.
4. Thornhill AR & Snow K (2002) *J. Mol. Diagn.* **4**, 11–29.
5. Gianaroli L, Magli MC, Ferraretti AP & Munne S (1999) *Fertil. Steril.* **72**, 837–844.
6. Wells D, Escudero T, Levy B, Hirschhorn K, Delhanty JD & Munne S (2002) *Fertil. Steril.* **78**, 543–549.
7. Wilton L, Williamson R, McBain J, Edgar D & Voullaire L (2001) *N. Engl. J. Med.* **345**, 1537–1541.
8. Verlinsky Y, Rechitsky S, Schoolcraft W, Strom C & Kuliev A (2001) *JAMA*, **285**, 3130–3133.
- ★ 9. Fiorentino F, Biricik A, Karadayi H, *et al.* (2004) *Mol. Hum. Reprod.* **10**, 445–460.
10. Fiorentino F, Kahraman S, Karadayi, *et al.* (2005) *Eur. J. Hum. Genet.* (in press).
11. Verlinsky Y & Kuliev A (2003) *Reprod. Biomed. Online*, **7**, 145–150.
12. Coutelle C, Williams C, Handyside A, Hardy K, Winston R & Williamson R (1989) *BMJ*, **299**, 22–24.
13. Holding C & Monk M (1989) *Lancet*, **2**, 532–535.
14. Findlay I, Matthews PL, Mulcahy BK & Mitchelson K (2001) *Mol. Cell. Endocrinol.* **183** (Suppl. 1), S5–12.
15. Findlay I, Quirke P, Hall J & Rutherford A (1996) *J. Assist. Reprod. Genet.* **13**, 96–103.
16. Fiorentino F, Magli MC, Podini D, *et al.* (2003) *Mol. Hum. Reprod.* **9**, 399–410.
17. Bermudez MG, Piyamongkol W, Tomaz S, Dudman E, Sherlock JK & Wells D (2003) *Prenat. Diagn.* **23**, 669–677.
18. Kuo HC, Ogilvie CM & Handyside AH (1998) *J. Assist. Reprod. Genet.* **15**, 276–280.
19. Lewis CM, Pinel T, Whittaker JC & Handyside AH (2001) *Hum. Reprod.* **16**, 43–50.
20. Snabes MC, Chong SS, Subramanian SB, Kristjansson K, DiSepio D & Hughes MR (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 6181–6185.
21. Zhang L, Cui X, Schmitt K, Hubert R, Navidi W & Arnheim N. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 5847–5851.
22. Kristjansson K, Chong SS, van den Veyver IB, Subramanian S, Snabes MC & Hughes MR (1994) *Nat. Genet.* **6**, 19–23.
23. Ao A, Wells D, Handyside AH, Winston RM & Delhanty JD (1998) *J. Assist. Reprod. Genet.* **15**, 140–144.
24. Wells D, Sherlock JK, Handyside AH & Delhanty JD (1999) *Nucleic Acids Res.* **27**, 1214–1218.



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25. Dean FB, Hosono S, Fang L, *et al.* (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99**, 5261–5266.
- ★★ 26. Lasken RS & Egholm M (2003) *Trends Biotechnol.* **21**, 531–535.
27. Dean FB, Nelson JR, Giesler TL & Lasken RS (2001) *Genome Res.* **11**, 1095–1099.
28. Hosono S, Faruqi AF, Dean FB, *et al.* (2003) *Genome Res.* **13**, 954–964.
29. Lovmar L, Fredriksson M, Liljedahl U, Sigurdsson S & Syvanen AC (2003) *Nucleic Acids Res.* **31**, e129.
- ★ 30. Handyside AH, Robinson MD, Simpson RJ, *et al.* (2004) *Mol. Hum. Reprod.* **10**, 767–772.
31. Hellani A, Coskun S, Benkhalifa M, *et al.* (2004) *Mol. Hum. Reprod.* **10**, 847–852.
32. Hellani A, Coskun S, Tbakhi A & Al-Hassan S (2005) *Reprod. Biomed. Online*, **10**, 376–380.
33. Munne S, Sandalinas M, Escudero T, *et al.* (2003) *Reprod. Biomed. Online*, **7**, 91–97.
34. Verlinsky Y & Kuliev A (2003) *Fertil. Steril.* **80**, 869–70.



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