

Enrolling a Patient for Preimplantation Genetic Diagnosis

IVF-Reproductive Center Patient Referral

- Register the Couple:** Simply complete our “patients information form” for the couple. This acts as a physician’s order for us to enroll them into the program
- DNA Diagnostic Report:** You probably have a DNA diagnostic report(s) in the patient’s chart. For example, a cystic fibrosis mutation report defining the gene mutation(s). If you do not have one or the mutations are unknown, we can assist you in obtaining it through our genetic facility. We use these data to custom design the molecular probes necessary for their single-cell PGD testing.
- Schedule a sample trial:** PGD for single gene disorders is quite different from analysis for chromosomes, where DNA contamination or PCR inhibition is not a problem. Because of being a DNA based procedure, contamination can cause a misdiagnosis and PCR inhibition can determine a diagnosis failure. The biopsy/tubing procedure used at your IVF center should be evaluated before sending the first case by scheduling a **sample trial** (i.e. a mock PGD on spare blastomeres), in order to assure that the number of tubes with no cells is acceptable, a result can be obtained (no PCR inhibition) and contamination is not introduced into the specimens. We will provide feedback and suggestions for improved technique when indicated.

That’s it! This is all you have to do. What does GENOMA do next, once you have referred a case for PGD?

GENOMA Steps

- Collect and Review Genetic Information.**
Our Certified Genetic Counselors take a Genetic History and review the existing DNA Information.
- Design, Construct and Optimize Probes.**
EACH and EVERY PGD case is customized for each couple. We start immediately after receiving the necessary Blood/cheek swab samples.
- Notification that the Test is Ready.**
We contact you, the IVF Center, once the probes are constructed, optimized and certified for the couple’s PGD case.
- Our Laboratory then waits until you tell us you need us to test the biopsied samples.**

Information on preliminary PGD workup

A vast array of genetic diseases can be analyzed by PGD, but first we must develop, optimize and validate the specific probes that will be used.

To validate a diagnostic single cell PCR protocol, before clinical application, extensive preclinical trials on single lymphocytes or cheek cells are necessary, in order to evaluate single cell amplification efficiencies and ADO rates for all the primers to be used in the procedure.

The pre-PGD workup for each couple includes blood sample (2-5 ml from both partners and affected children or family members, if any, see paragraph "Prescription for Drawing Blood", page 5) analysis of the prospective parents for mutation verification and informativeness for the polymorphic markers included in each assay. Additional family members may also be tested as the individual situation warrants. In some cases we also inquire for collection of cheek cells. The collection of blood and cheek cells allows preliminary analyses to be performed, which are essential to confirm that our PGD techniques will be applicable.

Once the samples are received, preliminary testing can be performed within a few weeks. Our test development and validation is performed under stringent conditions and highly skilled staff.

The probes we build for PGD families are custom-designed. GENOMA PGD lab is known globally for his ability to test for some of the rarest genetic conditions.

For many of the more common single gene diseases GENOMA can perform the necessary validation in about 4 weeks. For genetic diseases with variable mutations the development, optimization and validation procedures can take 1-2 months. Remember, there is no such thing as off-the-shelf PGD. A pre-PGD evaluation must be performed before the cycle begins for all patients.

TECHNICAL DETAILS ON DEVELOPMENT OF PGD PROTOCOLS

Using blood samples and buccal cells, Genoma develops patient-specific PGD protocols which shall detect, for mutation-specific genetic disease, whether the tested embryos are non-affected, carrier, or affected by such disease, based on analysis of one to two cells from such embryo of the mutation and linkage markers.

The PGD protocols includes: (a) primers for the mutation(s), and (b) primers for at least 2 STRs linked to the relevant gene. Wherever possible, the linked markers will be tetranucleotides, to avoid artifacts at single cell level, and preferably located one upstream and one downstream to the gene.

In every case, where possible, the STRs will be fully informative (*i.e.*, 4 different alleles, father a/b and mother c/d) for the patients under study. Where this is not possible, markers with father (a/a) and mother (b/c) will be preferably selected, rather than father (a/b) mother (c/c), or father (a/b) mother (b/c). Where none of the above conditions are not possible, the PGD protocols includes at least two or more semi-informative linked STR markers (di- or tetranucleotide repeat).

Genoma shall also provide a report which includes details of the amplification rate of each PCR (mutation and markers), and the ADO rate found for each PCR. Genoma shall determine this information by analyzing at least 50 isolated buccal cells or single lymphocytes from both carriers of the mutation (*i.e.*, the patients).

Samples necessary for PGD protocols development are the following:

- DNA or Blood samples from each partner of the couple;
- Buccal swabs from each carrier; and
- DNA or Blood samples from affected children of the couple (if any) or other carrier/affected members of the families.

Documents necessary for PGD protocols development are the following:

- Genetic report containing a clear description of the gene(s) and the mutation(s) involved (if known); and
- Genetic counseling containing as much information as possible on the transmission of the specific disease (and mutations) in the families (*i.e.* genealogical trees, etc.).

Genoma will complete each PGD protocol within three months (90 days) of its receipt of all samples necessary for the development of same. For existing PGD protocols, the turnaround time will be 30-60 days.

Patient Information form for Preimplantation Genetic Diagnosis pre-clinical work up

Patient : Last _____ First _____
By Convention, the Woman

Date of Birth 19 ____ ____ ____ Genetically Affected; "Carrier"; "Non carrier"
Year Month Day (Has Gene Mutation) (Heterozygote) (No Gene Mutation)

Patient : Last _____ First _____
The Male Partner

Date of Birth 19 ____ ____ ____ Genetically Affected; "Carrier"; "Non carrier"
Year Month Day (Has Gene Mutation) (Heterozygote) (No Gene Mutation)

Affected Relative: Last _____ First _____
If Any

Date of Birth ____ ____ ____ Relationship to Patient _____
Year Month Day (Son, Sister, Mother, Aunt, Nephew, with this Genetic Disorder)

Affected Relative: Last _____ First _____
If Any

Date of Birth ____ ____ ____ Relationship to Patient _____
Year Month Day (Son, Sister, Mother, Aunt, Nephew, with this Genetic Disorder)

Unaffected Relative: Last _____ First _____
If Any

Date of Birth ____ ____ ____ Relationship to Patient _____
Year Month Day (Son, Sister, Mother, Aunt, Nephew, with this Genetic Disorder)

Unaffected Relative: Last _____ First _____
If Any

Date of Birth ____ ____ ____ Relationship to Patient _____
Year Month Day (Son, Sister, Mother, Aunt, Nephew, with this Genetic Disorder)

Genetic Disorder of Concern: _____
(e.g. Cystic Fibrosis, Fragile X)

Indication for PGD: _____
(e.g. Cystic Fibrosis, Fragile X, Beta Thalassaemia combined with HLA matching)

Other Indication: _____
(e.g. Advanced maternal age, recurrent miscarriages, repeated implantation failure, PGS, Chromosomal Translocation)

Case Summary: _____
(Brief description of the case)

Pedigree:

Request:

- | | |
|---|--|
| <input type="checkbox"/> Mutation(s) screening | <input type="checkbox"/> PGD Set Up (single gene defect) |
| <input type="checkbox"/> PGD Set Up (HLA matching) | <input type="checkbox"/> PGD Set Up (Aneuploidy screening) |
| <input type="checkbox"/> PGD Set Up (Translocation) | <input type="checkbox"/> Other (please specify) |
-

Clinic Name: _____

Address: _____

PGD Coordinator with whom we can communicate: _____

Phone: _____
(Or Work Mobile)

Fax: _____

Best Email: _____
VERY important for us to interact with you and coordinate your case

Please fax this form and the DNA diagnostic report(s) that define the gene mutation (if you have it) to us at +390664492025 or e-mail it to the following address: Biricik@laboratoriogenoma.it Call us at +39068811270 if you have any questions.

Date: _____

Prescription for Drawing Blood

This is a prescription/request for a purple-top (lavender / **EDTA**) vacutainer tube of blood (**2-5 ml**) to be sent to us for DNA testing. The individual does not need to be fasting.

Please place the individual's name and date of birth on the side of the tube. The sample should not be centrifuged or processed, and does not need to be refrigerated unless shipment departure will be delayed 48-72 hours after the sample is collected. Actual shipment can be at ambient temperature. Please do not send on a Friday when the sample(s) may be delayed on a hot/cold loading dock somewhere; just wait until the following Monday; put in refrigerator until shipping the next week.

Use FedEx, DHL or similar courier. The less expensive 2-day delivery is acceptable. Package the sample(s) such that they will not break on transport (Styrofoam/bubblewrap), ideally in separate zip-lock type bags in case one does break. These blood samples will be used to laser capture individual lymphocytes for single-cell molecular testing, and/or for isolating DNA.

We encourage you to contact us at any time with questions or concerns while preparing blood samples.

Please use the address of the Laboratories:

GENOMA - Molecular Genetics Laboratory
Via di Castel Giubileo, 11 00138 ROME - ITALY
Tel. : + 39068811270 (6 lines PBX) Fax : +390664492025
e-mail: info@laboratorioigenoma.eu
web: www.laboratorioigenoma.eu

Blood / Buccal Swab Submission Form

Submit with Samples

Simply complete the few fields on this form and send it along with the blood/buccal swab samples. Then, simply print two copies of the form. One copy is for your records and one copy should accompany the samples.

	Last Name	First Name	Date of Birth	Male / Female	Type of sample	Date of Collection
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

Genetic Disorder of Concern: _____

Your Clinic Center name: _____

City, State: _____

Phone: _____

Send to: **GENOMA - Molecular Genetics Laboratory**

Via di Castel Giubileo, 11 00138 ROME – ITALY

Tel. : + 39068811270 (6 lines PBX) Fax : +390664492025

Samples' and documents' check list

- | | |
|---|--|
| <input type="checkbox"/> Blood sample from the Male Partner | <input type="checkbox"/> Blood sample from the Female Partner |
| <input type="checkbox"/> Blood sample from the Affected Child of the couple (if any) | <input type="checkbox"/> Blood sample from the Affected member(s) of the family (if applicable) |
| <input type="checkbox"/> Blood sample from family members (for linkage studies only) | <input type="checkbox"/> Buccal swab from the male and/or female partners and/or the affected children (if any) carrying the disease causing mutation(s) |
| <input type="checkbox"/> Genetic report including indication on mutation(s) identified* | <input type="checkbox"/> Genetic counseling including a clinical diagnosis and indication on the gene involved (if the mutations are unknown) |
| <input type="checkbox"/> Blood / Buccal Swab Submission Form | <input type="checkbox"/> Patient Information form |

* You probably have a DNA diagnostic report(s) in the patient's chart. For example, a cystic fibrosis mutation report defining the gene mutation(s). If you do not have one or the mutations are unknown, we can assist you in obtaining it through our genetic facility. We use these data to custom design the molecular probes necessary for their single-cell PGD testing.

PREIMPLANTATION GENETIC DIAGNOSIS (PGD) PRICE LIST

The cost of the procedure is divided into **Set-Up** portion and **Clinical PGD** portion. The set-up fee consists in the optimization of the system for the family under investigation and is a one-time cost. The cost for clinical PGD for one IVF cycle is the cost for our laboratory to perform genetic testing on the polar bodies and/or blastomeres for one genetic condition. If we perform testing for more than one condition (for example: HLA typing and beta-thalassemia), the laboratory set-up and PGD costs will increase.

PGD FOR SINGLE GENE DISORDERS

- Laboratory set-up fee* **€800 - 1.500**
(for one genetic condition)
- PGD for one IVF cycle **€2.000**
(for one genetic condition)

PREIMPLANTATION HLA MATCHING

- Laboratory set-up fee **€1.200**
- Preimplantation HLA matching for one IVF cycle **€2.000**

PGD FOR SINGLE GENE DISORDERS COMBINED WITH HLA MATCHING

- Laboratory set-up fee **€2000**
- PGD for one IVF cycle **€2.500**
(HLA matching + one genetic condition)

To include into the above PGD protocols also screening for **aneuploidies** (performed by PCR analysis):

- **Chromosomes 21, X, Y** **€500**
- **Chromosomes 21, 18, 13, X, Y (advanced maternal age - AMA)** **€1000**
- **Chromosomes 13, 14, 15, 16, 18, 21, 22, X, Y**
(advanced maternal age - AMA repeated implantation failure – RIF;
recurrent abortion - RM) **€1500**

PGD FOR ANEUPLOIDY SCREENING (PGS)

- PGD for one IVF cycle **€2.000**
(Chromosomes 13, 14, 15, 16, 18, 21, 22, X, Y)

PGD FOR CHROMOSOME TRANSLOCATION

- Laboratory set-up fee **€1.200**
- PGD for one IVF cycle **€2.000**

* Please note: the *laboratory set-up fee* will vary depending upon the genetic condition and specific mutations involved. Our genetic counsellors can provide you the cost for your patients/family.