OVERVIEW ON TRANSPORT PGD

GENOMA provides PGD analysis to IVF centers, including PGD of aneuploidy for advanced maternal age (such as Down syndrome), repeated IVF failure, recurrent spontaneous abortions, chromosome translocations, as well as PGD for single gene defects (such as Cystic fibrosis, ß-Thalassemia), PGD for HLA matching, PGD for late on-set disorders (such us Huntington) PGD for inherited predisposition to cancer (such us Retinoblastoma, Neurofibromatosis, Li-Fraumeni syndrome) and many other genetic conditions.
We also provide embryologist that can perform embryo biopsy and cell processing.
Patients interested in PGD services can contact an IVF center for which we provide services or directly contact our genetic counselor for an in depth discussion of your case.
GENOMA accepts PGD cases from any IVF clinic around the world. Following biopsy on day 3 of embryo growth, one cell is removed. Individual blastomeres are then placed inside a test tube for PCR testing or spread on slides in preparation for FISH testing, and shipped to our Center for analysis. We use a **special courier service**, available for all European countries, able to deliver cells within **3-6 h** from their shipment.
We accept samples throughout the year, and we can complete the diagnosis within 6-8 hours from receipt in the laboratory, for aneuploidy screening or translocation, or approximately within 24 hours for single gene disorders. Other applications, e.g. preimplantation HLA matching might require over 24h to achieve the results, that will be available certainly in time for a day-4 or day 5 transfer.

Sending single cells for a sample trial or clinical PGD

We encourage you to contact us at any time with questions or concerns while preparing blood samples, preparing for blastomere biopsy or during the biopsy.

Remember, PGD for single gene disorders is quite different from analysis for chromosomes, where DNA contamination is not a problem. Because of being a DNA based procedure, contamination can cause a misdiagnosis. The biopsy technique used at your IVF center should be evaluated to assure that a result can be obtained and contamination is not introduced into the specimen. Before sending your first case, schedule a **sample trial** that we can analyze. We will provide feedback and suggestions for improved technique when indicated.

The method of entering the zona pellucida has not been shown to affect the analysis, slit, acid Tyrodes, or laser are all acceptable. It is important to visually confirm that an intact blastomere has been placed into the lyses buffer. If the blastomere is disrupted during biopsy, wash or transfer into lyses buffer a second blastomere should be biopsied, placed in a separate tube and labeled (as a second cell). The embryo should be marked as possibly contaminated.

Whenever you have a question or concern, do not hesitate to contact GENOMA so.

GENOMA s.r.l.
CHIAMATA GRATUITA NUMEROVERDE 800-501651
Sede legale e Studi Medici : 00198 Roma - via Po,102
Tel. : + 390685304150 - +390685358425 **Fax :** +390685344693
Laboratori e Studi Medici : 00138 Roma - Via di Castel Giubileo, 11
Tel. : + 39068811270 (6 linee PBX) **Fax :** +390664492025
C.F. e P. Iva : 07363481008 - REA : 1028514 - Iscr. Reg. Impr. 18118/2003
Web : www.laboratoriogenoma.eu **E- mail:** info@laboratoriogenoma.it

GUIDELINES FOR BLASTOMERES COLLECTION AND TRANSPORTATION

REAGENTS AND EQUIPMENT

- Attenuated glass Pasteur pipettes
- Sterile PCR Tubes (0.2 ml)*
- Alkaline Lysis Buffer* (store at -20°C; single use aliquots)
- Sterile Rack*
- Mineral Oil*
- Parafilm
- Sterile Gloves
- Mask
- Bonnet

*These materials will be provided by our Centre.

EMBRYO BIOPSY AND TRANSFER OF BLASTOMERES IN PCR TUBES

1. Embryo biopsy should be performed in sterile conditions in IVF lab.
2. The fundamental criteria for the selection of the blastomeres should be the presence of a clear nucleus and the maintenance of cell integrity.
3. The biopsy of 2 blastomeres from each embryo is advisable. If the embryo ≤6 cells stage biopsy can be performed for one cell only.
4. Biopsied blastomeres should be maintained in drops of Ca^{++} Mg^{++} free biopsy medium, before transferring them into PCR tubes.
5. The biologist performing the transfer of the blastomeres into the PCR tubes must use bonnet, mask and sterile gloves.

GENOMA s.r.l.
CHIAMATA GRATUITA NUMEROVERDE 800-501651
Sede legale e Studi Medici : 00198 Roma - via Po,102
Tel. : + 390685304150 - +390685358425 **Fax :** +390685344693
Laboratori e Studi Medici : 00138 Roma - Via di Castel Giubileo, 11
Tel. : + 39068811270 (6 linee PBX) **Fax :** +390664492025
C.F. e P. Iva : 07363481008 - REA : 1028514 - Iscr. Reg. Impr. 18118/2003
Web : www.laboratoriogenoma.eu **E- mail:** info@laboratoriogenoma.it

6. Attenuated glass Pasteur pipettes with 60-100µm diameter can be used for blastomeres transfer. These pipettes should be prepared before biopsy with a flame source and stored under UV until their use. Commercial pipettes are also available for this purpose. Minimum one pipette for each embryo is necessary.
7. After removal, each blastomere should be washed twice in drops of Ca^{++} Mg^{++} free medium, then should be carefully aspirated by using the glass pipette under stereo microscope with 2-3 µl of medium. Afterwards, blastomeres should gently transferred into sterile 0.2 ml PCR tubes, containing 5 µl of alkaline lysis buffer (provided by our Centre), under stereo or phase contrast inverted microscope and overlaid with one drop of PCR mineral oil (provided by our Centre).

IMPORTANT: Blastomeres should be transferred into PCR tubes with as less as possible amount of medium (**no more than 2-3µl**). An exceeding quantity of medium could determine PCR inhibition.

8. All steps should be double-checked, with a second biologist witnessing the correct labelling of dishes and tubes.
9. The pipettes must be changed after the transfer of blastomeres of each embryo (one pipette for each embryo should be used).
10. Each biopsied blastomere should be transferred into separate PCR tubes. Each tube should be labelled with the number of the embryo and a letter indicating the blastomere (for example, 1A for blastomere A of embryo 1; 1B for blastomere B of embryo 1). Use an indelible pen to label the tubes.
11. Tubes containing 1-2 µl of medium, collected from wash drop of each blastomere and from cell-free wash drop, should added to PCR tubes containing 5 µl of alkaline lysis buffer (provided by our Centre), to be used as negative (blank) controls (one blank per blastomere, and one final medium blank for the whole set). Add to the tubes one drop of PCR mineral oil (provided by our Centre).
12. Spin down the tubes using a microcentrifuge for few seconds and place them in the sterile rack.
13. Seal the rack with parafilm and place it in a safety box or envelope for transportation.
14. Remember to fill the PGD acceptance form

GENOMA s.r.l.
CHIAMATA GRATUITA NUMEROVERDE 800-501651
Sede legale e Studi Medici : 00198 Roma - via Po,102
Tel. : + 390685304150 - +390685358425 **Fax :** +390685344693
Laboratori e Studi Medici : 00138 Roma - Via di Castel Giubileo, 11
Tel. : + 39068811270 (6 linee PBX) **Fax :** +390664492025
C.F. e P. Iva : 07363481008 - REA : 1028514 - Iscr. Reg. Impr. 18118/2003
Web : www.laboratoriogenoma.eu **E- mail:** info@laboratoriogenoma.it

Suggestions for contamination control

Stringent precaution should be taken in order to minimize contamination occurrences. Gloves and dedicated lab coats should be worn during preparation of single cells Preparation of media and reagents should be carried out within a laminar flow cabinet located in a "clean" room restricted to those activity. Mineral oil pipetting should be performed using aerosol-resistant pipette tips and a dedicated pipette for this purpose.

Gloves should be changed very frequently. Hood surface, and pipetters should be cleaned with chlorine bleach or absolute ethanol and treated with UV light before uses.

BIOPSY TIPS

- One specimen tube open at a time.
- Avoid carry-over of lyses buffer to next cell. If cell lyses, change pipet tips and biopsy a second cell in a separate tube labeled accordingly.
- Do not use IVF oil.
- No stage warmer.
- Limit the number of embryos biopsied at one time to 5 to prevent evaporation of placement droplet and wash droplet.
- Limit the transfer of media into wash droplet.
- Keep the sample tubes in an upright orientation holds the DNA in a set position through shipping for accurate DNA isolation.

SHIPPING TIPS

- Check tube labels are readable before packing tubes.
- Include original informed consent forms (yours and ours).
- Include embryo biopsy worksheet.
- Keep a supply of cold shipping packets and Styrofoam containers that come with reagents.
- Avoid dry ice.
- The day before biopsy, request courier pickup an hour after expected completion time.
- Fax biopsy data worksheet ahead of shipment.
- Attach airline security letter on your letterhead to outside of box.

GENOMA s.r.l.
CHIAMATA GRATUITA NUMEROVERDE 800-501651
Sede legale e Studi Medici : 00198 Roma - via Po,102
Tel. : + 390685304150 - +390685358425 **Fax :** +390685344693
Laboratori e Studi Medici : 00138 Roma - Via di Castel Giubileo, 11
Tel. : + 39068811270 (6 linee PBX) **Fax :** +390664492025
C.F. e P. Iva : 07363481008 - REA : 1028514 - Iscr. Reg. Impr. 18118/2003
Web : www.laboratoriogenoma.eu **E- mail:** info@laboratoriogenoma.it