PG-Seq™ Core Panel Software

powered by Journey Genomics

User Guide

FOR USE WITH:
PG-Seq™ Core Panel

Compatible with Illumina® and Element Biosciences® platforms

PG-Seq™ Core Panel Software User Guide

This product is for research use only.
Not for use in diagnostic procedures.

1. Overview

The PG-Seq™ Core Panel software is designed to assist with the identification of specific genetic mutations in the following genes: *BRCA1, BRCA2*, *CFTR, DMD*, *F8*, *FMR1*, *GJB2* and *HBB* in the context of Preimplantation Genetic Testing (PGT) using Next Generation Sequencing (NGS). Utilizing the PG-Seq™ Core Panel kit technology, using whole genome amplified DNA (WGA) or genomic DNA as input, the kit provides all the reagents for library preparation hybridization, capture and data analysis for implementation of PGT-M on Illumina® and Element Biosciences® sequencing platforms. Following Illumina® sequencing or Element Biosciences®, the potential genetic mutations of the samples for the targeted genes are analyzed and visualized with the PG-Seq™ Core Panel software.

2. Revision History

Version	Date	Description
v1.0	March 2025	Release of PG-Seq™ Core Panel Software v1.0

3. Input Requirements

PG-Seq™ Core Panel software allows loading of gVCF, BAM and BAI file types obtained from FastQ files generated from Illumina® or Element Biosciences®. All these files need to be aligned against human (GRCh38) genome.

Note that the software will not process the following:

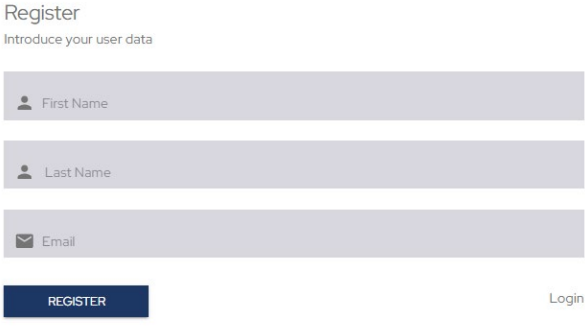
- Samples that have not been generated using the PG-Seq™ Core Panel kit assay.
- Corrupted gVCF or BAM/BAI files.
- A type or number of familiar samples which are not suitable to perform the informativity study (see appendix)

4. Computer Requirements

The PG-Seq™ Core Panel software is a cloud-based software. Only requirement is to have a stable and high-speed internet connection for accessing to the software without interruptions.

5. Access & Activation

1. Go to https://apps.journeygenomics.com/
2. Click on register.
3. Fill the form and press Register.



4. After registration process, will receive a password via email within 1-2 business days.

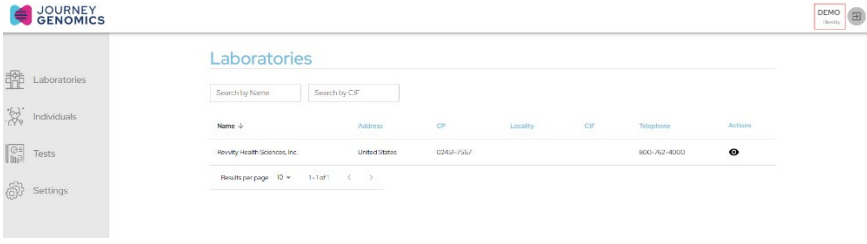
NOTE: Please contact Revvity at https://www.revvity.com/contact-us/technical-support if you are experiencing issues with access or activation of the software.

6. Algorithm Basics

The PG-Seq™ Core Panel software offers linkage analysis and direct testing inside each created project. Once the data is loaded, the software executes the following internal processes before the result visualization is displayed: the software reads the gVCF file to decide the positions that are suitable to be shown, different metrics of each position can be shown, such as coverage, quality or allele frequency, positions colored in grey correspond to positions with coverage and/or quality lower than the threshold. Once the quality control has been performed, the linkage analysis is conducted employing the "viable" positions remaining in the informativity study. In the final step, the embryo status is determined by tracking the non-affected or affected alleles. Also, sex determination is performed by analyzing different SNPs along the X and Y chromosomes.

7. Settings

1. To access to the software settings please click on the upper-right side of the window, where your username appears.

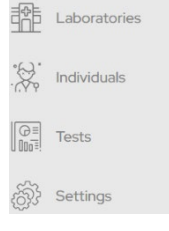


2. Once in your profile, user information can be changed. Specifically English or Spanish language can be chosen. Also, the password can be modified by pressing the Change Password button.

8. Display Options

The PG-Seq™ Core Panel Software is divided in different tabs:

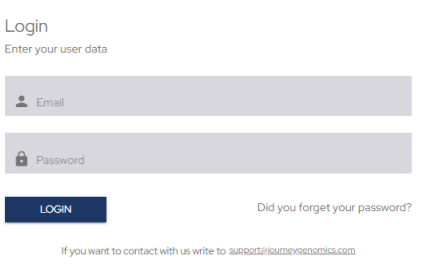
1. The Laboratories tab, that includes information about your institution.
2. The Individuals tab, that must be selected to introduce the individuals' information. For more information go to section 9.
3. The Tests tab must be selected to perform an analysis.
4. The Settings tab includes information about user profiles (Only available for Laboratory administrators).

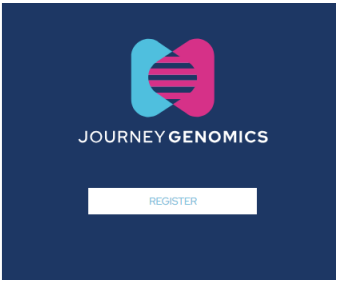


9. Launching the Software and Creating a Couple sheet

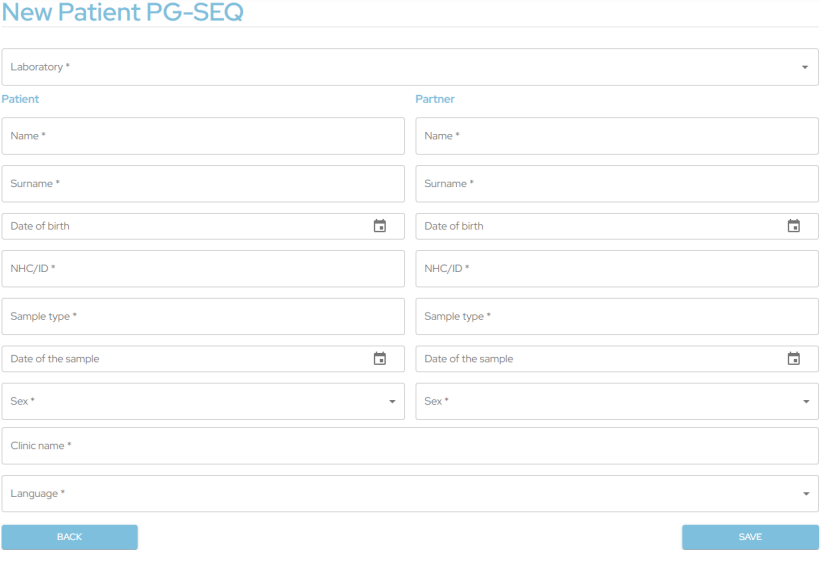
Note: “Couple” in this context refers to the progenitors of the embryo(s) we want to study.

1. Go to https://apps.journeygenomics.com/
2. Sign into the PG-Seq™ Core Panel Software.





3. In the Individuals tab, go to PG-Seq Core Panel and open the couple information sheet by clicking New button.
4. Fill out the form with the patient’s information and press Save.

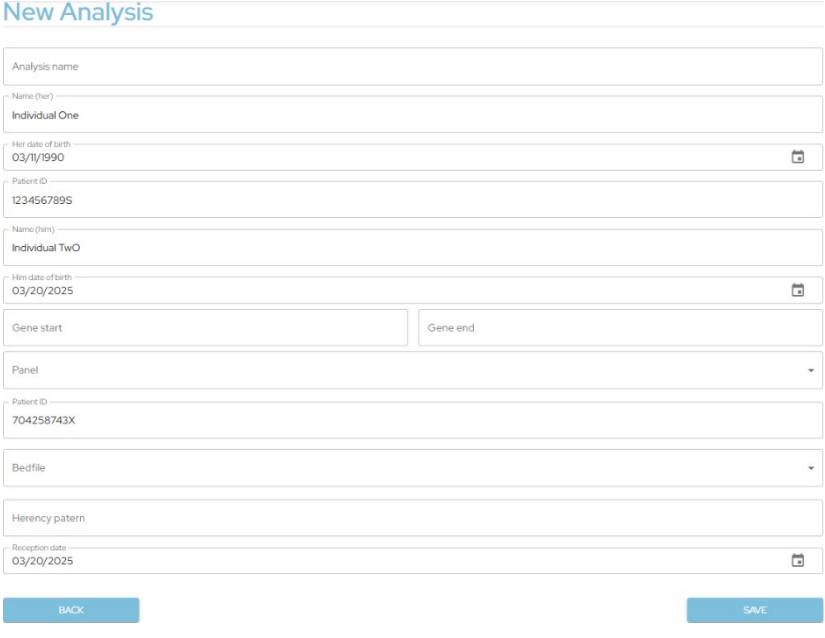


10. Launching an Informativity Study

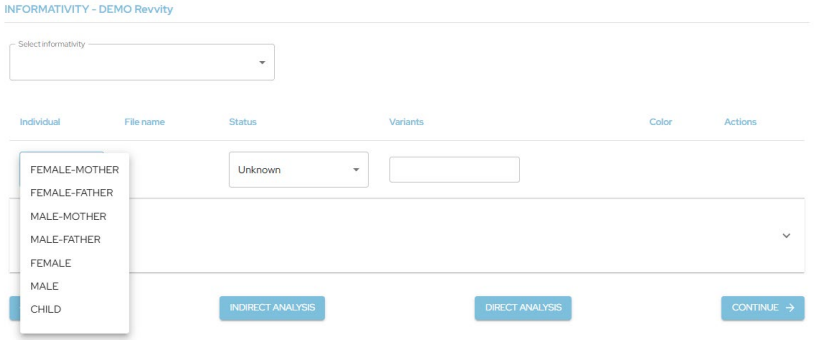
After a couple sheet has been created, all genetic data can be added to the analysis:

1. On the Tests tab, go to PG-Seq Core Panel and click New to create an analysis.
2. Select a patient for the analysis using the drop-down menu:

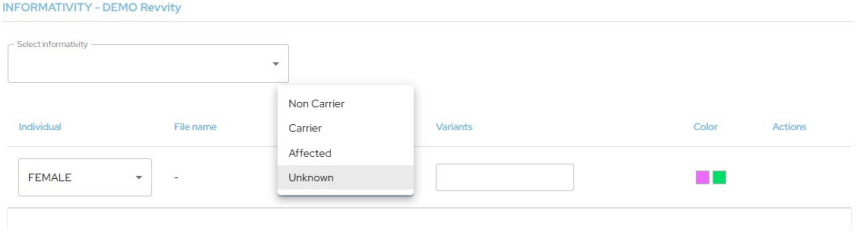
3. Fill out the form with genetic information and click Save. Note that the information that is shared with the couple information form is filled automatically.



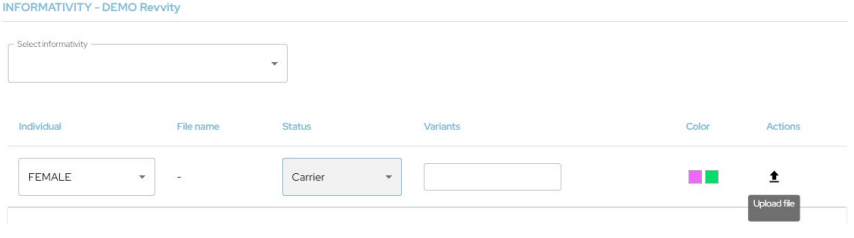
4. Next step is the Informativity module.
5. First select one Individual to be included on the informativity study.



6. Second, select the known Status of the individual. Note that the status can change depending on the gene selected according with the inheritance pattern of the disease. For autosomal recessive inheritance Non-carrier, Carrier and Affected status are displayed, but for autosomal dominant inheritance only Non-carrier and Affected status are displayed.



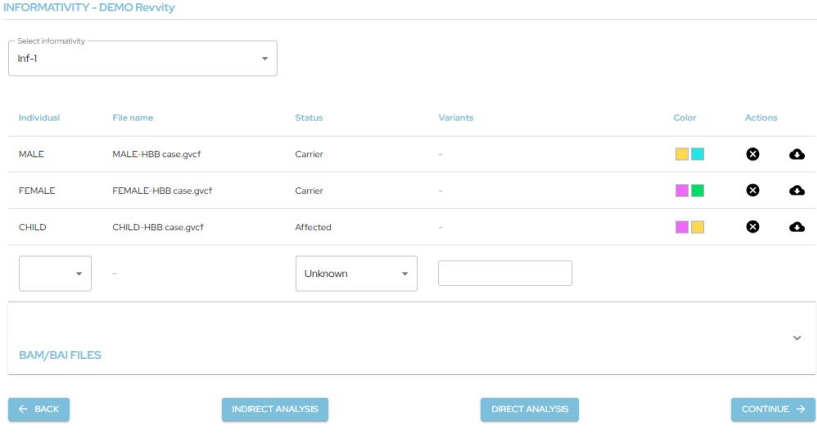
7. Finally upload the gVCF file of the individual by clicking on the arrow button under the actions column.



8. Repeat the previous step for every individual you want to be included in the analysis.
9. Click Indirect analysis to begin processing the informativity study.
10. The informativity study can be reviewed on a new window. Note that shaded positions correspond to key positions.

(*Optional*)
None informative and semi-informative SNPs can be displayed by clicking No info and Semi info buttons, respectively. In addition, quality and coverage of each SNP analysed can be shown by clicking Quality/Coverage button. Also, allele frequency can be shown by clicking Allele Frequency button.

11. To perform a direct testing analysis, upload the BAM/BAI file in the BAM/BAI FILES section and click Direct Analysis. A window opening the genomic viewer IGV will appear.

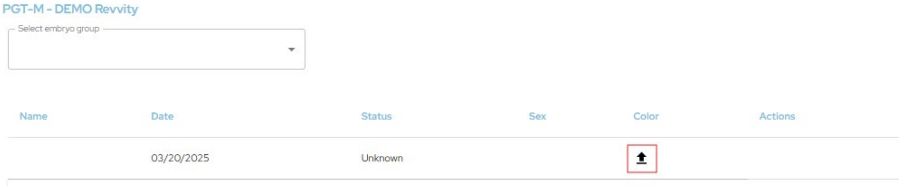


12. Once reviewed click OK and then Continue.
13. Write a name of the project and click Confirm to save the informativity analysis. If numerous informativity studies are performed, each informativity analysis should be saved with a different name, if not and only few modifications have been performed the same name can be used.

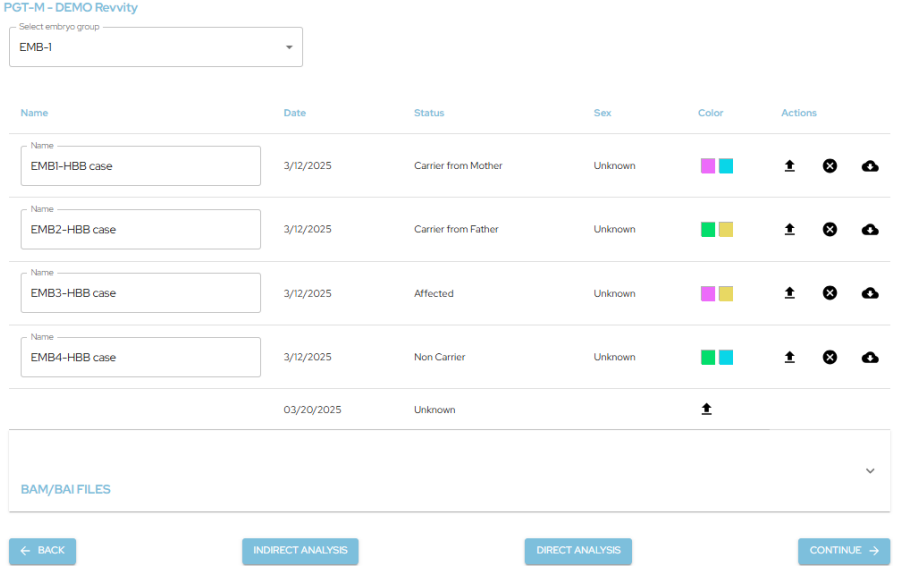
14. Once the informativity analysis is done and saved you can go to the PGT-M section to launch the PGT-M analysis (section 11) or directly download a report (section 12).

11. Launching a PGT-M Study

1. Upload as many gVCF files as embryos to be included in the analysis by clicking the arrow button.



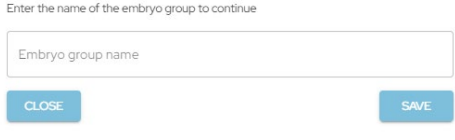
2. Once the data is completely uploaded, click Indirect Analysis.



3. The PGT-M study can be reviewed on a new window.

(*Optional*)
None informative and semi-informative SNPs can be displayed by clicking No info and Semi info buttons, respectively. In addition, quality and coverage of each SNP analysed can be shown by clicking Quality/Coverage button. Also, allele frequency can be shown by clicking Allele Frequency button.

4. To perform a direct testing analysis, upload the BAM/BAI file in the BAM/BAI FILES section and click Direct Analysis. A window opening the genomic viewer IGV will appear.
5. Once reviewed click OK and then Continue.
6. Write a name of the project and click Confirm to save the PGT-M analysis. If numerous PGT-M studies are performed, each informativity analysis should be saved with a different name, if not and only few modifications have been performed the same name can be used.



7. Once the PGT-M analysis is done and saved you can download a complete report (section 12).

12. Download a Results Report

After informativity/PGT-M study has been performed an independent report can be downloaded:

1. To download an informativity report, perform steps in section 10 and go directly to the step 4 of the analysis without introducing embryos' data. Here a preview of the final report is shown to confirm the information. To download the report, press Download Results button on the upper-left side of the window.
2. To download a PGT-M report, perform steps in section 10 and 11. On the step 4 of the analysis a preview of the final report is shown to confirm the information. To download the report, press Download Results button on the upper-left side of the window.

Note: for each report the header, the signature, the signer and his/her role can be completely customized according to each laboratory.

13. Recommendations and Cautions

- We recommend running the informativity samples first and see if there are enough informative SNPs to proceed with embryo testing.
- The region where your variant of interest is located can be marked by clicking on that position, if not, please select the closer position showed.
- Shaded positions correspond to key SNPs.
- We recommend for a more robust analysis to have at least three key SNPs on each side of the variant of interest in each allele.

14. Software Demo

For a software demonstration or technical support, please contact
https://www.revvity.com/contact-us/technical-support

Appendix A –General Overview of the Analysis Workflow

There are 4 steps in the analysis workflow:

1. Creation of a Project.
2. Informativity study: files and data of family members.
3. PGT-M study: files and data of the embryo(s) to be analyzed.
4. Results report (informativity and PGT-M).

1. Creation of a Project

On the first step of the analysis, we must create a project. After creation of the project, this data can be shown by clicking on the eye button under the *Actions* column of each analysis. if any modification is needed once the project has been created, please first contact support team to ensure the validity of the changes.

2. Informativity study: files and data of family members.

On the second step, the gVCF files of family members are uploaded here for analysis. The list the actions you can perform on this step are shown below.

1. Selection of the individual.

2. Selection of the status of the individual: "Non-carrier", "Carrier" and "Affected". Depending on whether the pathology is dominant, recessive or X linked, the options are established correspondingly (see appendix B).

3. Upload gVCF files.

4. Upload BAM/BAI files.

5. Process data and generate results (Indirect Analysis).

6. Load IGV genetic viewer (Direct Analysis).

7. Choose which individuals we want to be shown in the results.

3. PGT-M study: files and data of the embryo(s) to be analyzed

On the third step, the gVCF files of the embryos which are going to be analyzed are uploaded here for analysis. In terms of informativity study here we list the actions you can perform on this step.

1. Upload one or more embryo gVCF files.

4. Upload BAM/BAI files.

5. Process data and generate results (Indirect Analysis).

6. Load IGV genetic viewer (Direct Analysis).

7. Choose which individuals we want to be shown in the results.

4. Results Report

The final step is the results report that shows all the data regarding each independent analysis. Two different reports can be generated depending on the analysis performed. If only the informativity step is performed, an informativity report can be obtained by continuing directly to step 4 without uploading files in step 3. On the other hand, if both informativity and PGT-M studies are performed, a complete PGT-M report is generated. Both reports can be downloaded by clicking the download report button.

Appendix B– Samples Required for PG-Seq Core Panel Analysis

In this appendix we are going to describe the type and number of familiar samples required for getting conclusive results with the PG-Seq Core Analysis.

General Points

Generally, samples from the couple (progenitors of the embryo) and another additional family member for each mutation tested are required. It is recommended that all family members included were confirmed genetic carriers of the alteration. If they are not carriers they could also be included in the study, but its state should be completely known.

A healthy (non-carrier)/affected child is considered a family member, and he/she is the best option for obtaining successful results. If the child is only carrier (in recessive disorders), the origin of the alteration is always required to be known. If it is not possible, the sample should be discarded and a different sample from another family member should be used. If there are no child samples available, it is possible to perform the study with samples from other family members. In these cases, there are differences between recessive and dominant diseases.

Autosomal dominant disorders

In the case of disorders with dominant inheritance pattern, samples from the couple and a minimum of one sample from other affected family members are required (figure 1). For example, required sample from a grandparent (affected), and recommended from both grandparents, always in the affected family. Please note that, if the available sample comes from an affected member, the solution is clear. However, if the available sample is from a healthy individual, and we have no clinical confirmation that the other member (non-available) was a carrier, the risk can be high.

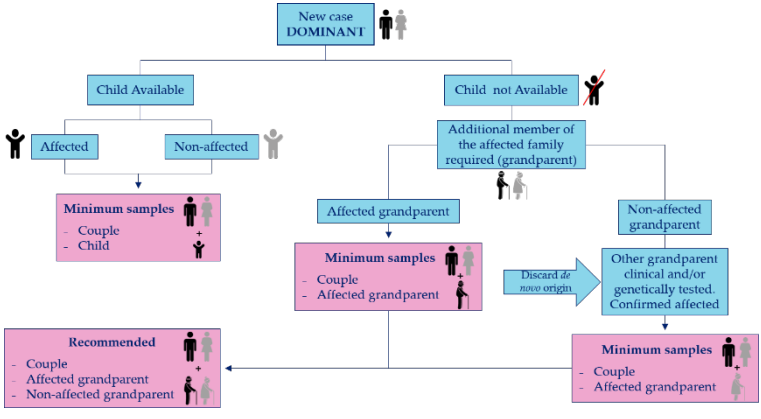


Figure 1. Diagram representing the minimum and recommended samples for a case of autosomal dominant disorder (affected male).

Autosomal recessive disorders

In disorders with recessive inheritance pattern, we will need more samples. We will need a minimum of one sample from each branch of the family (figure 2). Also, we need to be sure that, if only one member is available and the male/female-father/mother available is a non-carrier, the other family member may be genetically confirmed as a carrier.

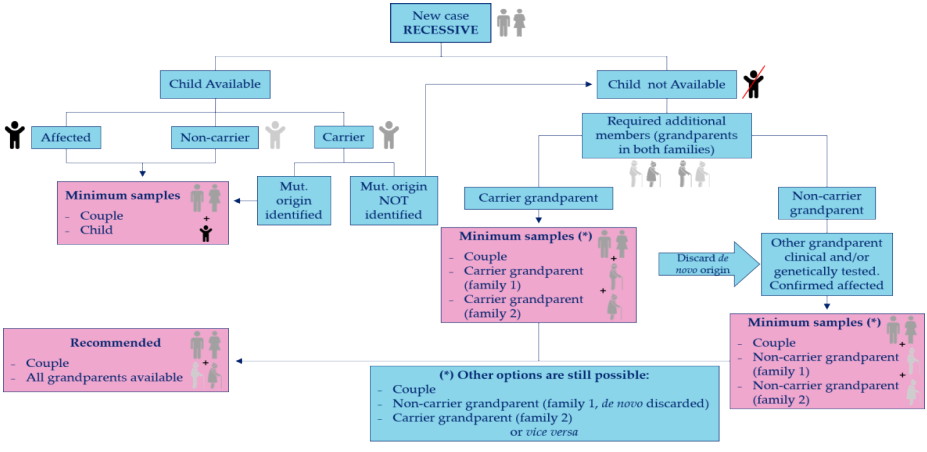


Figure 2. Diagram representing the minimum and recommended samples for a case of autosomal dominant disorder (carrier male and female).

X-linked disorders

In X-linked disorders, in a female carrier, we need samples from the female family branch, like in the case of dominant disorders (figure 3).

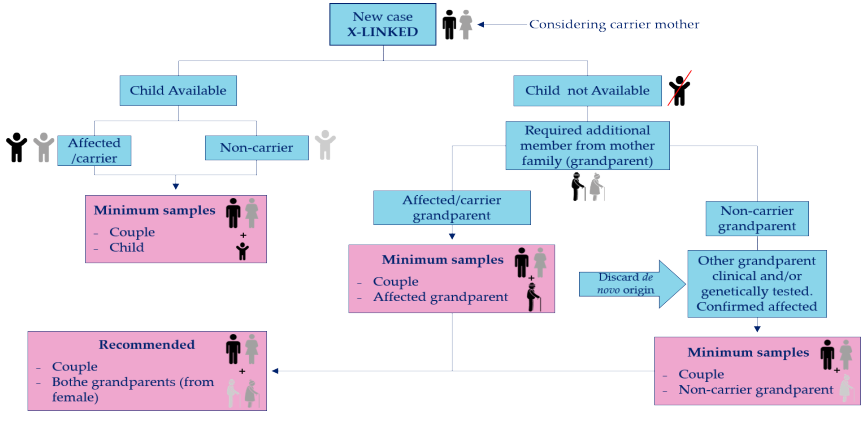


Figure 3. Diagram representing the minimum and recommended samples for a case of X-linked disorder (carrier female and affected male).

Appendix C– Glossary

Informativity Study: Analysis to determine if genetic markers can reliably track the inheritance of a pathogenic variant within a family. Requires DNA samples from affected/unaffected relatives to identify informative polymorphisms (STRs/SNPs) flanking the mutation.

Indirect Analysis: Linkage-based method using polymorphic markers (STRs/SNPs) near the disease gene to infer embryo haplotypes rather than directly testing the mutation itself. Given the minute amount of DNA from embryo, it reduces risks from allele dropout compared to direct methods.

Direct Analysis: Targeted detection of the variant of interest in embryo DNA, by NGS or other methods such as qPCR or Sanger sequencing.

www.revvity.com

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Rewity, Inc.
77 4th Avenue,
Waltham MA, 02451-
7567 USA

(BOO) 762-4000
www.rewity.com

For a complete listing of our global offices, visit www.revvity.com