# ReproHealth® Nexus Software

For use with PGD-SEQ™ kit

## USER GUIDE

Version number: 2.0

**For Research Use Only. Not for use in diagnostic procedures.**

# Contents

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

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You are responsible for the accurate execution of the protocols outlined in this Software User Guide, as well as for the analysis and interpretation of the resulting data. Journey Genomics does not guarantee any specific outcomes.

# About this guide

## Overview

The ReproHealth® Nexus Software is designed to assist with the constructions of family haplotypes with the aim of identifying the inheritance of carrier alleles in the context of Preimplantation Genetic Testing (PGT) for Monogenic disorders (PGT-M) using Next Generation Sequencing (NGS). More than 600 genes or regions can be analyzed by means of PGD-SEQ™ kit. Utilizing AmpliSeq™ technology, using whole genome amplified DNA (WGA) or genomic DNA as input, the kit provides all the reagents for library preparation and data analysis for implementation of PGT-M on Ion Torrent™ sequencing platforms. Following Ion Torrent™ sequencing, the potential genetic mutations of the samples for the targeted genes are analyzed and visualized with the ReproHealth® Nexus software.

## Revision history

| Revision | Date | Description |
|---|---|---|
| 1 | September 2019 | Release of PGD-SEQ™ Software v1.0 |
| 2 | May 2026 | Release of ReproHealth® Nexus Software v2.0 |

This User Guide is intended as a manual for the ReproHealth® Nexus Software v2.0

# Requirements

## Input requirements

The ReproHealth® Nexus software allows direct loading of VCF, BAM, and BAI file types from Ion Torrent™ pipeline software. All these files need to be aligned against human (GRCh37) genome.

Note that the software will not process the following:

- Samples that have not been generated using the PGD-SEQ™ kit assay
- Corrupted VCF or BAM/BAI files
- BAM/BAI files with incorrect format*
- The type or number of familiar samples is not suitable to perform the informativity study.

* The file name for both BAM/BAI files needs to be exactly the same. Additionally, if the file name is too long, it is usually shown as *bambaiFail.

## Computer requirements

Access to the ReproHealth® Nexus software is included as part of each kit in the range of PGD-SEQ™ Kits.

The ReproHealth® Nexus software is a cloud-based software. The only requirement is to have a stable and high-speed internet connection for accessing to the software without interruptions.

# Algorithm Basis for PGD-SEQ™ analysis

## Software algorithm basis

The ReproHealth® Nexus software offers linkage analysis to investigate familial allele inheritance patterns. Linkage analysis is used to identify disease-causing variants by tracking the inheritance patterns of genetic markers, specifically SNPs, near the mutation of interest. This approach is based on the fact that markers that are physically close on a chromosome are more likely to be inherited together due to reduced probability of recombination events occurring between them. The key to successful linkage analysis lies in careful sample selection for study inclusion.

Once the data is loaded, the software executes the following internal processes before the result visualization is displayed. The software reads the VCF file in order 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 10 and 20, respectively. 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-carrier or carrier alleles. Also, sex determination is performed by analyzing different SNPs along the X and Y chromosomes.

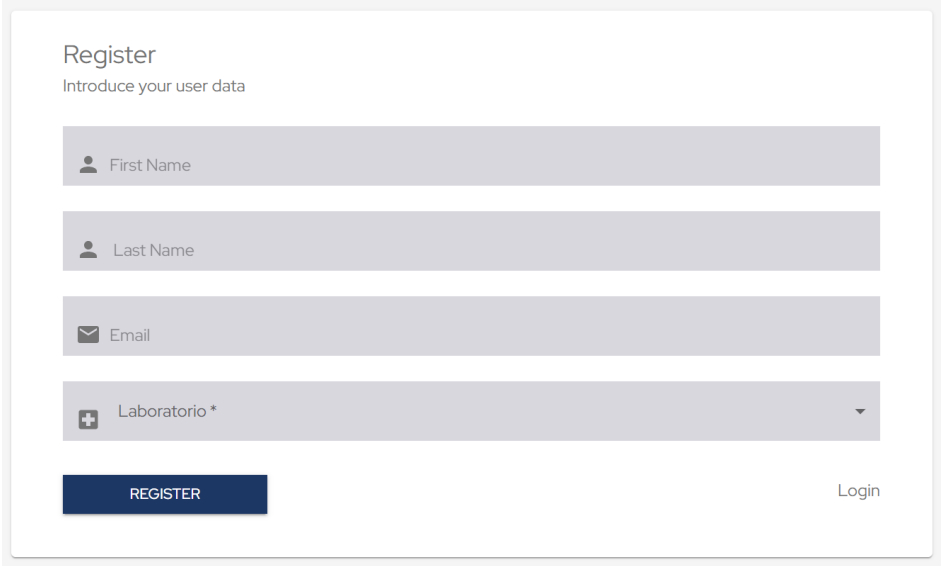
To aid in the categorization of results, we have incorporated a confidence score. This score is based on the key parameters considered when reviewing a PGT-M case, including:

- The number of informative markers flanking the variant on both alleles.
- The inheritance pattern of the disorder.
- The feasibility of direct variant analysis.

# Starting at ReproHealth® Nexus Software

## Access and activation

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

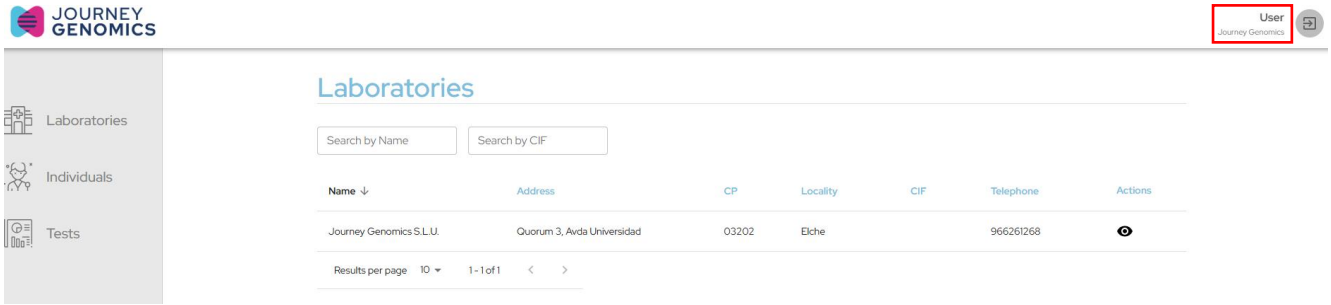



4. After registration process, Journey Genomics' team will validate your user, and you will receive a password via email.

**Note**: Please contact support@journeygenomics.com if you are experiencing issues with access or activation of your software account.

## Profile settings

To access the profile settings, please click on the upper-right side of the window, where your user's name appears.



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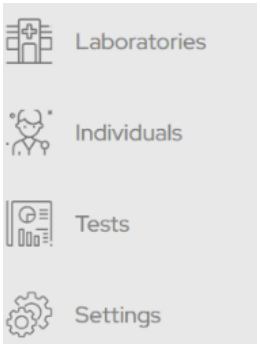
Once on your profile, user information can be changed. Concretely, the language of the software can be chosen between English and Spanish. Also, the password can be modified by pressing the Change Password button.


# User interface

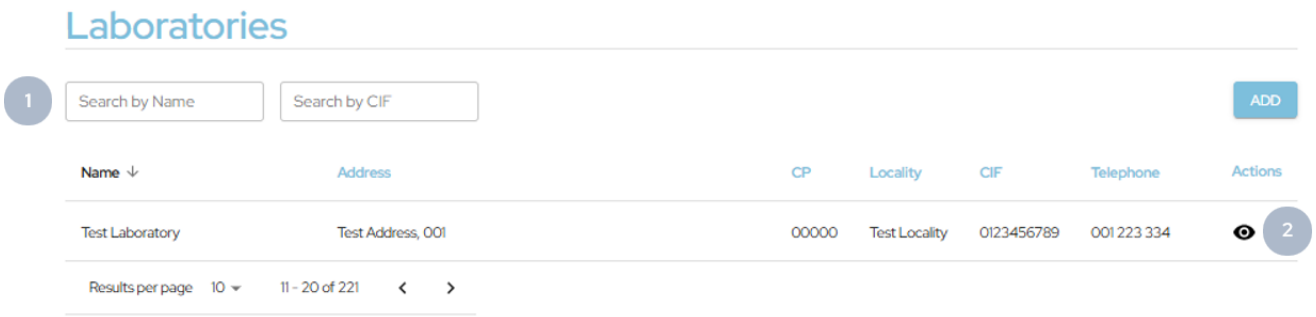
The ReproHealth® Nexus software is divided into different tabs.

1. Laboratories tab
2. Individuals tab
3. Tests tab
4. Settings tab




## Laboratories tab

The Laboratories tab includes information about your institution.




1. Search boxes
2. See button.



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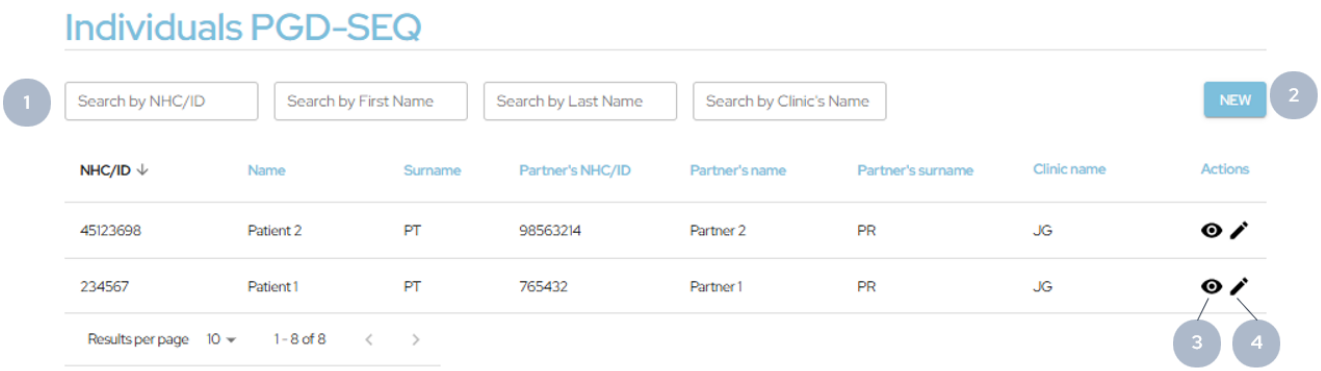
## Individuals tab

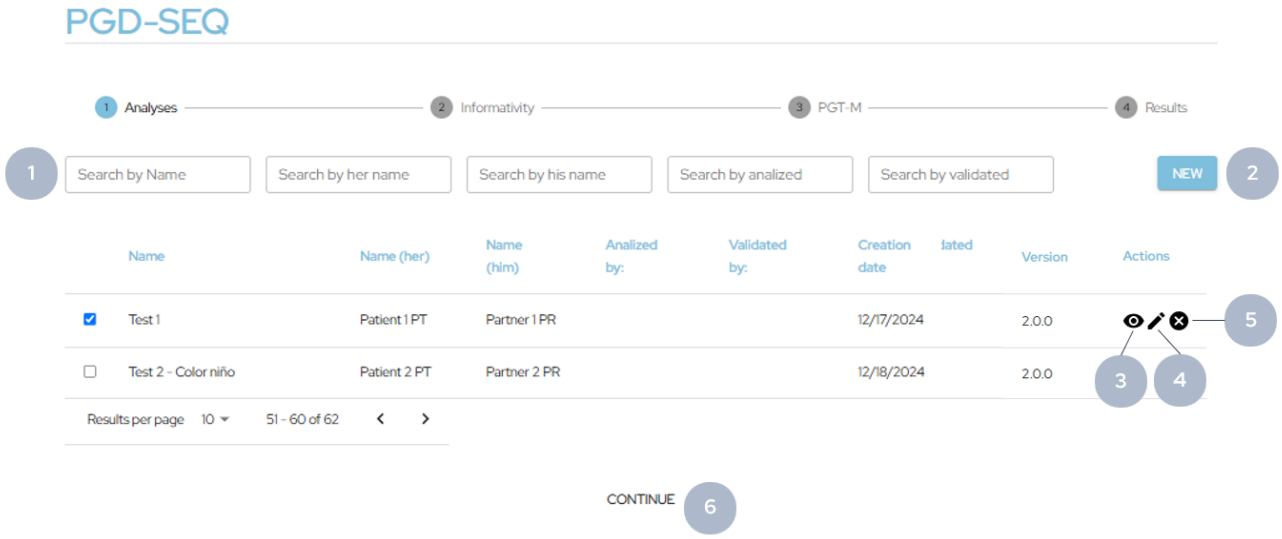
The Individuals tab has to be selected to introduce the individuals' information. For more information, go to section 5 (Data Management - Create a Couple Sheet, page 13).




1. Search boxes
2. New button. Allows the inclusion of new individuals.
3. See button. Inside this page, all projects associated with the individual can be seen.
4. Edit button. Allows the edition of individual's information.

## Test tab

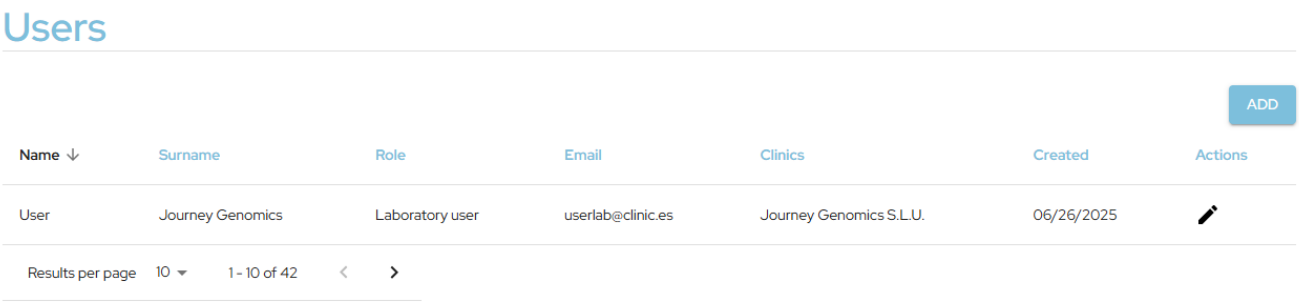
The Tests tab must be selected to perform an analysis.




1. Search boxes
2. New button. Allows the inclusion of a new PGD-SEQ test.
3. See button. Inside this page, the information of the project is shown.
4. Edit button. Allows the edition of individual's information.
5. Delete button.
6. Continue button. To proceed to informativity test of the selected project.

## Settings tab

The settings tab includes information about user profiles. Only available for Laboratory administrators.




## Users' roles and permissions

This section outlines the distinct user roles within the software and the specific permissions associated with each.

## Laboratory Administrator

The Laboratory Administrator role is designed for users who require comprehensive control over their laboratory's activities and user management within the platform and have the following permissions.

**Laboratories tab**

- View a list and access details of their own laboratory and related laboratories.

**Individuals tab**

- View the list and details of individuals belonging to their laboratory.
- Create new individuals.
- Edit the information of individuals belonging to their laboratory.

**Test tab**

- View the list and details of all projects associated with their laboratory.
- Create new projects.
- Edit all projects associated with their laboratory and generate reports.

**Settings tab > Users**

- View the list of all users associated with their laboratory.
- Create new users, associated with their laboratory and limited to the "Laboratory User" role.
- Edit user information, including passwords.
- Delete users, which includes a process for reassigning projects.

## Laboratory User

The Laboratory User role is intended for individuals who perform routine tasks within their laboratory, with specific limitations on project and user management.

**Laboratories tab**

- Users can view a list and access to details of their own laboratory.

**Individuals tab**

- Users can view a list of individuals belonging to their laboratory.
- Users can edit the information of individuals belonging to their laboratory.
- Users can view the details of individuals.
- Users can create new individuals.

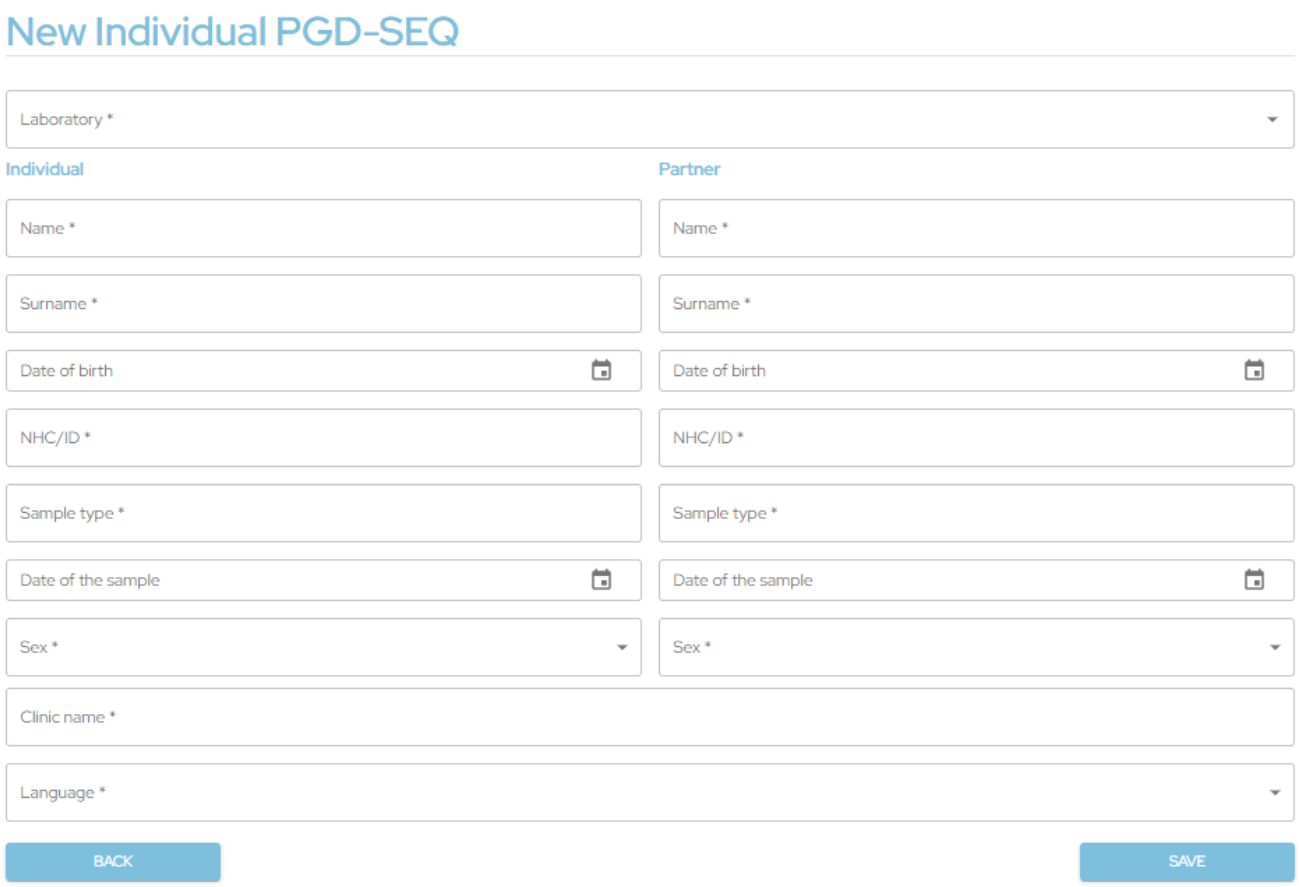
**Test tab**

- Users can view a list of all projects associated with their laboratory.
- Users can view the details of ALL projects associated with their laboratory.
- Users can edit projects that they have personally created and generate reports.
- Users can create new projects.

# Data Management

## Creating a Couple Sheet

1. In the Individuals tab, go to PGD-SEQ and open the couple information sheet by clicking New button.
2. Fill out the form with the patient's information and press Save.




*** Note**: The NHC/ID code must be unique for each patient.



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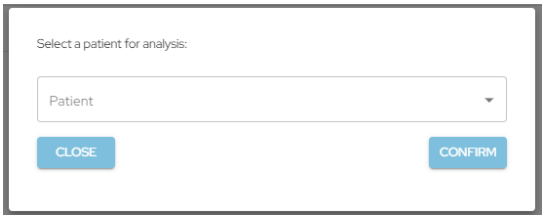
# Launch an Analysis

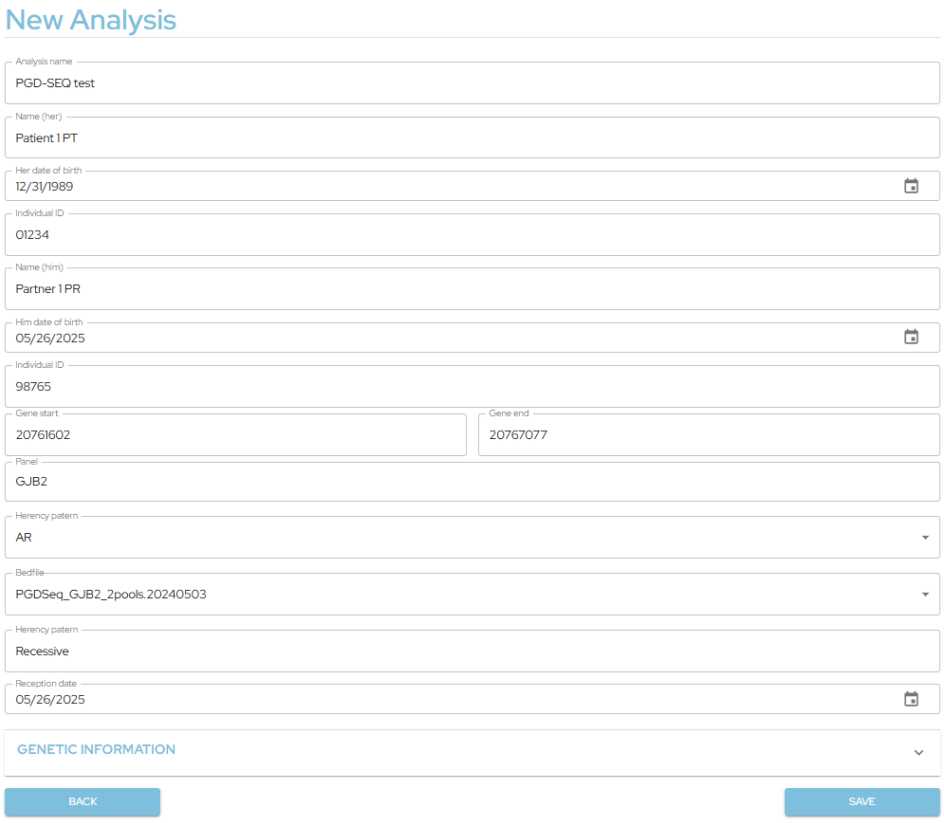
## Creating a project

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

1. On the Tests tab, go to PGD-SEQ section 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 most of the information previously included in the couple's sheet is filled automatically. Once the panel is selected, the genetic information section will be available.



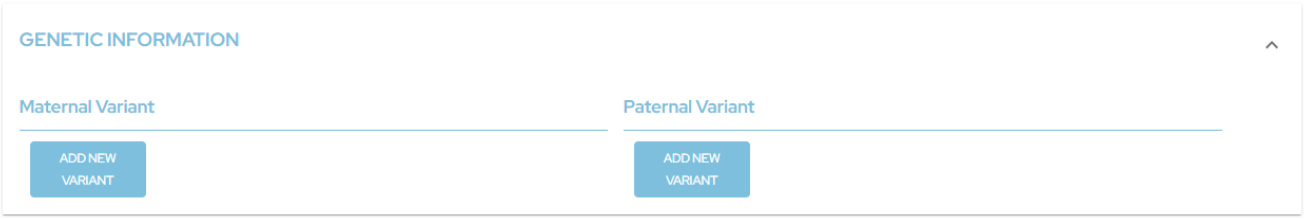

*** Note**: It is important to select the correct bedfile when creating the project.

4. Include all the genetic information available for each member of the couple, as described above.

## Genetic information section

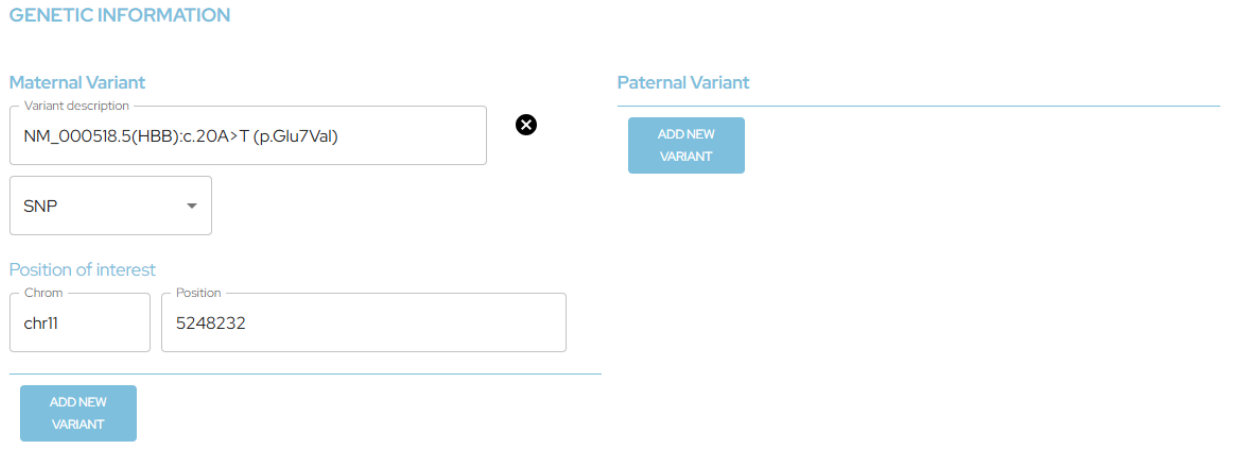
This section allows users to include specific genetic information relevant to their case. Including this information is not absolutely necessary, although providing these details can significantly improve the accuracy and precision of the analysis, so we highly recommend taking the time to input this information if you have it available.

Both the **Maternal Inheritance** and the **Paternal Inheritance** sections are available to list all the genetic variants that are present in the respective parent. For that, follow the steps listed above.

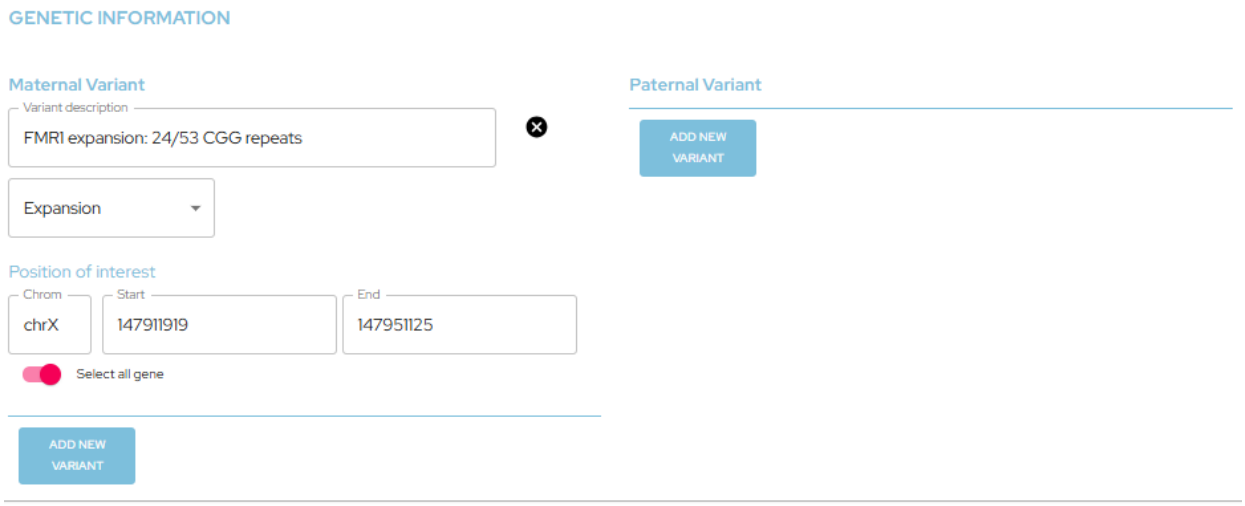



*** Note:** If any specific variant information is not entered here, the software will automatically use the entire gene as a reference point for linkage analysis. For region panels, the software will simply show you the total number of key positions within that region, without specifying whether they are upstream or downstream of any reference position. Additionally, if any information is not entered here, the Confidence Level associated to the Informativity and PGT-M analyses will not be calculated.

1. Fill variant description with variant information. It allows plain text so any information and format are valid.
2. Select the variant type in the drop-down list. All the different types of genetic variations that PGT-M can analyze, from single nucleotide polymorphisms (SNPs) and small insertions or deletions (indels) to larger deletions, duplications, and even expansions, can be selected.
3. Introduce chromosomic position of the variant. The importance of adding this genetic information is that the specific variant positions you provide will then become the reference points for linkage analysis.




*** Note:** If the exact location of the variant is unknown or undetermined, click on "**Select all gene**" option. Choosing this will automatically input the start and end points of the entire gene as the reference.

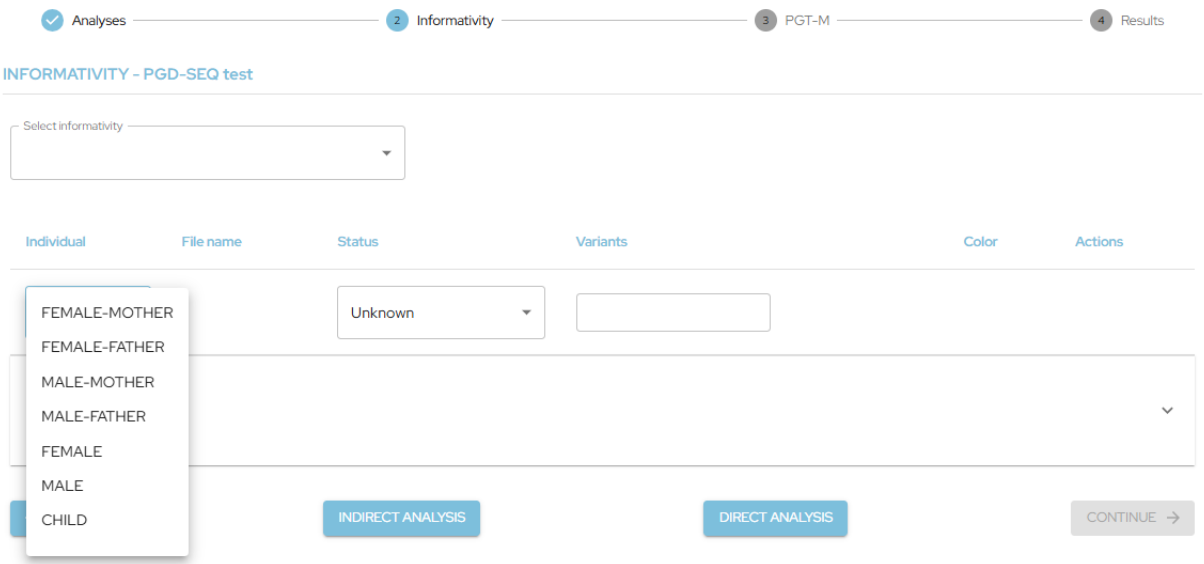



Multiple variants for each parent can be added. If more than one is included, the system allows you to specify whether these variants are in *cis* (on the same allele) or *trans* (on opposite alleles).

## Launch an Informativity Study

Once all relevant genetic information has been entered, it is important to link each family member's genetic status included in the analysis (in the Informativity step of the Test) to the specific variants listed under each inheritance. This ensures that the software understands who carries which genetic variant.

1. First, select one Individual to be included in the informativity study.

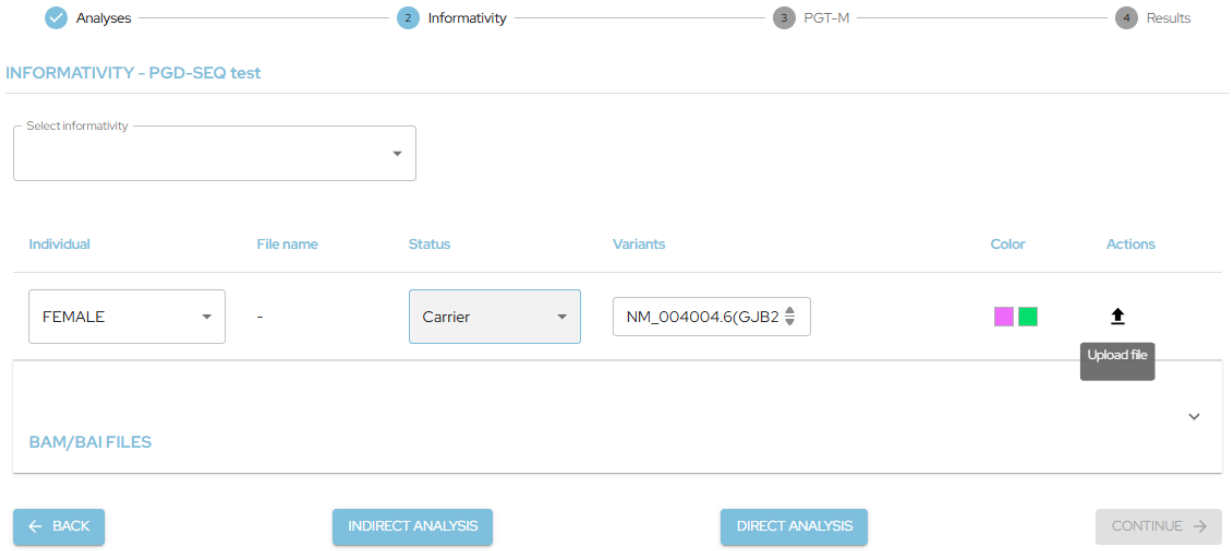



Second, select the known Status of the individual. Note that the status can change depending on the gene selected according with the inheritance pattern of the disease:

- For autosomal recessive inheritance, Non-carrier, Carrier, and Affected status are displayed.
- For autosomal dominant inheritance, only Non-carrier and Affected status are displayed.
- For X-linked inheritance, the status will depend on the individual selected.

Once the status is selected, the variant information will be filled based on the genetic information previously included.

Finally, upload the VCF file of the individual by clicking on the arrow button under the actions' column.



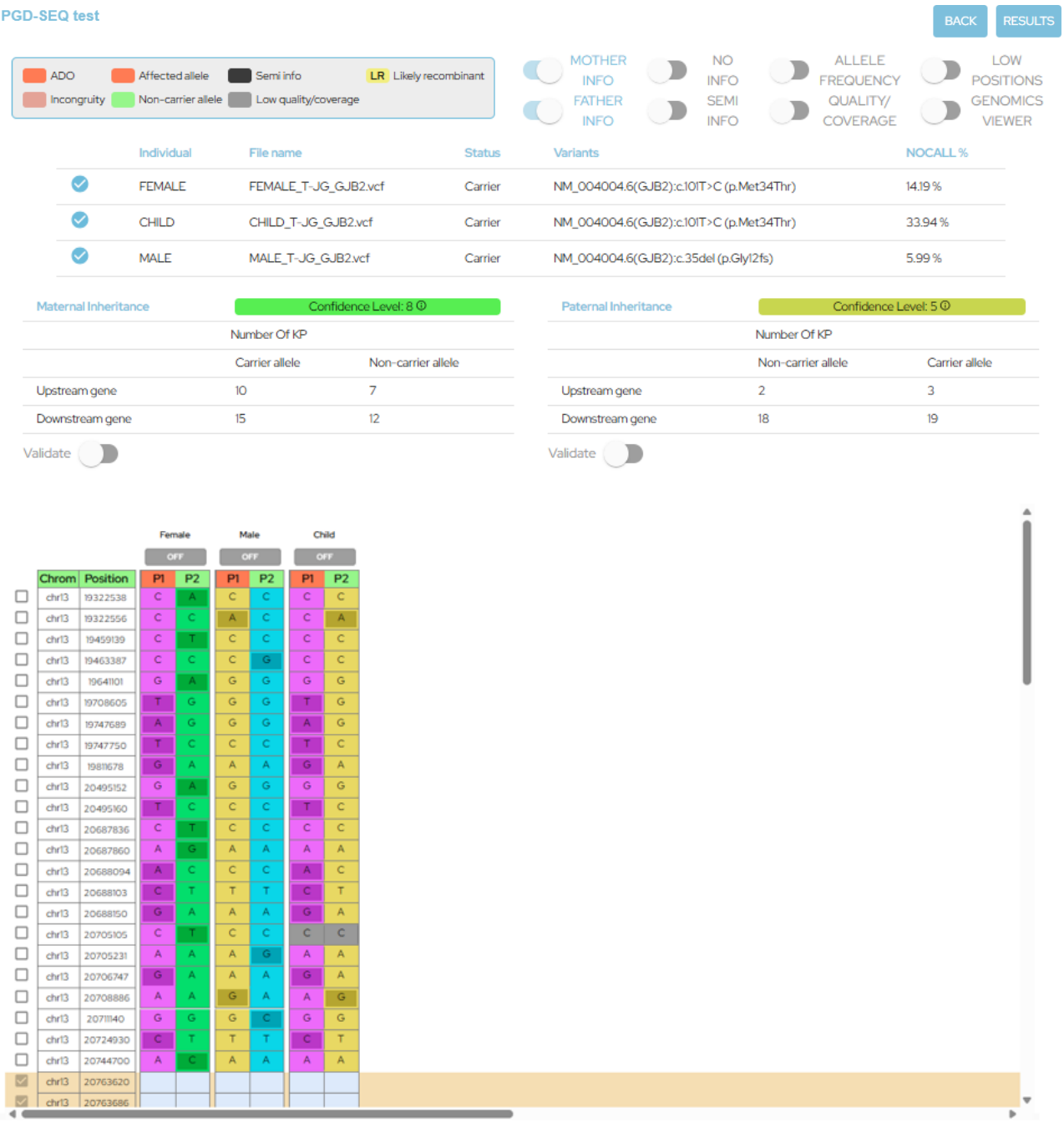

2. Repeat the previous step for every individual included in the analysis.
3. To perform a direct testing analysis, upload the BAM/BAI file in the BAM/BAI FILES section. By clicking the Direct Analysis button, the genomic viewer IGV will appear in a new page. ***Performing direct analysis is high recommended as it directly affects the confidence score calculated by the software***.

   *** Note**: The Direct analysis button becomes active when a variant meets the standards (is smaller than 15 bp), indicating that direct testing is viable. **Important**: An active button does not guarantee that the variant is covered by the panel. To ensure direct testing is available for a concrete panel and revision, please, reach out to support@journeygenomics.com.
4. Direct Analysis page will include a table with the family samples and the associated status based on the variant that is being analyzed. A dropdown associated with each variant will allow selecting the status analyzed by direct testing. All included variants can be selected in the upper dropdown.


*** Note**: Selecting the 'Inconclusive' status for a sample in the Direct testing tab indicates that direct analysis is not viable for that specific sample and variant.

Each sample settings for the genomic viewer can be adjusted using the wheel on the right-hand side.

5. Click Indirect analysis to begin processing the informativity study.
6. The informativity study can be reviewed in a new page. Note that shaded positions correspond to key positions (KP).




Results are displayed in tables for each maternal and paternal inheritances. Based on the number of KP for each allele on each side of the variant and viable direct testing, a Confidence Score is determined.

*** Note**: The number of KP will always be displayed, but the confidence scored will only be determined when genetic information is filled in the corresponding section when creating a new project.

**Optional:** The visualization of analysis results offers several optional features to customize the display and help the data analysis, including:

- Non-informative and semi-informative SNPs: These positions can be displayed by clicking the No info and Semi info buttons, respectively.
- Quality and Coverage: The quality and coverage values of each analysed SNP can be shown by clicking the Quality/Coverage button.
- Allele Frequency: This value can be shown by clicking the Allele Frequency button.

- Genomics Viewer (IGV): The positions of interest can be visualized in the IGV genomic viewer by activating the Genomics Viewer button.
- Low Position filter: Positions with low coverage/quality and non-amplified positions can be displayed using the Low Position filter.
- Hom/Het filter: The positions shown in the analysis can be filtered per sample using a filter that allows selection based on whether the position is heterozygous or homozygous in that specific sample.

7. Once reviewed, click BACK button and then Continue.

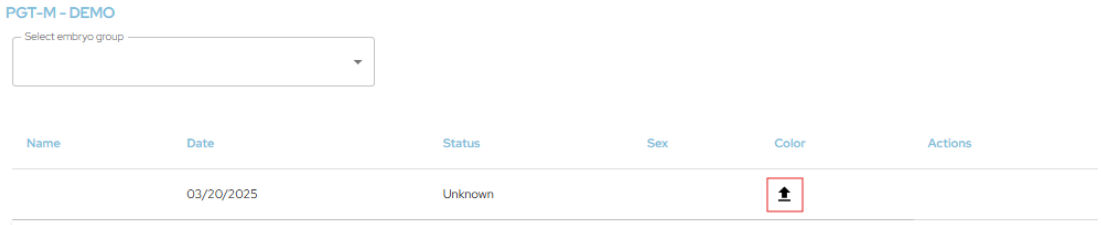
   **Optional**: To access the Technical Sheet of this analysis, you can select RESULTS button. Remember that the informativity test in that case will not be saved with an identification name unless clicking BACK button and then CONTINUE.

8. Write a name of the project and click Confirm to save the informativity analysis. If different informativity studies are performed for the same case, each informativity analysis should be saved with a different name; if only a few modifications are performed to the same samples, the same name can be used.


Once the informativity analysis is done and saved you can go to 'Launch a PGT-M Study' section to launch the PGT-M analysis or directly to 'Download a Results Report' section to download a results report.

## Launch a PGT-M Study

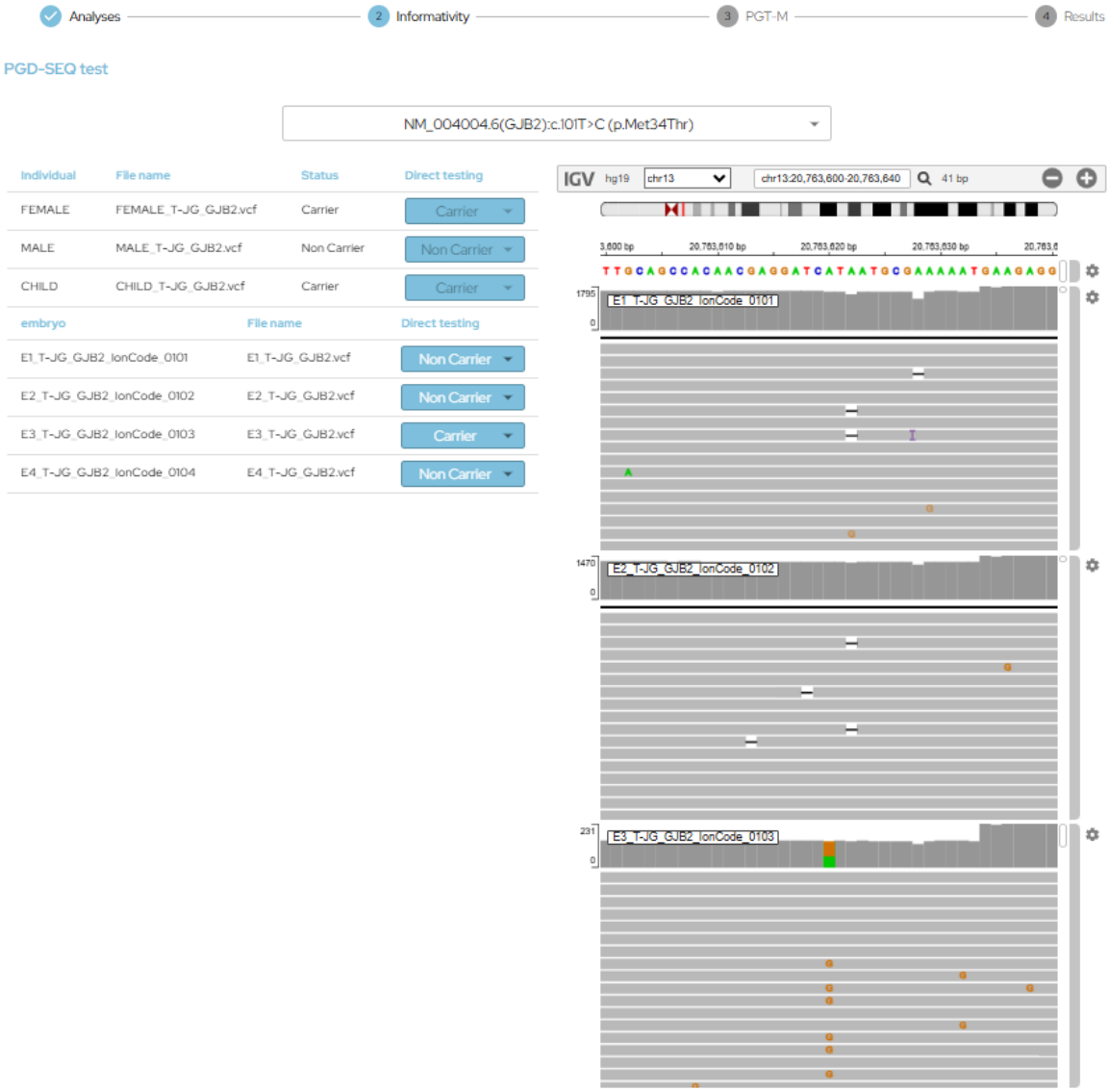
1. In the PGT-M step, upload such VCF files as embryos to be included in the analysis by clicking the arrow button.




2. To perform a direct testing analysis, upload the BAM/BAI file of each embryo sample in the BAM/BAI FILES section. The direct testing will be only available if any of the variants meets the required standards, as previously described. By clicking the Direct Analysis button, the genomic viewer IGV will appear in a new page. ***Performing direct analysis is high recommended as it directly affects the confidence score calculated by the software.***

   *** Note**: The Direct analysis button becomes active when a variant meets the standards (is smaller than 15 bp), indicating that direct testing is viable. **Important**: An active button does not guarantee that the variant is covered by the panel. To ensure direct testing is available for a concrete panel and revision, please, reach out to support@journeygenomics.com.

3. Direct Analysis page in PGT-M will include an additional table with the embryo samples. A dropdown associated with each variant will allow selecting the status analyzed by direct testing for each embryo sample. All included variants can be selected in the upper dropdown. Direct testing results for each sample should be selected in the associated dropdown.




4. Click Indirect analysis to begin processing the informativity study.
5. The informativity study can be reviewed in a new page.

## PGD-SEQ test

BACK RESULTS

ADO | Affected allele | Semi info | LR Likely recombinant
Incongruity | Non-carrier allele | Low quality/coverage

MOTHER INFO | NO INFO | ALLELE FREQUENCY | LOW POSITIONS
FATHER INFO | SEMI INFO | QUALITY/ COVERAGE | GENOMICS VIEWER

| | Individual | File name | Status | Variants | NOCALL % |
|---|---|---|---|---|---|
| ✓ | MALE | MALE_T-JG_GJB2_IonCode_0110.vcf | Carrier | Variante paterna: NM_004004.6(GJB2):c.35del (p.Gly12fs) | 14.88 % |
| ✓ | FEMALE | FEMALE_T-JG_GJB2_IonCode_0109.vcf | Carrier | NM_004004.6(GJB2):c.101T>C (p.Met34Thr) | 22.29 % |
| ✓ | CHILD | CHILD_T-JG_GJB2_IonCode_0111.vcf | Carrier | NM_004004.6(GJB2):c.101T>C (p.Met34Thr) | 40.23 % |

| | Sample Name | File name | Status | NOCALL % |
|---|---|---|---|---|
| ✓ | E1_T-JG_GJB2_IonCode_0101 | E1_T-JG_GJB2_IonCode_0101.vcf | Carrier from Father | 53.26 % |
| ✓ | E2_T-JG_GJB2_IonCode_0102 | E2_T-JG_GJB2_IonCode_0102.vcf | Non Carrier | 28.97 % |
| ✓ | E3_T-JG_GJB2_IonCode_0103 | E3_T-JG_GJB2_IonCode_0103.vcf | Carrier from Father | 27.7 % |
| ✓ | E4_T-JG_GJB2_IonCode_0104 | E4_T-JG_GJB2_IonCode_0104.vcf | Carrier from Mother | 42.1 % |

### Maternal Inheritance

| | E1_T-JG_GJB2_IonCode_0101 | E2_T-JG_GJB2_IonCode_0102 | E3_T-JG_GJB2_IonCode_0103 | E4_T-JG_GJB2_IonCode_0104 |
|---|---|---|---|---|
| Suggested status | Non-carrier allele | Non-carrier allele | Non-carrier allele | Maternal carrier |
| Above variant | 6 | 7 | 6 | 10 |
| Below variant | 6 | 14 | 13 | 15 |
| Confidence Level | Confidence Level: 7 ⓘ | Confidence Level: 7 ⓘ | Confidence Level: 7 ⓘ | Confidence Level: 7 ⓘ |
| Validate | Validate | Validate | Validate | Validate |

### Paternal Inheritance

| | E1_T-JG_GJB2_IonCode_0101 | E2_T-JG_GJB2_IonCode_0102 | E3_T-JG_GJB2_IonCode_0103 | E4_T-JG_GJB2_IonCode_0104 |
|---|---|---|---|---|
| Suggested status | Paternal carrier | Non-carrier allele | Paternal carrier | Non-carrier allele |
| Above variant | 1 | 2 | 2 | 2 |
| Below variant | 16 | 19 | 20 | 18 |
| Confidence Level | Confidence Level: 4 ⓘ | Confidence Level: 4 ⓘ | Confidence Level: 4 ⓘ | Confidence Level: 4 ⓘ |
| validate | Validate | Validate | Validate | Validate |

| | | | Female | | Male | | Child | | E1 T-JG GJB2 IonCode 0101 | | E2 T-JG GJB2 IonCode 0102 | | E3 T-JG GJB2 IonCode 0103 | | E4 T-JG GJB2 IonCode 0104 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | OFF | | OFF | | OFF | | OFF | | OFF | | OFF | | OFF | |
| | Chrom | Position | P1 | P2 | P1 | P2 | P1 | P2 | P1 | P2 | P1 | P2 | P1 | P2 | P1 | P2 |
| ☐ | chr13 | 19322538 | C | A | C | C | C | C | A | C | A | C | A | C | C | C |
| ☐ | chr13 | 19322556 | C | C | A | C | C | A | C | C | C | A | C | C | C | A |
| ☐ | chr13 | 19459139 | C | T | C | C | C | C | T | C | T | C | T | C | C | C |
| ☐ | chr13 | 19463387 | C | C | C | G | C | C | C | G | C | C | C | C | C | C |
| ☐ | chr13 | 19641101 | G | A | G | G | G | G | A | G | A | G | - | - | G | G |
| ☐ | chr13 | 19708605 | T | G | G | G | T | G | G | G | G | G | G | G | T | G |
| ☐ | chr13 | 19747689 | A | G | G | G | A | G | G | G | G | G | G | G | A | G |
| ☐ | chr13 | 19747750 | T | C | C | C | T | C | C | C | C | C | C | C | T | C |
| ☐ | chr13 | 19811678 | G | A | A | A | G | A | A | A | A | A | A | A | G | A |
| ☐ | chr13 | 20495152 | G | A | G | G | G | G | A | G | A | G | A | G | G | G |
| ☐ | chr13 | 20495160 | T | C | C | C | T | C | C | C | C | C | C | C | T | C |
| ☐ | chr13 | 20687836 | C | T | C | C | C | C | T | C | T | C | T | C | C | C |
| ☐ | chr13 | 20687860 | A | G | A | A | A | A | G | A | G | A | G | A | A | A |
| ☐ | chr13 | 20688094 | A | C | C | C | A | C | C | C | C | C | C | C | A | C |
| ☐ | chr13 | 20688103 | C | T | T | T | C | T | T | T | T | T | T | T | C | T |
| ☐ | chr13 | 20688150 | G | A | A | A | G | A | A | A | A | A | A | A | G | A |
| ☐ | chr13 | 20705231 | A | A | A | G | A | A | - | - | A | A | A | G | - | - |
| ☐ | chr13 | 20706747 | G | A | A | A | G | A | A | A | A | A | A | A | G | A |



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Results are displayed in tables for each maternal and paternal inheritances. Based on the number of KP for the inherited allele by the embryo on each side of the variant and viable direct testing, a Confidence Score is determined.

*** Note**: The number of KP will always be displayed, but the confidence scored will only be determined when genetic information is filled in the corresponding section when creating a new project.

**Optional:** The visualization of analysis results offers several optional features to customize the display and help the data analysis, including:

- Non-informative and semi-informative SNPs: These positions can be displayed by clicking the No info and Semi info buttons, respectively.
- Quality and Coverage: The quality and coverage values of each analysed SNP can be shown by clicking the Quality/Coverage button.
- Allele Frequency: This value can be shown by clicking the Allele Frequency button.
- Genomics Viewer (IGV): The positions of interest can be visualized in the IGV genomic viewer by activating the Genomics Viewer button.
- Low Position filter: Positions with low coverage/quality and non-amplified positions can be displayed using the Low Position filter.
- Hom/Het filter: The positions shown in the analysis can be filtered per sample using a filter that allows selection based on whether the position is heterozygous or homozygous in that specific sample.

6. Once reviewed, click BACK button and then Continue.

   **Optional**: To access the Technical Sheet of this analysis, you can select RESULTS button. Remember that the PGT-M test in that case will not be saved with an identification name unless clicking BACK button and then CONTINUE.

7. Write the name of the project and click Confirm to save the PGT-M analysis. If different PGT-M studies are performed for the same case, each PGT-M analysis should be saved with a different name; if only a few modifications are performed to the same samples, the same name can be used.

Once the PGT-M analysis is done and saved, you can download a complete report.

## Download a Results Report

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

- To download an informativity report, perform steps in 'Launch an Informativity Study' section and go directly to the step 4 of the analysis without introducing embryos' data. Here a preview of the final report is shown in order to confirm the information. To download the report, press Download Results button on the upper-left side of the window.
- To download a PGT-M report, perform steps in 'Launch an Informativity Study' and 'Launch a PGT-M Study' sections. On the step 4 of the analysis a preview of the final report is shown in order 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. Please send all this information to support@journeygenomics.com before starting to use the software.

## Download a Technical Sheet

After informativity/PGT-M study has been performed an independent technical sheet can be downloaded. This document will include the different tables with the indirect analysis and direct testing results. Both informativity and PGT-M technical sheets can be downloaded by following the next steps:

- To download an informativity technical sheet, perform steps in 'Launch an Informativity Study' section and press Results button after indirect analysis without introducing embryos' data. Here a preview of the technical sheet is shown in order to confirm the information. Take into account that only the validated tables will be included in the document. To download the technical sheet, press Download Results button on the upper-left side of the window.
- To download a PGT-M technical sheet, perform steps in 'Launch an Informativity Study' and 'Launch a PGT-M study' sections. After performing indirect testing on PGT-M section, press Results button. Here a preview of the technical sheet is shown in order to confirm the information. Take into account that only the validated tables will be included in the document. To download the technical sheet, press Download Results button on the upper-left side of the window.

*** Note**: Each result obtained in the direct analysis and indirect analysis must be validated in order to be included in the Technical Sheet.

# 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 this position is not present, 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.
- We highly recommend to introduce genetic information for a more accurate analysis.

# Appendix A

## Selecting samples for PGT-M

Sample selection must be performed before processing samples. As a general recommendation, the ideal scenario is to include the couple seeking PGT-M, and a previous child (whether affected, carrier *, or non-carrier) or the parents of the couple. This combination allows for the most informative analysis of inheritance patterns. However, when this ideal scenario is not feasible, we propose the following minimum sample requirements, distinguishing between the three Mendelian inheritance patterns: autosomal recessive, autosomal dominant and X-linked inheritances.

A critical consideration in sample selection for PGT-M studies is ensuring that each sample has undergone prior genetic testing to determine its status (non-carrier, carrier, or affected). This pre-screening is essential for the correct interpretation of the linkage analysis results.

When using an unaffected family member for linkage analysis, it's essential to rule out the possibility that the variant is *de novo* in the IVF couple. If the variant originated spontaneously in one of the parents, it would not be linked to an inherited haplotype in the unaffected family members, potentially leading to misleading results.

**Autosomal dominant inheritance**:

- The couple.
- At least one parent of the affected individual (proband).

This combination allows for the identification of the disease-associated haplotype in the affected individual and its transmission pattern.

**Autosomal recessive inheritance**:

- The couple
- At least one parent of the carrier member of the couple. If both members are carriers, at least one parent from each side of the couple.

This setup enables the tracing of carrier haplotypes from both parental lineages, crucial for identifying compound heterozygosity or homozygosity in affected offspring.

**X-linked inheritance**:

- The couple.
- One parent of the carrier female.

This combination allows tracking of the X-linked haplotype through the maternal line.

*** Note**: In autosomal recessive inheritance, if both parents are carriers of the same variant, a carrier child cannot be used to perform linkage analysis. Only affected or non-carrier individuals provide unambiguous information.

## Special cases

*Informativity with a child belonging to only one member of the couple*

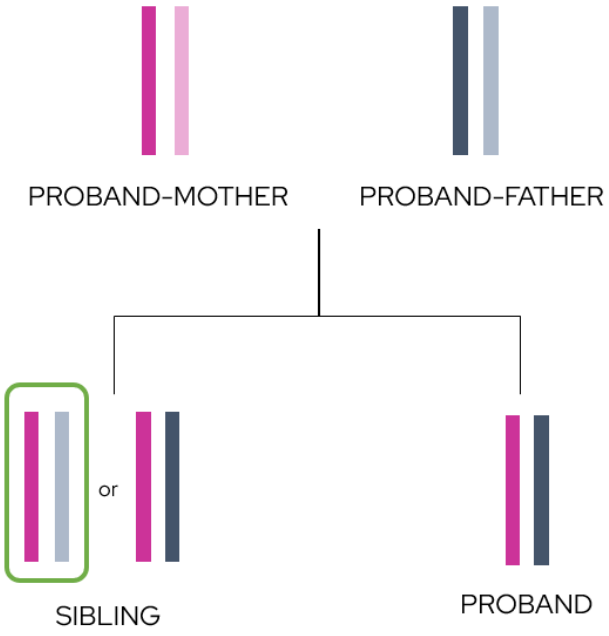
In cases where the available family member is a child only belonging to one member of the couple, this sample cannot be used as a child sample for autosomal inheritance because the allelic data from such a sample does not meet the criteria for a trio-based analysis with the couple in question.

The rationale for this approach is based on allelic sharing; the child has inherited a haplotype from the proband but shares no genetic material with the proband's partner. This single line of inheritance means the child's data can only be used to confirm the set of alleles inherited from the proband. From an analytical point, this functional role is equivalent to that of a parental sample for the proband, so this sample can be included with that role.

An exception to this rule occurs in the analysis of X-linked inheritance involving a male child. In such cases, the sample can be used as a standard child sample because the male is hemizygous for the X chromosome, possessing only a single X chromosome that is entirely inherited from the mother.

*Informativity with individual siblings*

When utilizing a carrier/affected sibling sample for familial analysis, it is important to take into account that this type of sample has 50% chance of being informative. This occurs if the sibling has inherited either the same two parental haplotypes as the proband, therefore, it is impossible to reliably track the co-inheritance of specific alleles, thus preventing the assignment of the pathogenic variant to the correct parental chromosome.




An exception to this rule occurs in the analysis of X-linked inheritance involving an affected brother. In such cases, the sample can be used as a female-father sample because the male is hemizygous for the X chromosome, possessing only a single X chromosome that is entirely shared with the proband.

*Informativity without family members (direct testing only)*

This analytical approach applies only to variants that meet the requirements to be suitable for direct testing: they must be SNPs or indels smaller than 15 bp. A minimum of six embryo samples is required to reduce the risk

of diagnostic errors derived from Allele Dropout (ADO). Following biopsy processing, direct variant analysis is conducted on all samples (see *Direct testing of embryo samples* on page 18). Based on these results, one embryo is selected to serve as an internal reference, analogous to a child sample, for the subsequent linkage analysis.

It is important to note that a higher frequency of genotypic incongruities between markers is often observed in embryo samples. Therefore, it is strongly recommended to select a carrier/affected embryo as the reference, as an apparently non-carrier embryo may be a misdiagnosed carrier due to ADO of the pathogenic allele.

Additionally, in cases of autosomal recessive inheritance where both partners are carriers of the identical variant, a carrier (heterozygous) embryo cannot be used as a reference sample. This is because the parental origin of the pathogenic allele in the embryo cannot be determined by direct testing, which prevents the accurate phasing of the disease-linked haplotype.