BRCA 1 & BRCA 2

Instructions for Use

Version 01
REF: BRCA1-HYB-001-RU / BRCA2-HYB-001-RU
For Research Use Only

August 2016
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1. BACKGROUND INFORMATION

Hereditary breast and ovarian cancer (HBOC) is an autosomal dominant cancer predisposition syndrome caused by germline BRCA1 or BRCA2 mutations. A mutation in one of these two highly penetrant genes confers a 10 to 20 times increased relative risk of developing a breast cancer compared to the general female population, translating into 70 – 80% risk of developing breast cancer and a 39-40% risk to develop ovarian cancer by age 70 [1,2]. Similarly, male BRCA1 mutation carriers have a cumulative breast cancer risk of 1.2% by age 70 [3]. 10 to 15% of the hereditary breast and ovarian cancer cases are imputable to large rearrangements, including deletions and duplications of large genomic regions, mainly in BRCA1 but also in BRCA2 genes. Molecular Combing has been successfully employed to detect large rearrangements in BRCA1 and BRCA2 [4-9].

2. INTENDED USE

The FiberProbes® BRCA1 & BRCA2 are designed to detect and characterize large rearrangements in the BRCA1 and BRCA2 genes by fluorescent hybridization on combed DNA extracted from blood samples and prepared according to the Molecular Combing procedures [10-14]. The FiberProbes® BRCA1 & BRCA2 are intended to be used for Research Use Only.

Important note: In addition to the FiberProbes® BRCA1 & BRCA2, the use of the FiberVision® scanner and the FiberStudio® software is highly recommended for the image acquisition and for detection, review and analysis of the hybridized FiberProbes® BRCA1 & BRCA2 signals, respectively (see the Instructions for Use for FiberVision® scanner and FiberStudio® software).
3. PRINCIPLE OF THE BRCA ASSAY

Molecular Combing allows the direct visualization and analysis of single DNA molecules. It enables DNA (extracted from blood samples for the BRCA assay: Figure 1, step 1) to be stretched uniformly and irreversibly attached to a treated glass coverslip (Figure 1, step 2). The presence of specific genomic sequences (BRCA1 and BRCA2 genes in the test material) can be detected on stretched DNA by hybridization with labeled polynucleotides (FiberProbes® BRCA1 & BRCA2: Figure 1, step 3) and visualized by fluorescence microscopy, ideally with the FiberVision® scanner (Figure 1, step 4), as it is described in the corresponding Instructions for Use provided by Genomic Vision. The constant stretching factor allows the direct measurement of the probe length and the physical cartography of the region of interest. For the BRCA assay, the characterization of the BRCA1 and BRCA2 genes in the test material is ideally determined with the FiberStudio® software (Figure 1, step 5) as described in the corresponding Instructions for Use provided by Genomic Vision.
4. PRODUCT CONTENT AND DESCRIPTION

The FiberProbes® BRCA1 & BRCA2 set contains 5 x 10 µl per vial for BRCA1 probes and 5 x 10 µl per vial for BRCA2 probes (2 tests per vial, 10 tests in total). BRCA1 and BRCA2 probes must be mixed before use. One test is defined as sufficient for one hybridization with combined BRCA1 and BRCA2 probes of combed DNA onto coverslips (22 x 22 mm area).

The FiberProbes® BRCA1 & BRCA2 contain fluorescein-, digoxygenin- and biotin-labeled polynucleotides that are intended to be used in combination with specific reagents (see Reagents and materials recommended but not provided) that allow their detection in green, blue and red fluorescent signals.

Labeled polynucleotides are premixed with blocking DNA in formamide-free hybridization buffer (Saline-Sodium Citrate (SSC), Sodium Dodecyl Sulfate (SDS), Sodium Lauroyl Sarcosinate and BlockingAid™ blocking solution).

5. STORAGE AND HANDLING

Upon arrival, the product must be stored between -25°C and -10°C, protected from the light until the expiry date printed on the label. Improper storage of the product can destroy or impair the performance of the product and consequently the assay should not be performed from such reagent since it may affect the result of the assay.

Handle all reagents and slides containing fluorophores in reduced light to prevent photobleaching.

Once thawed and prior to opening, the vials should be briefly centrifuged to ensure the contents are collected at the bottom of the vials.

Once opened, the remaining content of the vial can be frozen again and stored between -25°C and -10°C up to 6 months.
6. WARNINGS AND PRECAUTIONS

For Research Use Only. For professional use only.
Carefully read the instructions before use.
Do not use the product after the expiry date.

Hybridization of FiberProbes® BRCA1 & BRCA2 should be ideally performed using coverslips with very long genomic DNA molecules linearly stretched at high density (Figure 2, pictures 4 and 5) extracted from fresh blood samples. Using coverslips with genomic DNA at lower density (Figure 2, pictures 1-3) or short, wavy or tangled DNA molecules may affect the result of the test.

Figure 2 – Density of combed genomic DNA

As some substances contained in this product (in low concentrations and volumes) could be harmful for health, handle the reagent with care and wear appropriate personal protective equipment. See the Material Safety Data Sheet (MSDS) for safety information.

6.1. Reagents and materials recommended but not provided for the hybridization and the detection of the “FiberProbes® BRCA1 & BRCA2”

IMPORTANT: Since the FiberProbes® BRCA1 & BRCA2 have been evaluated and validated using the reagents and materials listed below, we recommend the use of these referenced reagents and materials for an optimal result.

The FiberProbes® BRCA1 & BRCA2 were validated with the following accompanying reagents and materials for their detection as green, red, and blue fluorescent signals:
- Green fluorescent signal:
  o 0.5 mg Cy™3 IgG Fraction Monoclonal Mouse Anti-Fluorescein (FITC) (Jackson Immunoresearch, Ref: 200-162-037). To be reconstituted (see reagent preparation).
- Red fluorescent signal:
  - 0.1 mg/ml BD Horizon™ BV480 Streptavidin (Becton Dickinson, Ref: S 564876). Ready-to-use
- Blue fluorescent signal:
  - 0.5 mg Alexa Fluor® 647 IgG Fraction Monoclonal Mouse Anti-Digoxin (Jackson Immunoresearch, Ref: 200-602-156). To be reconstituted (see reagent preparation).
- CombiCoverslips™ (Genomic Vision, Ref: COV-001).
- FiberVision® scanner (Genomic Vision, Ref: SCN-001)
- FiberStudio® software (Genomic Vision, Ref: STW-001)

Other reagents and materials are:
- Autoclaved distilled water
- Deionized formamide
- 20X SSC
- 70%, 90% and 100% ethanol
- BlockAid™ blocking solution
- Tween® 20
- 1X PBS
- Variable micropipette (1 µl - 200 µl)
- Tweezers
- Microscope slide
- Co-denaturation and hybridization instrument (e.g. Hybridizer, Dako)
- Ceramic coverslip tray
- Humidified chamber
- Incubator at 37°C
- 250 ml-beaker

6.2. Preparation of the reagents for the hybridization and the detection of the FiberProbes® BRCA1 & BRCA2

*IMPORTANT: Use autoclaved distilled water for preparation of all stock and working solution.*

- Hybridization Washing Buffer (2X SSC solution): mix 100 ml of 20X SSC and 900 ml of distilled water. Store at RT.
  *Note: The buffer must be warmed at 60°C prior to use.*
- Detection Washing Buffer (2X SSC/1% Tween): Mix 100 ml of 20X SSC and 10 ml of Tween® 20 with 890 ml of distilled water. To be prepared extemporaneously.
- Reconstitute the lyophilized reagents with autoclaved distilled water as follows:

<table>
<thead>
<tr>
<th></th>
<th>Quantity (mg)</th>
<th>Volume of distilled water to be added (ml)</th>
<th>Final concentration (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG Fraction Monoclonal Mouse Anti-Fluorescin (FITC)</td>
<td>0.5</td>
<td>0.35</td>
<td>1.43</td>
</tr>
<tr>
<td>Alexa Fluor® 647 IgG Fraction Monoclonal Mouse Anti-Digoxin</td>
<td>0.5</td>
<td>0.35</td>
<td>1.43</td>
</tr>
</tbody>
</table>

Prepare extemporaneously the mix of the detecting solution as follows (for one test):

<table>
<thead>
<tr>
<th>Volume (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>- 1.43 mg/ml Cy™3 IgG Fraction Monoclonal Mouse Anti-Fluorescin</td>
</tr>
<tr>
<td>- 0.1 mg/ml BD Horizon™ BV480 Streptavidin</td>
</tr>
<tr>
<td>- 1.43 mg/ml Alexa Fluor® 647 IgG Fraction Monoclonal Mouse Anti-Blocking™ Aid Solution</td>
</tr>
</tbody>
</table>

7. DETAILED PROCEDURE

7.1. Hybridization of the FiberProbes® BRCA1 & BRCA2 on combed DNA

IMPORTANT: all the steps where formamide is used should be performed under a fume hood.

1. Thaw the vial containing the “FiberProbes® BRCA1”, “FiberProbes® BRCA2” and the CombiCoverslips® with combed DNA on a ceramic coverslip tray for 10 min at room temperature.
2. Dehydrate the combed DNA CombiCoverslips® by dipping the ceramic coverslip tray 3 min at room temperature in successive baths of 70%, 90% and 100% ethanol.
3. Air dry the coverslips at room temperature for 10 min protecting from light.
4. For one test, transfer 5µl of the "FiberProbes® BRCA1“ and 5 µl of the "FiberProbes® BRCA2“ in a new microcentrifuge tube and add 10 µl of deionized formamide to the mix of FiberProbes® BRCA1 & BRCA2.
5. Mix well and incubate at 37°C for 30 min
6. Pipette 20 µl of the FiberProbes® BRCA1 & BRCA2/formamide mix on a microscope slide.
7. Avoiding trapped bubbles, set the coverslip on the drop of hybridization solution.
   Note: Carefully indicate the side of the CombiCoverslip® in contact with the hybridization solution to avoid confusion in the subsequent steps.
   Note: Lay down the CombiCoverslip® carefully using tweezers to avoid bubbles. Do not push and slide the CombiCoverslip™ once mounted, it will cause scratches on combed CombiCoverslip™. Adjust the position of CombiCoverslip™ by gentle touching the corner if needed.
   Note: Protect the hybridized CombiCoverslip™ from light from this step.
8. Co-denature the combed DNA on the CombiCoverslip™ and the FiberProbes® BRCA1 & BRCA2/formamide mix for 5 min at 90°C in the humidified chamber of a hands-free co-denaturation and hybridization instrument.
9. Incubate for 16-20 hrs at 37°C in the humidified chamber of the co-denaturation and hybridization instrument.
10. Remove the hybridized CombiCoverslip™ from the microscope slide and place on a ceramic coverslip tray in a 250 ml-beaker containing the pre-warmed (60°C) Hybridization Washing Buffer.
   Note: if the hybridized CombiCoverslip™ is stuck to the slide, add a drop of Hybridization Washing Buffer around the CombiCoverslip™ and wait until the CombiCoverslip™ floats. When removing CombiCoverslip™, slide its gently until one of corner is out on slide to avoid scratches, then gently “peel off” the CombiCoverslip™ with tweezers.
11. Wash the hybridized CombiCoverslip™ three times in pre-warmed (60°C) Hybridization Washing Buffer for 5 min each at 60°C.
   Note: Do not let the hybridized CombiCoverslip™ dry. It must be totally immersed in the solution.

7.2. Detection of the FiberProbes® BRCA1 & BRCA2

1. Pipette 20 µl of the layer of detecting solution on a microscope slide and set the hybridized CombiCoverslip™ on the drop of detecting solution.
   Note: Make sure to place the hybridized side of the CombiCoverslip™ in contact with the detecting solution.
2. Incubate the CombiCoverslip™ on the microscope slide for 20 min in a humidified chamber at 37 °C.
3. Remove the CombiCoverslip™ from the microscope slide and place it on a ceramic coverslip tray in a 250 ml-beaker containing the Detection Washing Buffer.

4. Wash the CombiCoverslip™ three times in the Detection Washing Buffer for 3 min each at room temperature with gentle agitation.

5. Wash the CombiCoverslip™ in 1xPBS for 3 min at room temperature with gentle agitation.

6. Dehydrate the CombiCoverslip™ by dipping the ceramic coverslip tray 3 min in successive baths of 70%, 90% and 100% ethanol.

7. Air dry the CombiCoverslip™ at room temperature for 10 min protecting from light.

8. The CombiCoverslip™ can be stored at 4°C protecting from light until observation.

7.3. Image acquisition of the fluorescent signals

For the BRCA assay, the acquisition of images of the hybridized coverslips must be ideally performed with the FiberVision® scanner, an automated image scanning system from Genomic Vision, equipped with a 40X objective and the following filters:

<table>
<thead>
<tr>
<th>Fluorophore</th>
<th>Excitation</th>
<th>Emission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green</td>
<td>543 ± 3 nm</td>
<td>588 ± 55 nm</td>
</tr>
<tr>
<td>Red</td>
<td>434 ± 17 nm</td>
<td>515 ± 23 nm</td>
</tr>
<tr>
<td>Blue</td>
<td>640 ± 14 nm</td>
<td>700 ± 35 nm</td>
</tr>
</tbody>
</table>

The hybridization signals appear as specific multicolor fluorescent signal arrays also called Genomic Morse Code (GMC):

- The complete BRCA1 GMC covers a genomic region of 195 kb and is composed of 16 signals of a distinct color (green, red, or blue). Each signal is composed of one or two single DNA probes.
- The complete BRCA2 GMC covers a genomic region of 144 kb and is composed of 13 signals of a distinct color (green, red, or blue). Each signal is composed of 2 to 4 single DNA probes.

7.4. Review of the BRCA1 and BRCA2 fluorescent signals

The FiberStudio® Software is dedicated to the analysis of Molecular Combing results (see Instructions for Use of the FiberStudio® Software). This software is designed to perform the detection, signal measurements, review and analysis of hybridization ROIs (region of interest) on combed DNA in a streamlined, computer-assisted process. Using raw images generated by the FiberVision® scanner and fed by an image server, it takes the user all the ways for the edition of a report displaying statistical information useful for analyzing the presence of large rearrangements in the BRCA1 and BRCA2 genes.
This chapter presents the instructions for the review of images generated after hybridization of the FiberProbes® BRCA1 & BRCA2. It describes signal interpretation rules enabling the constitution of a dataset compatible with large rearrangement detection in genes related to breast and ovarian hereditary cancer (i.e., BRCA1 and BRCA2).

Lexicography:

- **Signal**: Fluorescent marker of one color generated by probe hybridization and immunodetection using antibodies coupled to fluorochromes.
- **ROI**: A sequence of signals detected by the FiberStudio® software.
- **Gap**: A space between two fluorescent signals.
- **Segment**: A measurable fluorescent signal.
- **Specific signal**: A fluorescent signal corresponding to intended hybridization, as opposed to fluorescence caused by experimental noise.
- **Specific ROI**: A ROI containing specific signals.
- **Pre-report**: Intermediary interface page in FiberStudio® where the user selects ROI images to appear in the final PDF report.

7.4.1. Introduction to review

**Important**: Before proceeding with the complete review, it is highly recommended to investigate the first 20 ROIs to determine whether a second scan of the coverslip is necessary (for example, in case of out-of-focus images).

Once the scan quality has been validated, the complete set of ROIs should be reviewed without modifying the zoom level.

The review of signals automatically detected and measured consists in:

- The validation or discard of ROIs.
- The correction of measure tab positions and signal color labels.

The protocol scheme for manual review is presented in Figure 5.

A ROI is validated when it is composed of at least 3 specific and distinct signals. In contrast, ROIs that are either composed of either non-specific signals, or less than 3 distinct signals are discarded. Section **ROI validation and discard** describes and illustrates the rules for assessing ROI quality.

All specific signals of validated ROIs should be measured and associated to a label (see Sections **Signal measurements** and **Signal annotation** for further details).
7.4.2. Rules for review

7.4.2.1. ROI validation and discard

ROIs corresponding to one of the following categories are considered of validated signals (see example 1):
- ROIs containing at least 3 signals of red, blue and/or green colors (do not validate ROIs only composed of signals with mixed colors, i.e., cyan, magenta, yellow or white)
- Blurred ROIs
- U-shaped ROIs, only when the branches do not overlap
- ROIs containing sparse but aligned signals.

All ROIs that do not fill these requirements should be discarded (see example 2 for illustrations of such ROIs).
When a ROI is automatically detected as 2 distinct ROIs, please proceed with the following protocol:
- Extend the 1st ROI until it covers all specific signals
- Discard the 2nd ROI

**Example 1: ROIs to validate**

<table>
<thead>
<tr>
<th>Blurred ROIs</th>
<th>ROIs with at least 3 distinct signals</th>
<th>ROIs with sparse signals</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Blurred ROIs" /></td>
<td><img src="image2" alt="ROIs with at least 3 distinct signals" /></td>
<td><img src="image3" alt="ROIs with sparse signals" /></td>
</tr>
</tbody>
</table>
### Instructions for Use

**BRCA1 & BRCA2**

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**Example 2: ROIs to discard**

<table>
<thead>
<tr>
<th>ROIs with non-specific signals</th>
<th><img src="image1.png" alt="Example image 1" /></th>
</tr>
</thead>
<tbody>
<tr>
<td>Noisy or beaded ROIs</td>
<td><img src="image2.png" alt="Example image 2" /></td>
</tr>
<tr>
<td>ROIs with overlapping U-branches or with not aligned signals</td>
<td><img src="image3.png" alt="Example image 3" /></td>
</tr>
</tbody>
</table>

On these images, the overhead ROI is the one that should be validated.
7.4.2.2. Signal measurements

A measurement is obtained by placing tabs at the beginning and at the end of the signals of interest. To modify the positions of the tabs, please refer to the Instructions for Use of the FiberStudio® software. The rules to decide which signal has to be measured are the following:

- Every segment, i.e., signal corresponding to one of the following definitions:
  - A continuous fluorescent line (see example 3),
  - A set of fluorescent dots not separated by a complete disappearance of fluorescent signal (see example 4),
  - A set of at least 3 fluorescent dots, aligned and close to each other (see example 5).
- Dots extending a segment:
  - These dots are located next to a segment with the same color (see example 6). They must be included into the segment measurement.
  - When the dots and the segment of the same color are separated by another dot or signal of a different color, none of the dots should be measured (see example 7).
- Other types of signals are ignored (isolated dots, sets of only 2 dots... see examples 8 et 9).

**Example 3:**

Continuous fluorescent lines: to measure

**Example 4:**

Set of dots non separated by fluorescence disappearance: To measure
Example 5:

At least 3 close and aligned dots: to measure

Example 6:

Red dot and red segment separated by a green dot: do not measure red and green dots

Example 7:

Red dot extending a red segment: include in segment measurement

Example 8:

Isolated dots: do not measure

Example 9:

Dot within a blue segment: do not measure

It’s possible to modify tab baseline in order to align the maximum number of segments (the segments should be aligned onto the baseline, not above or under). When the position of the tabs have to be modified, they should be placed as close to segments as possible. All segments are measured.

To assign tabs (add, delete, modify), please refer to the Instructions for Use of the FiberStudio® software.

7.4.2.3. Signal annotation

Each signal has to be annotated with a label. The rules for label annotation are the follows:
- Red, blue and green segments are associated to the corresponding color labels ("R" for red, "B" for blue, "G" for green).
- Gaps (i.e., non-fluorescent measurements) are associated to the label ".".
- Segments of mixed colors (i.e., cyan, magenta, yellow and white) are associated to the label "." (see example 10).

**Example 10:**

![Image showing magenta and cyan signals labeled as gaps](image)

Magenta and cyan signals labelled as gaps

To annotate the label (add, delete, modify), please refer to the Instructions for Use of the FiberStudio® software.

Once the review is completed, it needs to be validated (either by the user, or by an independent validator). After every validated review, the automatic analysis for the detection of anomalies in BRCA1 or BRCA2 gene can be directly launched (see section 7.5). The final results are summarized in the PDF file report. The raw data of the review are also automatically saved in Excel file on the FiberStudio® server for other analysis.

7.5. **Data analysis**

Once the review is completed, it needs to be validated (either by the user, or by an independent validator). After every validated review, the automatic analysis for the detection of anomalies in BRCA1 or BRCA2 gene can be directly launched (see section 7.5.1). The final results are summarized in the PDF file report. The raw data of the review are also automatically saved in Excel file on the FiberStudio® server for other analysis (see section 7.5.2).

7.5.1. **Report description**

This chapter describes the contents of the report files and gives guidelines and recommendations for results interpretation.

Once the automatic analysis of the dataset is launched, a first interface page appears with partial analysis information and a complete list of ROI images, ordered according to analysis
results (see section ROI images). The user selects a subset of ROIs images to be in the final report and then launch the report generation.

An example of FiberStudio® BRCA PDF report is shown in Figure 6. It is structured in different blocks, each described in details below.

Figure 6- Example of BRCA report in FiberStudio®

7.5.2. PDF report description

7.5.2.1. General information
This block displays the following pieces of information:

1. The date
2. The patient ID
3. The numbers of all coverslips used for the report creation
4. The names of the users that worked on the manual review of the coverslips used to create the report
5. The names of the users that validated the manual review of the coverslips used to create the report
6. The name of the user that updated the pre-report and saved the PDF report creation

7.5.2.2. Summary of anomaly detection

An example for the block “Summary of anomaly detection” is displayed in Figure 8.
Figure 8 - Example of block “Summary of anomaly detection”

This block summarizes the data set and the results of anomaly detection algorithm:

1. The GMCs of BRCA1 and BRCA2 regions are presented with exon positions. The parts of the GMC that do not reach a sufficient coverage (see Section Erreur ! Source du renvoi introuvable.) are highlighted in gray.

2. A summary of detection results with all the statistically significant detections (see Section Erreur ! Source du renvoi introuvable. for definition of statistically significant). For each GMC, when no significant detection has been found, a resolution value is shown, computed only for GMC segments and gaps sufficiently covered.

Recommendations:

In case some parts of the GMCs are not sufficiently covered (e.g., gray areas of GMCs in Figure 8), it is recommended to increase the data set either by either a user detection of the ROI that may have been missed by the software (for user selection of ROI please refer to the instructions for use of FiberStudio® software) or by processing a new coverslip and integrating it to the existing data set.

7.5.2.3. Description of data

An example of the block “Description of data” is displayed in Figure 9. It contains information about data coverage:

Figure 9 - Example of block “Description of data”

1. The numbers of detected and validated ROIs for each GMC.
2. The number of measurements (coverage) for every segment of each GMC. The value is highlighted in green when the coverage is above 50, in red otherwise.

7.5.2.4. Details of anomaly detection

The blocks of "Details of anomaly detection" display the results of anomaly detection, performed independently for each BRCA gene. They provide additional information about all anomaly detections, whether they are statistically significant or not. At least one block is displayed per gene, the total number of blocks depending on the number of anomalies detected. Three types of block can be displayed:

- No anomaly
- Breakpoint
- Bimodality

*No anomaly block*

When no anomaly has been detected, a block such as the example given in Figure 10 is displayed.
The following details are given:

1. GMC of interest
2. Resolution of the anomaly analysis:
   Depending on the number of segment measurements and their variability, an anomaly resolution in kb (kilo base pair) is computed for the GMC part that comprises exons 1 to 15 of BRCA2 gene. Another resolution is computed for the GMC part that comprises exons 16 to 27 of BRCA2 gene and its upstream region.

For BRCA1, two resolutions are also displayed, one for the BRCA1 gene and one for its upstream region (see Figure 11).
Figure 11 - Resolution split for BRCA1 in case no anomaly is found

Unlike the resolution values described in the Section *Summary of anomaly detection*, these values are computed over all segments, whether they are sufficiently covered or not. A value “Inf” is returned when data coverage is not sufficient to detect anomalies with sensitivity higher than 90%.

3. GMC graphic with exon positions as well as segment and gap labels
4. Histogram of percentages of ROI endings:
   Each bar of the histogram represents, for all the ROIs containing at least one of the segments specified in the x-axis, the percentage of these ROIs ending in between the segments.

*Bimodality block*

For each bimodality detected on measurements of a GMC segment or gap, a block such as the example shown in Figure 12 is displayed.
Figure 12 - Example of block “Details of anomaly detection” in case of bimodality detection

It contains information such as:

1. GMC of interest
2. Characteristics of detected bimodality:
   - Segment which length measurements contain abnormal values
   - Anomaly size, estimated as the difference between the means of abnormal and normal length measurements.
   - Confidence percentage of the anomaly presence
3. GMC graphic with exon positions as well as segment and gap labels. The segment concerned by the anomaly is framed in black when the anomaly is significantly detected (see Section Erreur! Source du renvoi introuvable.) and in grey otherwise. In the case where the anomaly is not significantly detected, a sentence is printed below the GMC explaining the reason why the detection is not significant (either a lack of data or an anomaly size below resolution).
4. Histogram of segment length measurements:
The green bars correspond to the measurements with expected lengths (i.e., belonging to the normal group of ROIs), the red bars to the measurements with abnormal length (i.e., belonging to the abnormal group of ROIs).

5. Information about the two groups of ROIs
For each group, the number of ROIs is specified as well as the mean and standard deviation of the segment measurements. The p-value from the t-test on the equality between the measurement means of the two groups is also specified.

**Breakpoint block**

When at least one breakpoint is detected, a block such as the example shown in Figure 13 is displayed.

---

**Figure 13** - Example of block "Details of anomaly detection" in case of breakpoint detection
The following details are given:

1. GMC of interest
2. Information about the breakpoint detected:
   - Segments impacted by the anomaly: When one breakpoint is detected, the impacted segments are surrounding the breakpoint; When several breakpoints are detected, the impacted segments are the segments surrounding and in-between the breakpoints.
   - Size of anomaly, null in case of one breakpoint detected and equal to the length of the GMC corresponding to the impacted segments in case of several breakpoint detections.
   - Confidence percentage of the anomaly presence
3. GMC graphic with exon positions as well as segment and gap labels.
   The positions of the breakpoints are shown on the GMC with lightning bolts, black when the anomaly is significantly detected (see Section Erreur ! Source du renvoi introuvable.) and grey otherwise. In the case where the anomaly is not significantly detected, a sentence is printed below the GMC explaining the reason why the detection is not significant.
4. Histogram of percentages of ROI endings:
   Each bar of the histogram represents, for all the ROIs containing at least one of the segments specified in the x-axis, the percentage of these ROIs ending in between the segments.
   The value(s) corresponding to the breakpoint location(s) is (are) shown in red.
5. Information about the size of the group of normal ROIs and the group of abnormal ROIs
7.5.2.5. ROI images

In case of anomaly detection (see Sections *Bimodality block* and *Breakpoint block*), the report displays ROIs, and their id numbers from the normal (1) and abnormal (2) groups that were selected by the user.

In case of no anomaly detection (see Section *No anomaly block*), the report displays ROIs and their id numbers selected by the user from the whole set of reviewed ROIs.

7.5.2.5.1. Validation or cancellation

![Figure 14 - Example of ROI images in case an anomaly is detected](image)

Figure 15 - Comment section in report
Comments can be added to the report in the “Comments” section.

7.5.3. Excel file description

The Excel file contains the same detection results as the PDF report with some additional information on the data such as:

- Measurement values for every ROI, after global normalization with a factor depending on the stretching factor value and alignment to the expected GMCs.
- ROI characteristics for every ROI, such as stretching factor value and total length measurement.

A precise description of the Excel file is provided in its “Notes” sheet.

8. INTERPRETATION OF REPORT AND RECOMMENDATION

The report informs about detection of anomalies that can be linked to presence of large rearrangements in BRCA1 and/or BRCA2 genes. More precisely,

- The absence of detected anomaly is a good indicator for the absence of inversion or translocation as well as duplication and deletion above a certain resolution specified in the report (see section Summary of anomaly detection).

- The detection of a breakpoint is a good indicator for the presence of an inversion or a translocation.

- The detection of more than one breakpoint is a good indicator for the presence of a deletion of entire segments of the GMC.

- The detection of bimodality is a good indicator for the presence of a duplication or deletion in the gene regions. It indicates a deletion when the abnormal length measurements are below the expected ones, an insertion or duplication in the opposite case.

The performances of anomaly detection strongly depend on the quality of the data (i.e., the amount of data and their variability). Consequently, a confidence percentage is computed for any anomaly detection as well as a resolution value in case of no detection. These values should be taken into account during report interpretation.
At least 50 measures for every segment of each GMC have been defined as sufficient coverage for anomaly detection. Such coverage enables detection of bimodalities and breakpoints with more than 90% sensitivity. Below that coverage, no conclusive results about the presence of a large rearrangement can be provided by the software.

When the region of interest is not sufficiently covered (see Sections Summary of anomaly detection and Description of data for coverage information on report), the results are considered inconclusive and the following actions are recommended:

- If only a subset of ROIs detected automatically has been reviewed, increase this subset in order to accumulate more segment measurements by adding ROI that may have been missed by the software (for user selection of ROI, please refer to the instructions for use of FiberStudio® software)
- Process additional coverslips up to complete review and add it to the data set.

It is also recommend to consider an anomaly detection as statistically significant only when it respects the following rules:

- Its confidence percentage exceeds 90%
- Its coverage is sufficient (i.e., its sum of abnormal and normal ROIs exceeds 50)
- In case of bimodality, the estimated anomaly size is above 3kb.

The block “Summary of anomaly detection”, described in Summary of anomaly detection, summarizes information about whether or not the GMCs are sufficiently covered and the presence of anomalies significantly detected. We recommend to base report interpretation on this block.
9. **INTERFERENCE**

There is no interference. Hybridization of the FiberProbes® BRCA1 & BRCA2 may generate background that appears as dispersed fluorescent dots onto the CombiCoverslips™ but it does not generate unspecific multicolor fluorescent signal arrays that may interfere with the analysis and the results.

10. **RECOMMENDATIONS AND LIMITATIONS**

The FiberProbes® BRCA1 & BRCA2 are designed for Research Use Only. The FiberProbes® BRCA1 & BRCA2 cannot be used for diagnostic. Genomic Vision does not assume any responsibility for improper application of this product.

11. **TRAINING**

Training is mandatory for performing this assay. Genomic Vision will provide training in specimen preparation, assay procedure, and interpretation of Molecular Combing testing of BRCA assay for inexperienced users. It is also recommended that a laboratory that has previously received training but now has new personnel performing the assay request training for the new users.
12. REFERENCES


### 13. ORDERING INFORMATION AND RELATED PRODUCTS

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<tr>
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<td>Provided by 2 units</td>
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<td>Holder with 10 positions</td>
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</tr>
<tr>
<td>CombiCoverslips™</td>
<td>Box of 50 units</td>
<td>COV-001</td>
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To place an order, please contact us at sales@genomicvision.com or visit our website [http://www.genomicvision.com/gv-store/](http://www.genomicvision.com/gv-store/)
Our experts are available to answer your questions
http://www.genomicvision.com
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