FiberProbes®

For DNA Structural Analysis

Instructions For Use

Version 01
REF: ATH-HYB-001-RUO

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GENOMIC VISION

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1. INTENDED USE

The FiberProbes® are designed to specifically identify loci by fluorescent hybridization on combed DNA extracted from blood samples or cell lines and prepared according to the Molecular Combing procedures, in order to analyze DNA structural variation. The FiberProbes® are intended to be used for Research Use Only.

Any of the FiberProbes® available on our website can be combined together.

2. OVERVIEW OF THE ASSAY

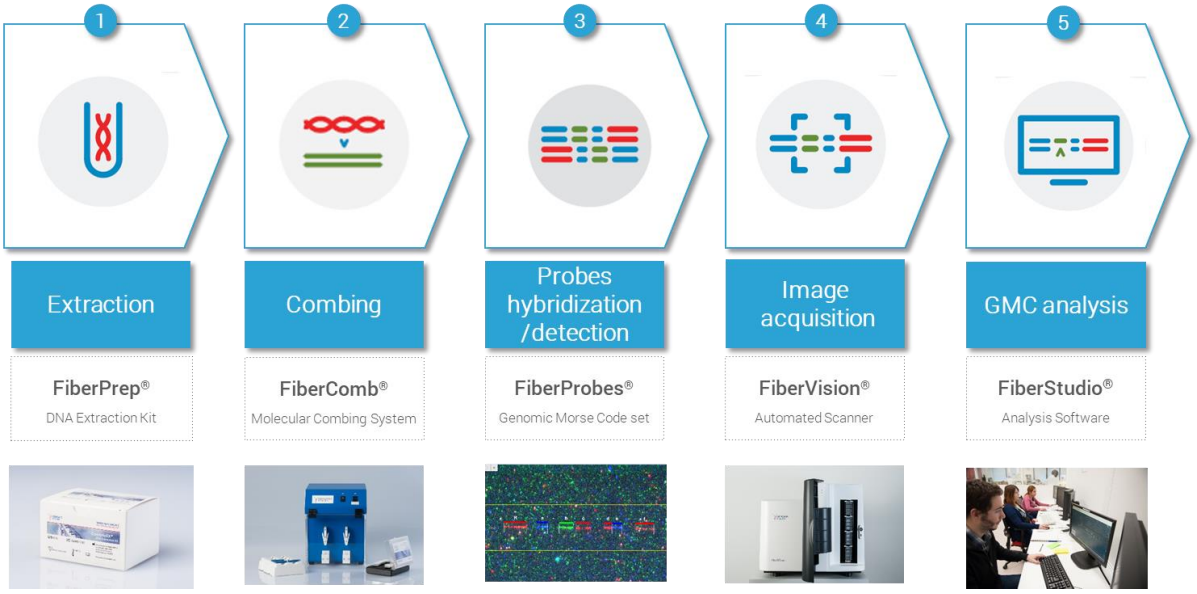


Figure 1 - Workflow of the FiberProbes® analysis on the Molecular Combing Platform

Molecular Combing allows the direct visualization and analysis of single DNA molecules. It enables DNA extracted from fresh blood cells or cell lines (step 1: FiberPrep® DNA Extraction Kit) to be uniformly and irreversibly stretched attached to a treated glass coverslip (step 2: FiberComb® Molecular Combing System) according to the protocols for "DNA extraction and Molecular Combing" that are provided by Genomic Vision. The labeled FiberProbes® will be hybridized on stretched DNA and revealed by immunodetection (step 3: FiberProbes® Hybridization/Detection). The genomic sequence identified by the FiberProbes® selected can then be detected and visualized by fluorescence microscopy (step 4: FiberVision® Automated Scanner) as it is described in the present Instructions for Use. The constant stretching factor

allows the direct measurement of the probe length and the physical cartography of the regions of interest. The images obtained can be analyzed on a dedicated software called FiberStudio® that is capable of detecting and analyzing all signals on a coverslip and "translating" the Genomic Morse Code into valuable information about gene modifications (step 5: FiberStudio® Analysis Software)

3. PRODUCT CONTENT AND DESCRIPTION

The FiberProbes® Assembly with the reference ATH-HYB-001-RUO contains the probes listed in the table below:

Probes	Genomic coordinate (NCBI36/hg18)	Color
DUX4-HYB-001-RUO	U85056.1: 24213-27507	Green
Chr_4-HYB-001-RUO	Chr4: 191159954-191177973	Red
Chr_10-HYB-RUO	Chr10: 135263045-135277945	Blue
Centro_4&10-HYB-001-RUO	Chr10: 135289200-135299096 Chr10: 135304359-135322974	Magenta Blue
Tel_qA-HYB-RUO	β-sat:135349215-135352590 Chr10: 135361156-135371004	Red Red
Tel_qB-HYB-RUO	Chr4: 191248888-191253373 Chr10: 135361156-135371004	Blue Red

Table 1 – Probes corresponding to the Assemby ATH-HYB-001-RUO

FiberProbes® set: 5 x 20 µl per vial (2 tests per vial, 10 tests in total). One test is defined as sufficient for one hybridization experiment of combed DNA on coverslips (22 x 22 mm area).

The FiberProbes® Assembly contain labeled polynucleotides that are intended to be used in combination with specific reagents (see Reagents and materials recommended but not provided – 6.1) that allow their detection in green, blue, red and magenta fluorescent signals.

Labeled polynucleotides are premixed with blocking DNA in formamide-free hybridization buffer.

4. STORAGE AND HANDLING

Upon arrival, the product must be stored between -25°C and -10°C protected from 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 test.

Handle all reagents and slides containing fluorophores in reduced light environment 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 for up to 6 months.

5. WARNINGS AND PRECAUTION

For Research Use Only. For professional use only. Carefully read the operating instructions before use.

Do not use the product after the expiry date.

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

5.1. Reagents and materials recommended but not provided for the hybridization and the detection of the FiberProbes® Assembly

IMPORTANT: Since the characteristics of the "FiberProbes® Assembly" have been evaluated and validated using the reagents and materials listed below, we recommend the use of these referenced reagents and materials for optimal results.

The "FiberProbes® Assembly" were validated with the following reagents and materials for their detection as green, red, blue and magenta fluorescent signals:

- 0.5 mg Cy™3 IgG Fraction Monoclonal Mouse Anti-Fluorescein (FITC) (Jackson Immunoresearch Ref: 200-162-037). To be reconstituted (see reagent preparation).
- 0.1 mg/ml BV480 Streptavidin (BD Biosciences, Ref: 564876). Ready-to-use.
- 0.5 mg Alexa Fluor® 647 IgG Fraction Monoclonal Mouse Anti-Digoxin (Jackson Immunoresearch Ref: 200-602-156). To be reconstituted (see reagent preparation).

Note: Magenta fluorescent signal is the result of the double labeling and detection with Blue and Red.

- CombiCoverslips™ (Genomic Vision, Ref: COV-001).

- FiberVision® Automated 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 rack
- Humidified chamber
- Incubator at 37°C
- 250 ml-beaker

5.2. Preparation of the reagents for the hybridization and the detection of the "FiberProbes® Assembly"

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

- 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:

	Quantity (mg)	Volume of distilled water to be added (ml)	Final concentration (mg/ml)
Cy™3 IgG Fraction Monoclonal Mouse Anti-Fluorescein (FITC)	0.5	0.350	1.43
Alexa Fluor® 647 IgG Fraction Monoclonal Mouse Anti-Digoxin	0.5	0.350	1.43

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

	Volume (µl)
- 1.43 mg/ml Cy™3 IgG Fraction Monoclonal Mouse Anti-Fluorescein (FITC)	0.8
- 0.1 mg/ml BV480 Streptavidin	0.8
- 1.43 mg/ml Alexa Fluor® 647 IgG Fraction Monoclonal Mouse Anti-Digoxin	0.8
- BlockAid™ Blocking Solution	17.6

6. ASSAY PROTOCOL

6.1. Hybridization of the "FiberProbes® Assembly" 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® Assembly" and the coverslips with combed DNA on a ceramic coverslip rack for 10 min at room temperature.
2. Dehydrate the combed DNA coverslips by dipping the ceramic coverslip rack 1 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 10 µl of the "FiberProbes® Assembly" in a new microcentrifuge tube and add 10 µl of deionized formamide to the "FiberProbes® Assembly"
5. Mix well and incubate at 37°C for 30 min
6. Pipette 20 µl of the probes/formamide mix and put it on a microscope slide.
7. Avoiding trapped bubbles, set the coverslip on the drop of hybridization solution.

Note: Carefully indicate the side of the coverslip in contact with the hybridization solution to avoid confusion in the subsequent steps.

Note: Lay down the coverslip carefully using tweezers to avoid bubbles. Do not move the coverslip once mounted, it will cause scratches on combed coverslips. Adjust the position of coverslip by gently touching the corner if needed.

Note: Protect the hybridized slides from light from this step.

8. Co-denature the combed DNA on the coverslip and the probes 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 coverslip from the microscope slide and place on a ceramic coverslip rack in a 250 mL-beaker containing the pre-warmed (60°C) Hybridization Washing Buffer.

Note: If the coverslip is stuck to the slide, dip the slide in the prewarmed 2XSSC buffer for a few seconds. This will help removing the coverslip. When removing the coverslips, gently slide them until one of corner is out on slide to avoid scratches, then gently "peel off" the coverslip with tweezers.

11. Wash the coverslip three times in pre-warmed (60°C) Hybridization Washing Buffer for 5 min each at 60°C.

Note: The coverslip must be totally immerged in the solution. Do not let the coverslip dry in between the washes.

6.2. Detection of the "FiberProbes® Assembly"

1. Pipette 20 µl of the detecting solution on a microscope slide and set the coverslip on the drop of detecting solution.

Note: Make sure to place the hybridized side of the coverslip in contact with the detecting solution.

2. Incubate the slide for 20 min in a humidified chamber at 37 °C.
3. Remove the coverslip from the microscope slide and place it on a ceramic coverslip rack in a 250 ml-beaker containing the Detection Washing Buffer.

4. Wash the coverslip three times in the Detection Washing Buffer for 3 min each at room temperature with gentle agitation.
5. Wash the coverslip in 1xPBS for 3 min at room temperature with gentle agitation.
6. Dehydrate the coverslip by dipping the ceramic coverslip rack 1 min in successive baths of 70%, 90% and 100% ethanol.
7. Air dry the coverslip at room temperature for 10 min protecting from light.
8. The coverslip can be stored at 4°C protecting from light until observation.

6.3. Visualization of the fluorescent signals

The coverslips hybridized with the FiberProbes® must be scanned with the FiberVision® Automated Scanner, an automated image scanning system developed by Genomic Vision equipped with a 40X objective and the following filters:

Fluorophore	Excitation peak (nm)	Emission peak (nm)
Cy3	543 ± 3 nm	588 ± 55 nm
BV 480	434 ± 17 nm	515 ± 23 nm
Cy5	640 ± 14 nm	700 ± 70 nm

The hybridization signals appear as multicolor fluorescent signal arrays detected as follows:

- DUX4-HYB-001-RUO

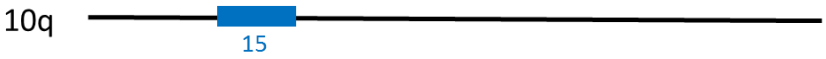
The DUX4 FiberProbes® will identify the 3.3kb sequence of the microsatellite named D4Z4. The fluorescent signals will appear in green with a variable size depending on the number of the repeated units of the analyzed sample.

- Chr4_HYB-001-RUO

The Chr4 FiberProbes® will be identified as an 18kb-red signal on chromosome 4 (Chr4: 191159954-191177973; NCBI36/hg18)

- Chr10_HYB-001-RUO

The Chr10 FiberProbes® will be identified as a 15kb-blue signal on chromosome 10 (Chr10: 135263045-135277945; NCBI36/hg18)



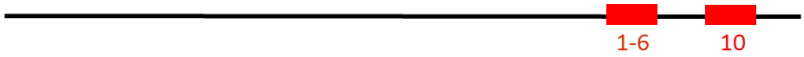
- Centro_4&10-HYB-001-RUO

The Centro_4&10 FiberProbes® will be identified as a 10kb-magenta signal, a 5kb space and a 20kb-blue signal on chromosome 10 (Chr10: 135289200-135322974; NCBI36/hg18).

As there is a homologous region on chromosome 4 the same fluorescent signal will also be observed on this genomic region (Chr4: 191184317-191218428; NCBI36/hg18)

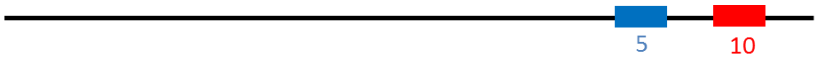
- Tel_qA-HYB-RUO

The Tel-qA FiberProbes® will appear as a 2 telomeric red signals, one from 1 to 6Kb corresponding to the β-satellite region and one of 10Kb.



- Tel_qB-HYB-RUO

The Tel-qB FiberProbes® will appear as a 2 telomeric signals, a 5kb-blue signal and a 10Kb-red signal.



6.4. Detection and analysis of hybridizations signals

The FiberStudio® Software is specifically developed by Genomic Vision for the analysis of Molecular Combing results. It acquires high resolution multi-color images of the whole surface of the coverslip with a handsoff workflow in only one hour.

When the scan of a coverslip is finished, the images are transferred to the FiberStudio® database.

The FiberStudio® Software extracts hundreds of signals and analyzes data to generate easily interpretable, accurate results.

Please refer to User Manual of "FiberStudio® Software" for the procedure of analysis of detected signals.

7. LIMITATIONS

The "FiberProbes® Assembly" are designed for Research Use Only. Genomic Vision does not assume any responsibility for improper application of this product.

8. ORDERING INFORMATION AND RELATED PRODUCTS

Product	Contents	Reference
FiberProbes® Assembly	10 hybridizations	ATH-HYB-001-RUO
FiberStudio® Software	Licence	SWT-001
FiberPrep® DNA Extraction Kit	100 extractions	EXT-001
FiberComb® Molecular Combing System	Combing Device	MCS-001
FiberVision Scanner®	Automated Scanner	SCN-001
Coverslip Clip Holder	Holder with 2 positions	CLI-001
Disposable Reservoirs	Pack of 10 units	RES-001
Reservoir Supports	Provided by 2 units	SUP-001
Bench Reservoir Holder	Holder with 10 positions	POR-001
Silanized Coverslips	Box of 50 units	COV-001

To place an order, please contact us at sales@genomicvision.com
or visit our website http://www.genomicvision.com/gv-store/

9. DISPOSAL INSTRUCTIONS

The "FiberProbes® Assembly" and the empty vials of "FiberProbes® Assembly" have to be eliminated after use in biological waste trash bin according to the national laws.

Our experts are available to answer your questions
http://www.genomicvision.com
support@genomicvision.com

Patent

This product or the use of this product is subject to proprietary rights (EP2007/059299, IB2009/007197). The Molecular Combing technology and products are covered by patents (FR2716206, FR 2716263, FR 2737574, FR 2755149) owned by Genomic Vision S.A.

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BlockAid is a trademark of Molecular Probes, Inc.
Tween is a registered trademark of Croda International PLC

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