FiberComb®
Molecular Combing System

RÉF produit: MCS-001-RUO

Instructions for Use
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Please read through and familiarize yourself with the contents of the instructions for use before using the system for the first time.

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Genomic Vision reserves the right to make technical improvements to this instrument and documentation without prior notice as part of a continuous program of product development. This manual supersedes all previous editions.

About this Instructions for Use

This manual describes the use of the FiberComb® Molecular Combing System and Accessories.

It is intended to provide the information necessary to:

- install the FiberComb® Molecular Combing System
- set up and use the FiberComb® Molecular Combing System
- service the FiberComb® Molecular Combing System

The manual also describes the characteristics and specifications of the FiberComb® Molecular Combing System.

Please read this manual carefully before using the instrument.
SAFETY PRECAUTIONS

The FiberComb® Molecular Combing System is a device operating from a 24V– supply produced by an external power supply, delivered with the instrument.

For the correct operation of this instrument, it is imperative that no other supply voltage be used. Failure to heed this warning may cause incorrect operation and/or damage to the instrument.

The external power supply provided with the instrument is a universal unit, featuring interchangeable AC input clips, able to operate at any frequency and voltage. The output lead, equipped with a MiniDIN plug, must be connected to the FiberComb® Molecular Combing System.

Connection of one instrument per wall socket is highly recommended to limit the risk of voltage surge.

The external supply power is used for disconnecting device: it must be accessible to unplug the instrument from the main power in case of problems.

Because of the low input voltage, there is no risk of electric shock inside the FiberComb® Molecular Combing System. Nevertheless, all usual precautions must be taken when using liquids in the vicinity of the instrument or the external power supply:

- In case of liquid spillage, the instrument and/or the external power supply must be immediately disconnected from the wall socket and dried. The spilt liquid must be cleaned up as quickly as possible.
- If a biohazard liquid is concerned, the nearest local distributor must be contacted, and must be advised of the health risks associated with the liquid.
- If liquid penetrates to the interior of the instrument or its external power supply, serious damage may be caused. The instrument MUST NOT be connected to the wall socket until it has been inspected by a qualified Technician or Service Engineer.
INCORRECT OPERATION

Use of this instrument in any manner different from that described in the instructions for use may alter or invalidate the warranty provided with the instrument.

Genomic Vision shall not be held responsible in the event that use of this instrument in any manner different from that described in this instructions for use. If the instrument is used in any manner different that is specified by Genomic Vision, the protection provided by the equipment may be compromised.
SYMBOLS

The following symbols may be found in various places on the Molecular Combing System and its power supply, or in this Instructions for Use:

- Direct Current (DC)
- Alternating Current (AC)
- Fuse
- Catalogue number
- Serial number
- Manufacturer
- Manufacture date
- Temperature limitation
Humidity limitation

New waste

Consult instructions for use

Keep dry

Fragile

Top of the package in the direction of arrows

Power On

Power Off

Warning!

If you have any concerns about the safety of this instrument, please contact your local distributor.

MAINTENANCE PROCEDURES MUST BE CARRIED OUT ONLY BY A QUALIFIED TECHNICIAN OR SERVICE ENGINEER.
1. BACKGROUND INFORMATION

Molecular Combing is a process whereby single DNA molecules bind by their extremities to a silanised surface and are then uniformly stretched and aligned by a receding air/water interface [1].

Molecular Combing is a simple and reproducible fiber stretching technique [1, 2]. A chemically modified glass coverslip (CombiCoverslip™) is dipped into a buffered DNA solution (Figure 1 - Principles of DNA stretching by Molecular Combing; Step A). DNA fibers bind to the chemically modified, hydrophobic surface by one or both of their extremities in a pH-dependent manner (Step B). When the CombiCoverslip™ is pulled out with a slow and constant speed \( v = 300 \mu m.s^{-1} \) [3], the receding meniscus stretches the anchored DNA molecules onto the CombiCoverslip™ as it applies a constant perpendicular force on them (Step C). This rapid process results in irreversibly fixed DNA fibers and has the major advantage—in comparison to other known fiber-stretching techniques—that DNA fibers are aligned in parallel all over the surface (Step D). The stretching factor is constant \( 1 \mu m = 2 \text{ kb} \) [1, 2], so that internal size standards are not necessary once calculated under the same lab conditions.

![Figure 1 - Principles of DNA stretching by Molecular Combing](image-url)
Molecular Combing of DNA combined with immunofluorescent detection is one of the most efficient techniques for stretching and visualizing single DNA fibers. Indeed, hybridization of combinations of fluorescent probes of different sizes and colors, each one specific to a certain sequence of the region of interest, results in specific hybridization patterns of “dashes and dots” consisting the Genomic Morse Codes (GMC) (Figure 2, A). Any change in the order, the size of the probes or the spacing between probes can be directly visualized and measured on combed DNA to map target genes and structural variations (SV) or large genomic rearrangements such as deletion, amplification or inversion (Figure 2, B).

Since its initial description over 20 years ago [1, 2], Molecular Combing has been used for drawing high-resolution physical maps of genomic regions [3] or for the detection of chromosomal rearrangements such as deletions, amplifications [4], copy number variations [5] as well as viral integration [6, 7]. It is also widely used to study DNA replication and genome stability [8-10].
Molecular combing can also be used to study DNA replication. Analysis of DNA replication is rendered possible by sequential labelling of the DNA being replicated with the modified nucleotides. Depending on the labelling used, the sequences replicated early or late in each DNA molecule can be distinguished as fluorescent segments alternating between green and red. The association of combing techniques with immunofluorescent technologies for combed DNA enables the direct visualization of active replication and to study the progress of replication on long DNA segments.

**Illustration 3— Signals from two pulse techniques, one with iododeoxyuridine (IdU) and the other with 5-chloro-deoxyuridine (CldU)**

2. **INTENDED USE**

The FiberComb® Molecular Combing System, together with its accessories (Disposable DNA reservoirs and CombiCoverslips™), is an automated instrument that stretches single DNA molecules onto specially-treated glass surfaces.
3. PRODUCT DESCRIPTION

The FiberComb® Molecular Combing System consists of two sub-assemblies operating simultaneously; each of them is able to:

- hold one or two 22 mm square glass coverslips (CombiCoverslips™)
- plunge these CombiCoverslips™ into the Disposable DNA reservoirs containing the DNA solution being analyzed
- maintain the CombiCoverslips™ in the solution for the required duration
- withdraw the CombiCoverslips™ from the reservoir at a slow, constant speed

These operations are controlled and monitored by a microcontroller.

3.1. System Components

The following chart lists all available parts or replacement parts:

<table>
<thead>
<tr>
<th>Description</th>
<th>Catalog Number</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>FiberComb® Molecular Combing System</td>
<td>ADMCS: Cannot be ordered separately</td>
<td>1</td>
</tr>
<tr>
<td>External Power Supply</td>
<td>ADE4002: Cannot be ordered separately</td>
<td>1</td>
</tr>
<tr>
<td>User Manual</td>
<td>Available on demand, or on-line: <a href="http://www.genomicvision.com">www.genomicvision.com</a></td>
<td>1</td>
</tr>
<tr>
<td>CombiCoverslip™ Holder</td>
<td>CLI-001</td>
<td>2</td>
</tr>
<tr>
<td>Reservoir Supports</td>
<td>SUP-001</td>
<td>2</td>
</tr>
<tr>
<td>Pack of 10 Disposable DNA Reservoirs</td>
<td>RES-001</td>
<td>1</td>
</tr>
<tr>
<td>Reservoir Bench Holder</td>
<td>POR-001</td>
<td>1</td>
</tr>
<tr>
<td>Box of 50 CombiCoverslips™</td>
<td>COV-002-RUO *</td>
<td>1</td>
</tr>
</tbody>
</table>
Each part can be ordered separately, upon referencing the description, catalog number and quantity required. The instrument must not use other accessories or external power supply than the accessories distributed by Genomic Vision.

3.2. Technical Specifications of the FiberComb® Molecular Combing System

3.2.1. Front Views
Figure 5 – Front View - Cover open

Disposable reservoir
3.2.2. Rear View

Figure 6 – Rear View

Power input connector
3.2.3. Conformity to Standards

The instrument conforms:
- to directive 89/336/CEE, modified by 92/31/CEE, 93/68/CEE and, 2014/30/UE (Electromagnetic Compatibility)
- to the low voltage directive 73/23/CEE, modified by 93/68/CEE and, 2014/35/UE (Electrical Safety)

3.2.4. General Specifications

Dimensions: 205 mm (W) x 260 mm (H) x 210 mm (D)
8.1in. (W) x 10.2in. (H) x 8.3in. (D)
Weight (without external power supply): 4.1 kg
9.0 lbs

3.2.5. Electrical Specifications

Power Consumption: 12W max.
Voltage: 24V~
Current: 0.5A
Fuse: 630 mAT

3.2.6. Electrical Specifications of External Power Supply

Power Input Requirements: 100 - 240V~ @ 50-60Hz
Input Current: 0.5A (RMS) max. @ 120V~
0.25A (RMS) max. @ 240V~
Output Voltage: 24V~
Output Current: 0.625 A max.
Length of Power Supply Cable: 2 m (approx.)
AC Input Clips: Europe, UK, USA/Canada and Japan/Australia
Output Connector: 8-pin MiniDIN
3.2.7. Functional Specifications: performance

- **User Interface:**
  - ON/OFF switch
  - Green LED for presence of voltage
  - Start/Stop push-button
  - 3-color (red/green/orange) LED for instrument status

- **Operating Environment:**
  - Indoor use
  - Temperature: 18 – 30 °C
  - Humidity: 15 – 85 % R.H.
  - Altitude: maximum 2000 m
  - Pollution degree: 2
  - Overvoltage category: II
  - Supply voltage fluctuation: +/- 10

- **Combing:**
  - Height: 18 mm
  - Speed:
    - Adjustable for calibration (internal adjustment)
    - Minimum: ~220 µm/s
    - Maximum: ~1100 µm/s
    - Preset to 300 µm/s
    - Precision: ±5 %

- **Soaking Time:**
  - Adjustable for calibration (internal adjustment)
  - Limits of adjustment:
    - Minimum: 0 minutes 0 seconds
    - Maximum: 42 minutes 30 seconds
  - Preset to 5 minutes
  - Resolution: 10 seconds
  - Precision: ± 10 seconds
3.3. Accessories of the FiberComb® Molecular Combing System

- **Coverslip Holder:**
  - Model: 2-slot coverslip holder
  - Material: Polyoxyméthylène (POM)
  - Inter-coverslip distance (2-slot model): 1.5 mm

- **CombiCoverslips™:**
  - Size: 22 x 22 mm
  - Thickness: #1.5 HP (High precision thickness)
  - Vinilsilane Coated Coverslips for Molecular Combing

- **Disposable Reservoir:**
  - Volume: 4000 mm³
  - Material: PP (base) / Elastomere (cap)

- **Reservoir holder:**
  - Material: POM

- **Reservoir Bench holder:**
  - Material: PS Choc
4. STORAGE AND HANDLING

Handle the instruments and its accessories with care and wear appropriate personal protective equipment (gloves, lab coat).

4.1. CombiCoverslips™

Upon arrival, the CombiCoverslips™ must be stored between +15 °C and +25 °C protected from the light until the expiry date printed on the label. Improper storage of the product can damage the chemical surface treatment or impair the performance of the product since it may affect the result of the test.

Once opened, the remaining content of the box can be stored between +15 °C and +25 °C for a maximum of 6 months.

Do not use CombiCoverslips™ after the expiry date indicated onto the label.

The CombiCoverslips™ are single-use accessories of the FiberComb® Molecular Combing System instrument, and thus should not be re-used.

**Warning:** CombiCoverslips™ are made of very thin glass and thus may be easily broken, and may provoke cut injuries if handled without care. To minimize the risk of cut, always wear protective gloves when using the CombiCoverslips™.

4.2. Disposable DNA reservoirs

The Disposable DNA reservoirs must be stored between +2 °C and +40 °C until the expiry date printed in the label. DNA solutions contained in the Disposable DNA reservoirs should be used as soon as possible after extraction, and should not be stored more than seven (7) days inside the disposable DNA reservoirs.

Do not use Disposable DNA reservoirs after the expiry date indicated onto the label.
The Disposable DNA reservoirs are single-use accessories of the FiberComb® Molecular Combing System instrument, and thus Disposable DNA reservoirs can be only be used for one DNA solution.
5. WARNINGS AND PRECAUTIONS

For professional use only. Carefully read the operating instructions before use.

6. INTERFERENCE

Vibrations may impact the quality of DNA combing and therefore the diagnostic performances. It is highly recommended to install the instruments away from any source of vibrations.

It is important to handle Disposable DNA reservoirs and CombiCoverslips™ with caution and use gloves to avoid contaminating analysis samples.

7. INSTALLATION

On delivery, carefully inspect the carton box. Unpack the FiberComb® Molecular Combing System and its accompanying items and inspect them in the presence of the shipper. If any damage or anomaly is observed, make an immediate report and claim to the shipper.

7.1. Unpacking and Installation

1. Open the box by cutting the adhesive tape.
2. Remove the Instructions for Use and the accessory box.
3. Grasp the instrument from the bottom and lift it out from the carton (refer to Figure 7).
4. Remove the packing foams and the foam film surrounding the device (refer to Figure 7). Be careful to not let the instrument fall.

5. Place the instrument on a flat, level surface that is free from vibrations.

6. Chose a location for the system that is away from direct sunlight and is relatively dust free. Do not install the FiberComb® Molecular Combing System or the external power supply close to a source of heat.

7. Maintain a minimum clearance of 10cm (4in.) from the backside to ensure that the power input connector is readily available to the operator.

8. Open the aerosol cover.
9. Remove the piece of foam blocking the CombiCoverslip™ Holder and the reservoir supports while blocking the CombiCoverslip™ Holder (refer to Figure 8). Store the foam block in the accessory box for future re-use.

![Figure 8 – Removing the Piece of Foam](image)

10. Remove the plastic protection of the aerosol cover.

11. Clean the exterior surface of FiberComb® Molecular Combing System and Accessories before the first use as indicated in section 12.1 – Instrument cleaning.

12. Close the aerosol cover.

13. Tilt the instrument to the side and unscrew the shipping lock (refer to Figure 9). This screw locks all moving parts in place during shipment and must be backed off prior to powering on the instrument. Back off the screw to the maximum possible.

**DO NOT TRY TO FORCE OUT THE SHIPPING LOCK COMPLETELY!**
Figure 9 – Unscrewing the Shipping Lock

**IMPORTANT:** if you move the device, it is necessary to screw the shipping lock before transport.

14. Unpack the accessory box and check its contents against the list below (Figure 10 – Accessory Box) for completeness. Contact your local distributor if any item is missing or damaged.

1. Instructions for Use (x1)

2. External Power Supply (x1)

3. Additional AC Input Clips (x4)
7.2. Set Up

The FiberComb® Molecular Combing System is delivered with a separate external power supply (Figure 11). This is a universal unit, featuring interchangeable AC input clips (Figure 12), able to operate at any frequency and voltage (100 - 240 V~ @ 50-60 Hz). It comes equipped with a European clip.

For use with other standard sockets, proceed as follows:

- Press the button 1 and slide out the AC input clip currently installed 2.
- Slide in the desired AC input clip 3.
- Keep sliding until the clip locks to the button.

Electrical installation of the instrument and power supply must be performed according to local regulation and standards.

In order to avoid the use of extension leads with multiple connections, which may disturb operation, the instrument must be installed close to a wall socket. Parallel connection of several instruments could induce dysfunction of the FiberComb® Molecular Combing System.
The instrument must be installed on a flat horizontal surface away from all devices that may cause vibrations.

Connect the external power supply to the FiberComb® Molecular Combing System: plug the power supply output lead to the MiniDIN power connector on the instrument rear panel (refer to Figure 13).

![Figure 13 – Connecting the External Power Supply](image)

The MiniDIN connector is keyed. It must be inserted with the mark being visible from the top (refer to Figure 14).
If insertion of the MiniDIN connector is difficult, do not force it and verify the connector orientation.

Connect the external power supply to the wall socket.

7.3. Calibration

After connection to the supply source, calibrate the combing (upward) speed and soaking time of the FiberComb® Molecular Combing System:

A) Start an operating cycle and measure the soaking time and the combing time.

For standard use, the soaking time must be 5 minutes ± 10 seconds and the combing time (upward movement) must be 1 minute and 40 seconds ± 10 seconds.

B) If the measured time is different from the expected time, calibrate the FiberComb® Molecular Combing System as follows:

1. Turn off the main power switch.
2. Disconnect the external power supply from the instrument.
3. Remove the four screws securing the rear panel using an Allen wrench of 2.5 mm.
4. Remove the cover by pulling it to the rear.
5. Hold the counterweight with one hand and, with the other, remove the two fastening screws (refer to Figure 15 – Internal View) using an Allen wrench of 2 mm.
6. Remove the counterweight completely.
7. Turn the screws localized on the potentiometers P1 and P3 with a screwdriver as follow (refer to Figure 16 – CPU Board):

![Figure 15 – Internal View](image-url)
Soaking time adjustment: turn the screw localized on the potentiometer P3 (approximately ¼ turn):

- In a clockwise direction → increases the soaking time
- In the counter-clockwise direction → decreases the soaking time
**Combing speed adjustment**: turn the screw localized on the potentiometer P1 (approximately ¼ turn):
- In a clockwise direction → increases the combing time
- In the counter-clockwise direction → decreases the combing time

8. Turn the power switch on.

9. Measure again the soaking time and the combing time

   If the measured durations correspond to a soaking time of 5 minutes ± 10 seconds and to a combing time of 1 minute and 40 seconds ± 10 seconds, the instrument is calibrated, you can replace the counterweight and close the device. If it is not the case, restart the calibration process according to the instructions described above.

The calibration has to be performed each time the FiberComb® Molecular Combing System is moved from its location, and whenever the soaking time or combing (upward) speed is non-conforming to the specifications mentioned above.

### 8. OPERATING MODE

#### 8.1. Control Panel Description

All instrument functions are controlled from the instrument front panel, which includes:

- **ON/OFF Switch** (labeled I/O) to apply or remove power from the instrument.
- **START/STOP Push-Button** (press and release to operate) used to:
  - start an operating cycle
  - temporarily interrupt a cycle
  - restart or terminate an interrupted cycle
  - acknowledge the end of a cycle
  - in case of an error, acknowledge the error

- **Green Indicator** (above the ON/OFF switch): when lit, indicates that voltage is present.
3-Color Indicator (above the START/STOP push-button) to indicate the instrument status, as shown in the table below:

<table>
<thead>
<tr>
<th>3-Color Indicator Status</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous Green</td>
<td>Stand-by state: instrument is available for use</td>
</tr>
<tr>
<td>Continuous Red</td>
<td>Working state: a sequence is running</td>
</tr>
<tr>
<td>Continuous Orange</td>
<td>Wait state: an operating cycle has been completed and the instrument is waiting for an acknowledgment from the operator</td>
</tr>
<tr>
<td>Alternately Flashing Red &amp; Green</td>
<td>Interrupted state: the operator has temporarily interrupted the current cycle</td>
</tr>
<tr>
<td>Flashing Red</td>
<td>Error state: an error condition has been encountered</td>
</tr>
</tbody>
</table>

Audible Alarm (buzzer) to warn the operator of the end of cycle.

8.2. Instrument Power On

Turn the power switch on. The indicator located above the ON/OFF switch lights (green) and the instrument initializes: the coverslip holder assembly moves up to its home position.

If it is already at the home position, no movement occurs, but the indicator located above the START/STOP push-button turns green. The instrument is ready for use.

If it is not at the home position, the motor runs to move the coverslip holder assembly upwards until it reaches the home position. During this initialization phase, the indicator located above the START/STOP push-button shows red, indicating that a sequence is running. When the home position is reached, the indicator turns green. The instrument is ready for use.
8.3. Installation of Disposable Reservoirs

Take a disposable DNA reservoir, remove the cap, add carefully the buffered DNA solution into the reservoir and place the Disposable DNA reservoir gently into the reservoir support (Figure 17).

Figure 17 – Installing a Disposable Reservoir onto its Support
8.4. Installation of Reservoir Holders

Reservoirs supports are fitted at the bottom of the instrument front panel. They are held in place by two magnets located in the front part of each reservoir support (Figure 18 – Installing a Reservoir Support).
1. Engage the reservoir support with the horizontal pair of metal rods (refer to Figure 18).
2. Push it gently until it touches the front panel.
8.5. Installation of CombiCoverslip

With the 2-slot coverslip holder model, it is possible to install glass coverslips (CombiCoverslips™) separately in each slot, i.e. on each side of the coverslip holder (Figure 19 – Installing CombiCoverslips™).

**IMPORTANT:** For a more reliable result, only one CombiCoverslip™ on the coverslip holder should be installed. The number pre-engraved on the CombiCoverslips™ enables to ensure traceability of analysis.

1. Press the spring clip to open the bottom of the holder

2. Insert the CombiCoverslip™ in the slot with putting the engraving upwards (refer to Figure 19):
o Bring the rear corner of the CombiCoverslip™ into contact with the coverslip holder stop (see detail 1). At the same time, make sure that the CombiCoverslip™ is in flat contact with vertical surface 2 (coverslip holder central part).

o Bring the front part of the CombiCoverslip™ in contact with the small horizontal surface, located on the front of the coverslip holder (see detail 3).

o Confirm that the rear corner of the CombiCoverslip™ is still in the correct position.

3. When the CombiCoverslip™ is in place, release the spring clip.

*Note 1: The CombiCoverslips™ are sensitive to light and temperature. Install them on the 2-slot coverslip holder just before combing. After combing, keep them protected from light.*
8.6. Installation of Coverslip Holders

Coverslip holders are installed on the upper part of the instrument front panel (Figure 20 – Installing a Coverslip Holder). They are held in place by two magnets, similarly to reservoir supports.

*Note:* this installation must only be carried out when the instrument is initialized and is in stand-by state, with the 3-color indicator showing continuous green.

1. Engage the holder with the horizontal pair of metal rods (refer to Figure 20).
2. Push it gently until it is held in place by the magnets placed on the holder.
3. Close the aerosol cover before using the START/STOP push-button.
8.7. Use of the START/STOP Push-Button

The function of the **START/STOP** push-button varies according to the status of the 3-color indicator. The actions produced by pressing the button are described in the table below:

<table>
<thead>
<tr>
<th>3-Color Indicator Status</th>
<th>Interpretation</th>
<th>Action of the START/STOP Push-Button</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous Green</td>
<td>Stand-by state: instrument is available for use</td>
<td><strong>Start</strong> of an operating cycle</td>
</tr>
<tr>
<td>Continuous Red</td>
<td>Working state: a sequence is running</td>
<td><strong>Temporary Stop</strong> (interruption)</td>
</tr>
<tr>
<td>Continuous Orange</td>
<td>Wait state: an operating cycle has been completed and the instrument is waiting for an acknowledgment from the operator</td>
<td><strong>Acknowledgment</strong> of the end of the cycle – the indicator returns to green</td>
</tr>
<tr>
<td>Alternately Flashing Red &amp; Green: Light is Green</td>
<td>Interrupted state: the operator has temporarily interrupted the current cycle</td>
<td><strong>Restart</strong> of the interrupted cycle – the indicator returns to red</td>
</tr>
<tr>
<td>Alternately Flashing Red &amp; Green: Light is Red</td>
<td>Interrupted state: the operator has temporarily interrupted the current cycle</td>
<td><strong>Permanent Stop</strong> of the interrupted cycle – the indicator returns to red</td>
</tr>
<tr>
<td>Flashing Red</td>
<td>Error state: an error condition has been encountered</td>
<td><strong>Acknowledgment</strong> of the error</td>
</tr>
</tbody>
</table>

**Summary:**

When the indicator is **green**, pressing the **START/STOP** button signifies **START**.

When the indicator is **red**, pressing the **START/STOP** button signifies **STOP**.
8.8. Starting an Operating Cycle

When the instrument is in stand-by state, the 3-color indicator shows continuous green. Press the **START/STOP** button to start an operating cycle.

The following table shows the successive phases of an operating cycle, in chronological order, with the corresponding colors of the indicator:

<table>
<thead>
<tr>
<th>Phase Number</th>
<th>3-Color Indicator Status</th>
<th>Phase Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Red</td>
<td>Downward movement of the CombiCoverslip(s)™ into the disposable DNA reservoir</td>
</tr>
<tr>
<td>2</td>
<td>Red</td>
<td>Waiting period for incubation (soaking time): typically five minutes</td>
</tr>
<tr>
<td>3</td>
<td>Red</td>
<td>Slow upward movement of the CombiCoverslip(s)™ at a speed of 300 µm/s typically (1 minute 40 seconds)</td>
</tr>
<tr>
<td>4</td>
<td>Orange</td>
<td>Signals the end of the cycle, waiting for operator acknowledgment – buzzer sounds intermittently</td>
</tr>
<tr>
<td>5</td>
<td>Green</td>
<td>Cycle complete: the operator has pressed the START/STOP button</td>
</tr>
</tbody>
</table>

*Note: during phase 1, when CombiCoverslips™ come close to the Disposable DNA reservoir, the downward movement is very slow. So, it is possible to verify if the coverslips enter correctly in the reservoir. If not, it is possible to interrupt the cycle (see below) and correct the position of the coverslips in the holder. If the CombiCoverslip™ has already touched the solution when the cycle is interrupted, discard it and replace by a new one, ensuring that it is well positioned. CombiCoverslips™ are not to be reused.*

8.9. Cycle Interruption – Restart – Termination
An operating cycle may be temporarily interrupted at any time, by simply pressing the START/STOP button during the course of the cycle (3-color indicator showing red).

The indicator goes out very briefly to acknowledge the button operation.

The color of the indicator then changes to red and green, alternately. Each color is shown for two seconds.

To terminate the interrupted cycle definitively, press the START/STOP button while the indicator shows red (the function of the button is then STOP). The instrument then returns to the home position, with the coverslip holder in the upper position. Once the home position has been reached, the indicator shows continuous green, and the instrument is ready for a new operating cycle.

To restart the interrupted operating cycle, press the START/STOP button while the indicator shows green (the function of the button is then START). The instrument then continues the operating cycle from where it was interrupted. The indicator shows continuous red to indicate that an operating cycle is running.

8.10. End of Cycle Acknowledgment

When an operating cycle has completed successfully, the 3-color indicator shows continuous orange and the buzzer sounds intermittently, to warn the operator.

The operator must then acknowledge the end of cycle, by pressing the START/STOP button. The buzzer stops sounding and the indicator shows continuous green.

Be careful at this step to not start again a combing cycle on a combed CombiCoverslip™. In a case of a double combing CombiCoverslip, do not heed the result.

8.11. Post-treatment of combed CombiCoverslips™
To avoid degrading the combed DNA, the CombiCoverslip™ should be handled avoiding contact with the surfaces.

8.11.1. Observation with a DNA intercalating dye

The density and quality of the combing of DNA molecules can be assessed visually using a fluorescent intercalating DNA, such as the YOYO® iodide-1 (see Figure 22: Adding a fluorescent inserting DNA for microscopic observation

1. Place a KIMTECH SCIENCE * Precision Wipes Tissue Wipers (not supplied) on a heating plate at 125 °C and place the combed CombiCoverslip™ on it for 3 minutes. Identify the face that is not in contact with the KIMTECH SCIENCE * Precision Wipes Tissue Wipers.
2. Add 15 µl of YOYO®-1 iodide solution (1µM) on a microscope slide.
   Note: YOYO®-1 iodide and ProLong ™ Antifade are not supplied with the instrument. The YOYO®-1 solution is prepared by mixing 1 µl of 1 mM YOYO-1 iodide with 1ml of ProLong ™ Antifade.
3. Place the CombiCoverslip™ on the YOYO-1 iodide solution with the side that was not in contact with the heating plate.
4. Directly observe the coverslips with an epi-fluorescence microscope equipped with appropriate filters (excitation = 491 nm; emission = 509 nm)
**Figure 21** - Addition of DNA’s fluorescent insert for microscopic observation

**Figure 22** – Density of combed DNA

Using CombiCoverslips™ with genomic DNA at lower density (Pictures 1-4) or short (Figure 23, Picture 1), wavy (Picture 2) or tangled (Picture 3) DNA molecules may affect the result of the test. In this case, it is recommended to extract DNA from a new plus or fresh blood.

**Figure 23** – Example of combed genomic DNA incompatible with *in vitro* diagnostic use

**IMPORTANT:** A CombiCoverslip™ visualized with the intercalating agent cannot be used to obtain a result. Hybridization or immunodetection has to be performed on another CombiCoverslip™ of the same batch, combed with the corresponding DNA solution.

### 8.11.2. Dehydration of the combed CombiCoverslips™

After the combing, the combed CombiCoverslips™ have to be preliminarily dehydrated by placing them in an incubator at 60 ° C

- For 4 hours for hybridization.
- For two hours for fluorescent immunodetection in replication analysis

After this step, the CombiCoverslips™ can be used or stored at -20 ° C protected from light.
8.12. Error Conditions

To detect any failure in the instrument (motor, sensor, etc.), each movement of the operating cycle is allocated a maximum time of execution.

If sensor information (indicating the completion of a movement) has not been received by the end of the allocated time, an error condition is signaled: the 3-color indicator flashes red intermittently (0.5 s ON, 0.5 s OFF, etc.) and the buzzer sounds intermittently (synchronized with the light).

Do not use a CombiCoverslip™ issued from an error cycle.

If the error appears during an operating cycle, and is due to an external cause which can be clearly identified and corrected, the operator can correct the problem and acknowledge the error condition by pressing the START/STOP button. The instrument then attempts to re-initialize, ready to start a new operating cycle.

If the attempt to initialize fails, the instrument remains in the error condition and cannot be used anymore. Contact your local distributor.
8.13. Instrument Cleaning

Before performing any maintenance procedure, turn the power switch off, disconnect the instrument from the external power supply and disconnect the external power supply from the wall socket.

The exterior surface of the instrument should be cleaned regularly. Use a sponge, chamois leather, or a soft cloth, dampened with a solution of distilled water and detergent (with the addition of 70 % alcohol if decontamination is required).

Never use chlorinated solvents, solvents based on hydrocarbons, or ether. Do not use abrasive detergent products.

8.14. Coverslip Holder and Reservoir Support Cleaning

The reservoir supports and the coverslip holders are made of POM. They may be cleaned with any product safe for use with POM (e.g. deionized water) and dried.

8.15. Calibration

The FiberComb® Molecular Combing System may be calibrated regularly, and whenever the soaking time or combing (upward) speed is non-conforming to the specifications.

Refer to chapter §10.3 Calibration.
9. **TRANSPORTATION AND RAISING**

Before transportation, screw the shipping lock under the instrument (refer to Figure 9). The transportation has to be done into the shipping packaging with packing foam. The transportation must be performed in accordance to the following conditions:

- Temperature: -20°C – + 40 °C
- Humidity: 15 – 90 % HR

Don’t let fall the instrument.

10. **TROUBLESHOOTING**

10.1. **Error Conditions**

Instrument error conditions arise when the time allocated for a phase of the operating cycle has elapsed, without recognition of one or more of the required sensor signals (see also section §8.1).

There are two possible causes:

- The motor does not turn or turns too slowly.
- One (or more) of the sensors has failed.

After any repair, the FiberComb® Molecular Combing System has to be calibrated. Refer to the §10.3 Calibration.

To identify the fault more precisely, first remove all accessories (coverslip holders, reservoir supports, etc.). Then remove the instrument cover as described in Section § 10.3 A.
10.1.1. Motor Does Not Turn or Turns Too Slowly

If operation is normal during the initialization phase and in the other phases when the motor turns at maximum speed, but the speed of upward movement is not correct, try to modify this speed. Refer to Section § 10.3 for additional instructions.

In other cases, follow the instructions below:

1. Check for the presence of the 24 V⎓ supply voltage: the green indicator (above the ON/OFF switch) should be lit.

2. Check for the presence of the 5 V⎓ logic voltage: the red LED D3 on the CPU board should be lit (refer to Figure 16).

3. Measure the 24 V⎓ on the CPU board. Connect a voltmeter (DC) between pin 3 (24V⎓) and pin 8 (GND) of test connector JP1. The value should be between 23V⎓ and 25Visclosed).
   If the measured value is out of range, check for correct insertion of the MiniDIN connector.
   If the connector is correctly inserted, replace the external power supply.

4. Measure the 5Visclosed) on the CPU board. Connect a voltmeter (DC) between pin 4 (5Visclosed) and pin 8 (GND) of test connector JP1. The value should be between 4.5Visclosed) and 5.5Visclosed).
   If the measured value is out of range, but the 24Visclosed) supply voltage is present and within range, it means that the CPU board failed and should be replaced.

5. Force the instrument to start a home movement (normally started when power is applied to the instrument). To do this, disconnect and re-connect the sensor CAPT2 to connector B3 (refer to Figure 16)

6. During the movement, observe the LED D4 (refer to Figure 16), which is connected in parallel with the motor.
   If the LED does not light, it means that the motor rotation command has not been generated.
   The CPU board failed and should be replaced.

7. If the LED does light, measure the voltage between pin 1 and pin 8 (GND) of test connector JP1. The value should be between 0.7 V and 0.8 V during the home movement.
If there is no voltage, it means that the motor acts like an open circuit. Check the electrical connections. If the connections are good, it is likely that the motor is defective and should be replaced.

8. If the problem persists, check if the cam is properly tightened on the motor shaft (two set screws). If necessary, remove the bracket which supports the motor assembly, by removing its three fastening nuts (refer to Figure 24 and Figure 25).

9. Make sure that there is no mechanical blockage or friction which prevents the motor from turning correctly. If necessary, remove the bracket which supports the motor assembly, by removing its three fastening nuts (refer to Figure 24 and Figure 25).

10. If the failure persists, it is possible that the motor turns correctly at high speed, but not at low speed. Check again for signs of motor jamming or seizing. Problems may be with the motor or the speed reducer (low motor torque, faulty reduction gear, etc.).

After any repair, the FiberComb® Molecular Combing System has to be calibrated. Refer to the § 10.3 Calibration.
Figure 24 – Mechanical Module

- Cam
- Movement control
- Sensor CAPT 1
- Fastening nuts for the motor assembly (2 of 3)

Figure 25 – Motor Assembly and its Supporting Bracket

- Speed reducer
- Motor
- Sensor CAPT 2
- Cam
10.1.2. Motor Turns Normally - Possible Sensor Problem

Check for correct operation of the two sensors which monitor motor movement.

The sensors used are slotted opto-electronic sensors, with an infra-red emitter on one side and a receiver on the other. The sensor output signal changes according to the sensor status:

- **Free**: the optical sensor is "on". Infra-red light from the emitter reaches the receiver.
- **Obstructed**: the optical sensor is "off". Infra-red light from the emitter cannot reach the receiver because of the presence of an object.

The status of each sensor is given on the CPU board by the two red LEDs D1 and D2 (refer to **Figure 16** for LED location and **Figure 24** and **Figure 25** for sensor locations):

- D1 indicates the status of sensor CAPT 1, connected to connector B4, and used to recognize the limit of the vertical movement.
- D2 indicates the status of sensor CAPT 2, connected to connector B3, and used to monitor motor rotation.

The following tables give the LED status relative to the sensor status:

<table>
<thead>
<tr>
<th>CAPT 1 Status</th>
<th>D1 Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>FREE</td>
<td>ON</td>
</tr>
<tr>
<td>Obstructed</td>
<td>OFF</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CAPT 2 Status</th>
<th>D2 Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>FREE</td>
<td>ON</td>
</tr>
<tr>
<td>Obstructed</td>
<td>OFF</td>
</tr>
</tbody>
</table>
1. Manually move the motor or use an external object (such as a screwdriver) to test sensor CAPT 1. Observe the status of corresponding LED D1. Replace the sensor if it doesn't give the right answer.

2. Repeat the operation to test sensor CAPT 2. Observe the status of corresponding LED D2. Replace the sensor if it doesn't give the right answer.

After any repair, the FiberComb® Molecular Combing System has to be calibrated. Refer to the § 10.3 Calibration.

10.2. Other Faults

10.2.1. Instrument Does Not Operate

1. Check for the presence of the 24V– supply voltage: the green indicator (above the ON/OFF switch) should be lit.

2. If the indicator is not lit, measure the 24 V– on the CPU board. Connect a voltmeter (DC) between pin 3 (24 V–) and pin 8 (GND) of test connector JP1. The value should be between 23 V– and 25 V–. If the measured value is out of range, check for correct insertion of the MiniDIN connector. If the connector is correctly inserted, replace the external power supply.

3. With the main power switch turned off, remove fuse F1 (type ATR 630mA slow blow) from the CPU board (refer to Figure 16). Use an ohmmeter to check the fuse condition. If the fuse is blown, it must be changed, but it will also be necessary to investigate the cause of the overload. If necessary, change the power supply.

After any repair, the FiberComb® Molecular Combing System has to be calibrated. Refer to the § 10.3 Calibration.
11. DISPOSAL INSTRUCTIONS

The CombiCoverslips™ and Disposable DNA reservoirs have to be eliminated after use in biological waste trash bin according to the national laws.

Once out of regular use, the FiberComb® Molecular Combing System has to be sent to Genomic Vision after decontamination in order to dispose it.
12. REFERENCES

Our experts are available to answer your questions

http://www.genomicvision.com

support@genomicvision.com

**Patent**
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