



# FiberPrep<sup>®</sup>

DNA Extraction Kit

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## DNA Extraction Protocol

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## 1. INTENDED USE

The FiberPrep® DNA Extraction kit is intended for the extraction of DNA from fresh blood samples or cultured cells for Molecular Combing applications. This kit is designed to deliver very high molecular weight DNA (average size: 400 kb).

## 2. STORAGE CONDITIONS AND TRANSPORTATION

The complete kit is divided into two parts according to the conditions of storage (+2°C to +8°C and -25°C to -10°C). Each element is necessary for the entire process of extraction.

## 3. PRODUCT USE LIMITATIONS

For general laboratory use.

## 4. INTRODUCTION

This FiberPrep® DNA Extraction kit has been designed to be used specifically for Molecular Combing, using silanized coverslips (Genomic Vision, Paris, France) and a FiberComb® Molecular Combing System (Genomic Vision, Paris, France).

Molecular Combing is a proprietary technology used for many applications and particularly for studying:

- DNA replication: physical characteristics and kinetic parameters of DNA replication can be studied (replication origin location, firing and fork velocity) by monitoring the incorporation of modified nucleotides in growing cells. This is a valuable tool for the evaluation of the efficacy of a certain class of anti-cancer molecules;
- Large genetic rearrangements: by use of specific labeled probes, rearrangements of a wide range of lengths (from 1 kb to several Mb) can be detected. This includes duplications, deletions, contractions or expansions and CNVs generally, as well as balanced translocations, inversions, etc.

The extraction step delivers high quality DNA solutions for Molecular Combing. The DNA is then stretched onto silanized coverslips using the FiberComb® Molecular Combing System. Several hundreds of single DNA molecules are stretched in a parallel way and with a constant stretching factor, which enables the correspondence between a physical measure and the DNA length (1  $\mu\text{m}$  = 2 kb). The hybridization and detection steps are followed by scanning with the FiberVision® automated scanner and analysis with the dedicated FiberStudio® software.

## 5. PRINCIPLE OF PROCEDURE

This protocol is used to purify and store genomic DNA molecules protected from mechanical stress. The extraction kit is designed for pelleted cells from either cultured eukaryotic cell lines or purified peripheral blood mononuclear cells (PBMC). After embedding cells in an agarose plug, proteins are digested by a proteinase and cell membranes are solubilized by a surfactant under inhibition of DNase activity. The treated DNA plugs are then ready to be used for the preparation of a high molecular weight DNA solution by agarase digestion.

## 6. KIT REAGENTS

- The +2-8°C part of the FiberPrep® DNA Extraction kit contains:

Buffers	Volume in the kit	Storage temperature
Buffer 2 (Plug Buffer)*	5 mL	+2°C to +8°C
Buffer 3 (Proteinase Buffer)	25 mL	+2°C to +8°C
Buffer 4 (Washing Buffer)	50 mL	+2°C to +8°C
Buffer 5 (Storage Buffer)	275 mL	+2°C to +8°C
Buffer 6 (Staining Buffer)	12 mL	+2°C to +8°C
Buffer 7 (Combing Buffer)**	250 mL	+2°C to +8°C
Component 3 (Proteinase)	3 mL	+2°C to +8°C

- The -20°C part of the FiberPrep® DNA Extraction kit contains:

Buffers	Volume in the kit	Storage temperature
Buffer 1 (Suspension Buffer)***	5 mL	-25°C to -10°C **
Component 7 (Agarase)	175 µL	-25°C to -10°C

\* Buffer 2 aliquots can be stored 1 month after first usage and should not be melted/refrigerated more than 10 times.

\*\* After opening of a Buffer 7 bottle, it should be stored at **+2-8°C for 6 months** maximum.

\*\*\* Buffer 1 is only used for extraction of DNA **from cultured cells**. If you extract your DNA **from blood**, please replace Buffer 1 by standard cell culture grade PBS (Phosphate-Buffered Saline). Once an aliquot of Buffer 1 is opened it should be stored at **+2-8°C for 1 month** maximum.

The FiberPrep® DNA Extraction Kit contains reagents to perform extractions on 50 samples, preparing 2 plugs per sample.

## 7. ADDITIONAL MATERIALS AND INSTRUMENTS REQUIRED

- FiberComb® Molecular Combing System (Genomic Vision, MCS-001)
- Silanized coverslips (Genomic Vision, COV-002-RUO or COV-002-IVD)
- Disposable DNA reservoirs (Genomic Vision, RES-001)
- Gel plug mold (Bio-Rad, Hercules, CA, USA, ref: 1703713)
- Water bath (42, 50, 68°C)
- Cell culture CO<sub>2</sub> incubator
- Counting chamber
- Test tube (15 mL and 50 mL)
- Round-bottom centrifuge tube (2 mL)
- Cell culture flask
- Centrifuge for cell samples
- Phosphate Buffer Saline (PBS) of cell culture grade (for extractions from blood samples)

Optional:

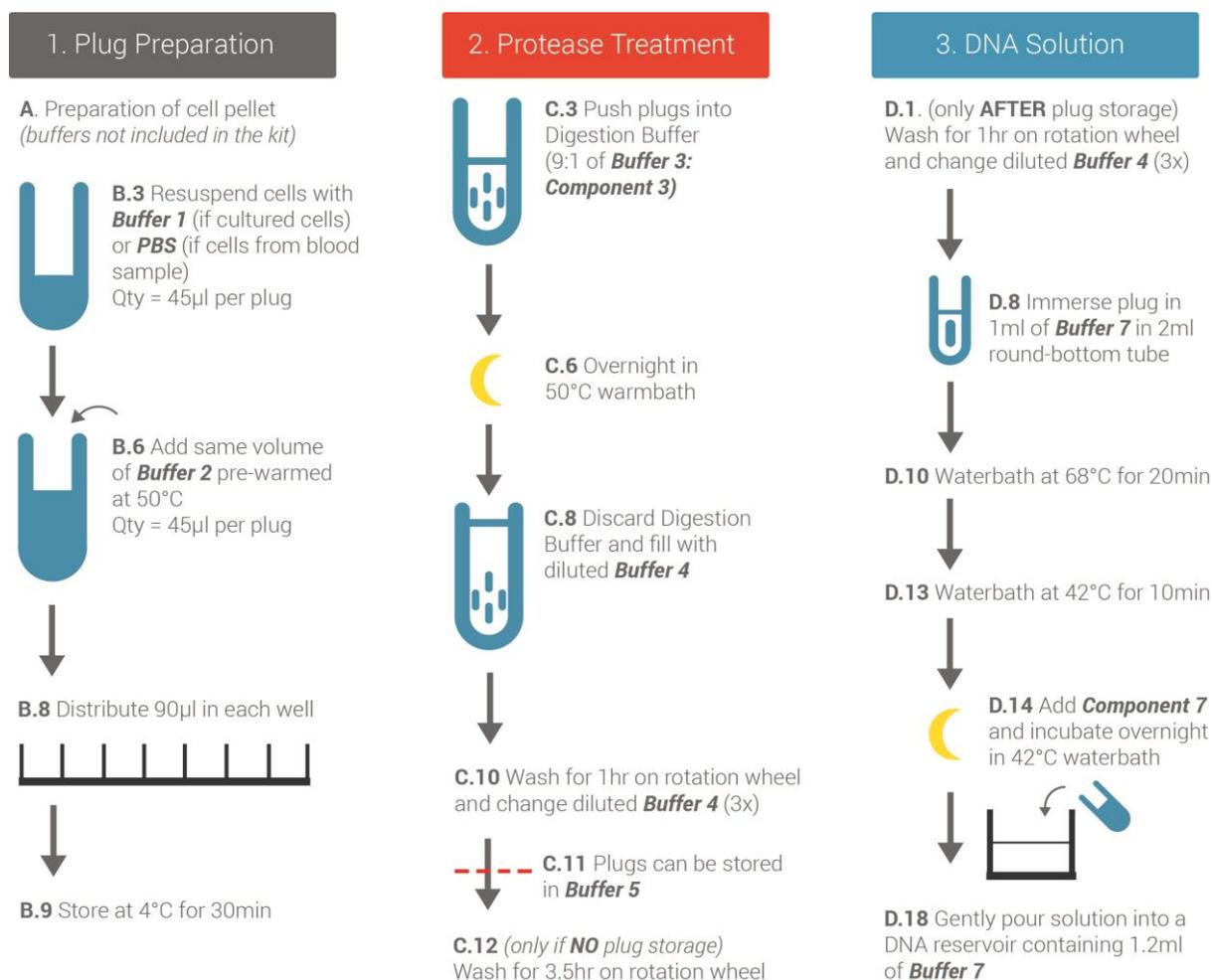
- YOYO-1 (Invitrogen)
- Mounting medium: Vectashield (Vector laboratories, USA), Slowfade (Invitrogen), Prolong (Invitrogen)

## 8. SAFETY PRECAUTIONS AND WARNINGS

- Wear a suitable lab coat, disposable gloves and protective goggles when handling reagents and samples. Thoroughly wash hands before and after handling them.
- Do not pipette by mouth.
- Samples and reagents of human origin as well as contaminated material and products must be discarded in a contaminated residue container.
- If liquid containing human material is spilled, clean the affected area with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

For more information, please consult the appropriate material safety data sheets (MSDS) on our website: [www.genomicvision.com](http://www.genomicvision.com).

## 9. ASSAY PROCEDURE



### 9.1. Preparation of cells

#### Before starting cell manipulation:

- Set two water baths at 68°C and 50°C.
- Melt the **Buffer 2** at 68°C for 10 min and check that the gel is completely melted. Homogenize the melted solution by inverting the tube and keep it at 50°C.

Respect safety measures associated with the type of cells you are manipulating. Cells should be considered intact until ESP treatment of cells is completed.

- For *cultured cells*, harvest your cells according to cell lines' specifications.
- For *blood samples*, best results are obtained with white blood cells purified by either red blood cell lysis or Ficoll® gradient centrifugation. Blood should be fresh or have a maximum storage time of 5 days at 4°C. This procedure is not validated for extraction from frozen blood. Tested anticoagulants are EDTA, ACD.

## 9.2. Plug preparation: Embedding cells into agarose plugs

*Caution: The generation of cell suspension and the subsequent casting of the plugs should be performed as rapidly as possible in order to minimize premature cell lysis*

1. Completely suspend the cell pellet with the appropriate volume of **Buffer 1** (for cultured cells) or **PBS** (for cells extracted from blood). 45 µL of cell suspension is needed for 1 plug.

The number of cells to be embedded in a plug depends on the downstream application:

- For replication studies using approximately **50,000 - 100,000 cells / plug** is recommended.
- For physical cartography (hybridization) applications, typically **500,000 – 1,000,000 cells / plug** is recommended.
- Optimal cell number has to be decided by users for their own applications.

*Example: If you counted 20 million cells total and want to prepare plugs with 1 million cells, final cell suspension volume is 900 µL (1 million cells / 45 µL). For the preparation of 5 plugs, use 247.5 µL (= 5.5 x 45 µL) of the cell suspension.*

2. Homogenize well by pipetting (10 times up and down).  
*Caution: It is important that any clumps of cells have been removed prior to embedding cells into the agarose plug.*
3. Warm the cell suspension to 50°C for 10 seconds.
4. Mix the suspension with an equal volume of **Buffer 2** at 50°C.
5. Homogenize the solution well at 50°C by pipetting (10 times up and down).  
*Caution: Make sure that the cell/Buffer 2 mix is homogeneous and no clumps are visible.*
6. Immediately dispense the mixture quickly into DNA plug molds. The volume of one well is approximately 90 µL. Keep the solution in the water bath while dispensing. Do not allow bubbles to form.
7. Set the DNA plug mold at 4°C (in a box to prevent the agarose gel from desiccating) for 30 min.  
*Caution: if the plugs are not well solidified or look too soft, incubate them 15 min longer at +4°C.*

## 9.3. Protein digestion treatment of plugs

1. Prepare the appropriate volume of ESP buffer by mixing **Buffer 3** and **Component 3** (9:1 volume). 250 µL of ESP buffer is necessary for 1 plug. This mix cannot be prepared in advance.

*Example: for 10 plugs, add 250 µL of **Component 3** to 2250 µL of **Buffer 3**.*

2. Dispense the required volume of ESP buffer in the appropriate tube(s). Plugs from the same origin may be incubated together.
  - If handling 3 plugs or less, a 15 mL-tube should be used.
  - If handling more than 3 plugs, a 50 mL-tube is preferred.
3. Push the solidified plug out into the ESP Buffer using a plastic plunger.
4. Warm up the tube containing the plug(s) at 50°C. Since the tubes contain small volumes of solution, make sure the tubes are held vertically in the water bath.
5. After 30 min at 50°C, gently swirl the tubes to homogenize the solution.
6. Keep the tubes at 50°C overnight (16-18 hours).
7. The following day, dilute enough **Buffer 4** in a 1:100 proportion with nuclease-free water for the following four washing steps. If possible, autoclave **diluted Buffer 4** in advance or use only recently diluted Buffer. Do not store it.
8. Transfer DNA plugs to 15 mL-tube(s) filled with **diluted Buffer 4** (max 3 plugs / tube) with a spatula. Be careful when manipulating the plugs.
9. Wash the plugs for 1 hour on a test tube rotator.
10. Change the **diluted Buffer 4** and wash again for 1 hour with rotation. Repeat twice (in total 3 washes).
  
11. *If you store the plugs*, transfer the plugs in:
  - 1 mL of **Buffer 5** in a 2 mL-round-bottom microtube if handling one plug,
  - 5 mL of **Buffer 5** in a 15 mL-tube for up to 3 plugs,
  - 10 mL of **Buffer 5** in a 50 mL-tube for a maximum of 10 plugs.Store the plugs at 4°C. Plugs can be stored for 1 year at 4 °C in **Buffer 5**.

or

12. *If you use this DNA plug immediately* for DNA solution preparation, change once again the **diluted Buffer 4** in the tube and wash for 3.5 hours with rotation. Then go directly to step 2 of the next section (9.4. Preparation of DNA solution from DNA plug).

#### 9.4. Preparation of DNA solution from DNA plug

*If DNA plug is freshly prepared, go to step 2.*

1. If DNA plug is stored in **Buffer 5**, wash the plug in 15 mL of **diluted Buffer 4** (1:100 proportion Buffer 4: Nuclease-free water) in a 15 mL-tube for 60 min at room temperature, on a test tube rotator. Repeat twice (in total 3 washes).
2. Using a spatula, transfer one washed plug to a 2 mL-round-bottom microtube.

### Optional - DNA Prestaining:

If you want to directly visualize combed DNA on silanized coverslips, you can pre-stain DNA into the plugs.

*Note: this option is not recommended as the presence of prestaining dye can increase the background level of the corresponding color which results in a worse contrast for replication or hybridization. Alternatively, DNA may be stained after combing.*

If you do not intend to pre-stain DNA, you may proceed directly to step 3. If you do want to perform the pre-staining, follow the steps below.

- Mix 100  $\mu\text{L}$  of **Buffer 6** + 0.3  $\mu\text{L}$  of 1 mM YOYO-1 (not included in the kit) and add to the tube containing the DNA plug.
- Check that the plug is completely immersed. If not, tap the tube gently on the bench until the plug drops to the bottom of the tube.
- Keep the tube at room temperature for 1 hour, protected from light. From this step on, the plugs and DNA solutions should be protected from light whenever possible.
- Set a water bath at 68°C.
- Pour the plug and solution out of the microtube onto plastic wrapping paper and then put the plug back into the microtube with a spatula.

*Start here from step 2 if you don't perform the pre-staining:*

3. Add 1 mL of **Buffer 7** to the microtube.
4. Check that the plug is completely immersed. If not, tap the tube gently on the bench until the plug drops to the bottom of the tube.
5. Set the tube on a fixed microtube holder and confirm that the microtube is not moved by current of water bath. Incubate at 68°C for 20 min.



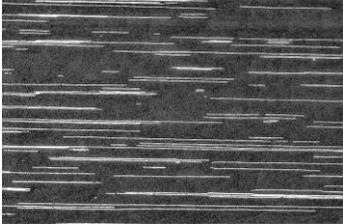
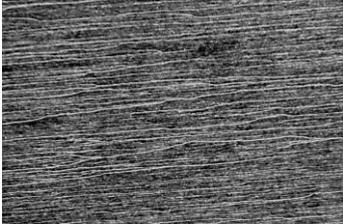
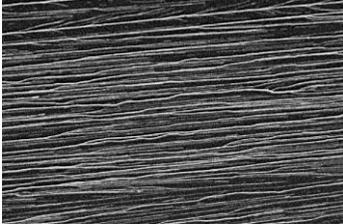
**Caution!** From this step on, manipulate DNA solutions with care, as DNA is no longer protected from mechanical shearing. Avoid any shaking, vibration, shock, etc.

6. During this incubation, equilibrate another water bath at 42°C.
7. After 20 min at 68°C, quickly transfer the microtube to the water bath at 42°C.  
**DO NOT** leave the microtube at room temperature between the two water baths.
8. Incubate the microtube at 42°C for 10 min. **NO SHAKING.**
9. Add 1.5  $\mu\text{L}$  of **component 7** to the microtube. **DO NOT MIX** the solution. Let the **component 7** diffuse spontaneously.
10. Incubate the microtube overnight (16-18 hours) at 42°C. **NO SHAKING.**

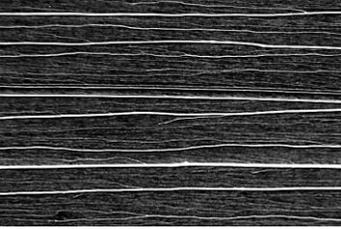
11. The following day, remove the microtube from the water bath.
12. Add 1200 µL of **Buffer 7** into a Disposable DNA Reservoir in advance.
13. **Gently** pour the DNA solution into the disposable DNA reservoir. Fill the reservoir with additional **Buffer 7** if necessary (up to a few millimeters below the top). The DNA solution is ready to be combed on a silanized coverslip with the FiberComb® Molecular Combing System.

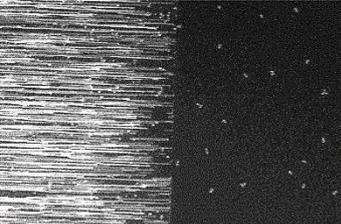
***Note:** the quality of DNA Extraction can be checked under a fluorescence microscope after the Molecular Combing step by mounting the coverslip with mounting medium. If the DNA is pre-stained (see section C.2. above – Preparation of DNA solution from plug), it can be directly visualized with filter sets adapted for Yoyo-1 fluorescence spectrum e.g. FITC. If no pre-staining is performed, it is still possible to include Yoyo-1 in the mounting medium (recommended concentration 1:10000) to stain combed DNA directly.*

## 10. TROUBLE SHOOTING GUIDE

Problem	Possible Cause	Suggestion
<b>1. Low combing density</b> = Low DNA concentration in the solution (low density of combed DNA on silanized coverslip)  	1.a) Low cell count	Make sure the cells were completely resuspended and homogenized when adding Buffer 1, and later on Buffer 2, by pipetting up and down at least 10 times.
	1.b) Loss of Proteinase activity	Check the Proteinase storage temperature and make sure to avoid any contamination sources when handling the Component 7. Use a new Component 7 aliquot.
	1.c) pH degradation of Buffer 7	Measure the pH of Buffer 7. If it is not 5.5, change Buffer 7.
<b>2. High combing density</b> = Too high DNA concentration in the solution (wavy or entangled molecules)  	2.a) DNA solution is too concentrated in the reservoir	Diluting solution with Buffer 7 is a possibility but may result in fiber breakage.
	2.b) High cell count	If initial material / sample is still available: decrease the number of cells used during plug preparation (prepare DNA plug with lower concentration of cells). If no initial material is available, use half or part of a plug.
<b>3. Linearity of DNA fibers</b> = Combed DNA shows wavy molecules on silanized coverslip  	3.a) Combing DNA solution while vibrating environment	Avoid any source of vibration close to MCS while combing.
	3.b) Insufficient plug washing	Washing time may be too short: try again with extended washing time.
	3.c) Problem (localized or not) on combing substrate (silanized coverslip)	Change substrate batch or surface.
	3.d) Mechanical stress after combing (during labeling process)	Comb a new silanized coverslip from the same solution and re-start the labeling process.
	3.e) pH degradation of Buffer 7	Cf. 1.c)

Problem	Possible Cause	Suggestion
<p>4.Short DNA fibers = Length of combed DNA on silanized coverslip is too short (DNA breakage)</p> 	4.a) Sharp handling of the DNA solution	From step 10 of section D (Preparation of DNA solution from DNA plug) the DNA is no longer protected from mechanical stress. Avoid any shaking, vibration or sharp movements when handling the DNA solutions.
	4.b) Insufficient plug washing	Washing timing may be too short: try again with extended washing time.
	4.c) Degraded starting sample material (cells)	If the starting material is not in optimal conditions (using old or frozen blood, for example), it is not possible to obtain a high quality DNA solution.
	4.d) Contamination with DNase	Avoid contamination sources and/or change buffers.
	4.e) Water bath temperature (> 68°C)	Check temperature of your water baths with a validated thermometer.
	4.f) Time of water bath (> 20 min)	Check duration of water baths steps.
	4.g) Vibrating source around DNA	cf. 4.a)
	4.h) Buffer 4 has been diluted with contaminated water and/or stored in diluted form.	Prepare a fresh solution of Buffer 4 and autoclave it. If it is not possible, always use recently diluted Buffer 4. Do not store it.
	4.i) Buffer 2 is too old or damaged and the gel can no longer protect the DNA from mechanical breaks.	Use a new bottle of Buffer 2 or a new kit.
	4.j) DNA is shortening during real time observation -> not inherently broken but breakage is induced during long exposure under microscope light.	Lower lamp intensity / power or reduce exposition time. Baking your coverslip in an oven at 60°C for 4 hr will reduce photobreaking. -> <b>Note:</b> the DNA solution quality is not the issue and can still be used for another combing for direct observation or hybridization (DNA molecules are not broken in solution).

Problem	Possible Cause	Suggestion
<p>5. DNA is not individualized = presence of very bright fiber bundles, network...</p> 	5.a) Non-efficient digestion of proteins during proteinase step	<p>Check the storage temperature and if the protocol has been followed carefully, or change Proteinase tube / Agarase tube.</p> <p>-&gt; <b>Caution:</b> storage temperature of the enzymes is critical (Trypsin, Proteinase and Agarase are thermosensitive)</p>
	5.b) Homogenization of cells during plug preparation is insufficient (cell aggregates)	Repeat plug preparation with extended mixing, check when cell counting that cells are isolated correctly and evenly distributed.
	5.c) Activity of Trypsin is degraded	Change Buffer 1 tube. Avoid multiple thawing/freezing of Buffer 1.
	5.d) Problem during cell harvesting (adherent cells)	Cf. 5.b)
	5.e) Defect localized on substrate	Change silanized coverslip or batch.
	5.f) DNA is not well distributed / homogenized in combing buffer solution / Suspension is not achieved	Repeat combing a day (or a few more days) later.
	5.g) DNA is too concentrated in solution	Cf. 2)
<p>6. Bad stability of DNA solution = Density (or length) is lowered over time / No DNA after 24 hr of solution storage</p>	6.a) Contamination of DNA solution with DNase or other DNA damaging agent (ex. metal ion...)	Change or autoclave materials that may be contaminated (spatula, tubes, pipette tips, ...)
	6.b) Vibration / shock of DNA solution during storage	Pay attention to avoid vibrations during storage and handling.
	6.c) Aging / natural degradation of DNA in solution	Recommendation is to comb DNA solution as soon as possible, storage in disposable reservoirs with Buffer 7 should not extend more than 7 days. It is recommended to store plugs rather than DNA solution of a specific sample, as plugs are more stable.
	6.d) Contamination of solution during combing	If possible, do all necessary combing in one session. We recommend to store plugs rather than combing the solution of a specific sample.

Problem	Possible Cause	Suggestion
7. Contrast of combed DNA = Low signal/noise ratio, signals are very low intensity and high background.	7.a) Premature aging of silanized coverslip or silanized coverslip defect	Repeat combing changing silanized coverslip.
	7.b) Mounting medium is not homogenized enough	Repeat combing and observation after homogenizing mounting medium (vortex).
	7.c) Mounting medium is too concentrated in marker (YOYO-1)	Prepare a new mounting medium making sure to have an adequate concentration.
8. Residues in solution = Visible colored or white residues (pellet or fiber) remain in the genomic DNA solution	8.a) Entangled DNA molecules	You may try to reach the residue using the tip of a pipette (autoclaved), and take it out of the solution. However, resulting DNA solution can be broken or still entangled.
	8.b) DNA aggregates due to a bad homogenization of cells during plug preparation	When possible, repeat plug preparation with extended mixing and check when cell counting that cells are isolated correctly and evenly distributed.
9. Defect on silanized coverslip not linked to DNA solution (white or black structure or stains, crystals, scratch, ...)		9.a) If defects are visible on combed coverslip, check if defects are also present at the limit of combing (where the surface has not been dipped into DNA solution) : if so, it may be defects coming from silanized coverslip
		9.b) Scratch performed during manipulation
10. Colored Buffer 1 -> normal colour is pink/reddish but it may turn yellow/purple depending on pH concentration or degradation	10.a) pH of solution is not correct	Do not use the Buffer 1. Use another aliquot.
	10.b) Buffer 1 has been left to ambient temperature instead of 4°C after first thawing of -20°C aliquot	Do not use the Buffer 1. Use another aliquot.
11. White precipitate in Buffer 7 (salt crystals, ...)	11.a) Water has evaporated from the Buffer 7, or temperature of DNA solution was excessively cooled (below 4°C) and precipitates have formed	Concentration in salts may not be correct. Do not use the Buffer 7. Use another aliquot.
12. Buffer 7 has turned slightly yellow	12.a) Inappropriate storage condition (Buffer 7 should be stored in refrigerator at 4°C)	Do not use the Buffer 7. Use another aliquot.

## 11. ORDERING INFORMATION & RELATED PRODUCTS

Product	Contents	Reference
FiberPrep® DNA extraction kit	100 extractions	EXT-001
FiberComb® Molecular Combing System	Combing device	MCS-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-002-RUO COV-002-IVD

To place an order, please contact us at [sales@genomicvision.com](mailto:sales@genomicvision.com)  
or visit our website <http://www.genomicvision.com/gv-store/>



Our experts are available to answer your questions

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### Intellectual Property Rights

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.

### Quality Control

The FiberPrep<sup>®</sup> DNA Extraction Kits undergo strict Quality Controls performed in Genomic Vision's laboratories. Should you nevertheless experience problems with the product, please contact the technical support team.

### Trademarks:

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