Chapter 22

Extended DNA Fibers for High-Resolution Mapping

Paul Fransz, José van de Belt, and Hans de Jong

Abstract

DNA fiber-FISH is an easy and simple light microscopic method to map unique and repeat sequences relative to each other at the molecular scale. A standard fluorescence microscope and a DNA labeling kit are sufficient to visualize DNA sequences from any tissue or organ. Despite the enormous progress of high-throughput sequencing technologies, DNA fiber-FISH remains a unique and indispensable tool to detect chromosomal rearrangements and to demonstrate differences between related species at high resolution. We discuss standard and alternative steps to easily prepare extended DNA fibers for high-resolution FISH mapping.

Key words Extended DNA fiber, Chromatin fiber, FISH, Physical mapping

1 Introduction

Microscopic mapping of DNA probes onto chromosomal targets has been a strong and efficient tool ever since the first in situ detection of nucleic acids by Gall and Pardue in 1969 [1]. This study involved localization of repetitive ribosomal DNA using autoradiographic detection of the radioactive RNA probes. During subsequent decades, the in situ hybridization protocol was improved in all possible aspects [2, 3]. The higher efficiency, sensitivity, and resolution made fluorescence in situ hybridization the default tool in molecular cytogenetics. A major step in further improving spatial microscopic resolution was the development of DNA fiber-FISH in human cells [4, 5] and in plants [6]. This technique enables to map sequences at kb resolution, which is extremely high compared to the 50 kb resolution for interphase-FISH and 100 kb for pachytene-FISH and 5 Mbp for metaphase FISH [7]. DNA fiber-FISH is used to map unique and repeat sequences relative to each other at the molecular scale without using sophisticated sequencing technologies (*see* Figs. 1 and 2 for mapping along extended DNA fibers from tomato and Arabidopsis). The technique is based on the histone-depletion methods to

Tony Heitkam and Sònia Garcia (eds.), *Plant Cytogenetics and Cytogenomics: Methods and Protocols*, Methods in Molecular Biology, vol. 2672, https://doi.org/10.1007/978-1-0716-3226-0_22,

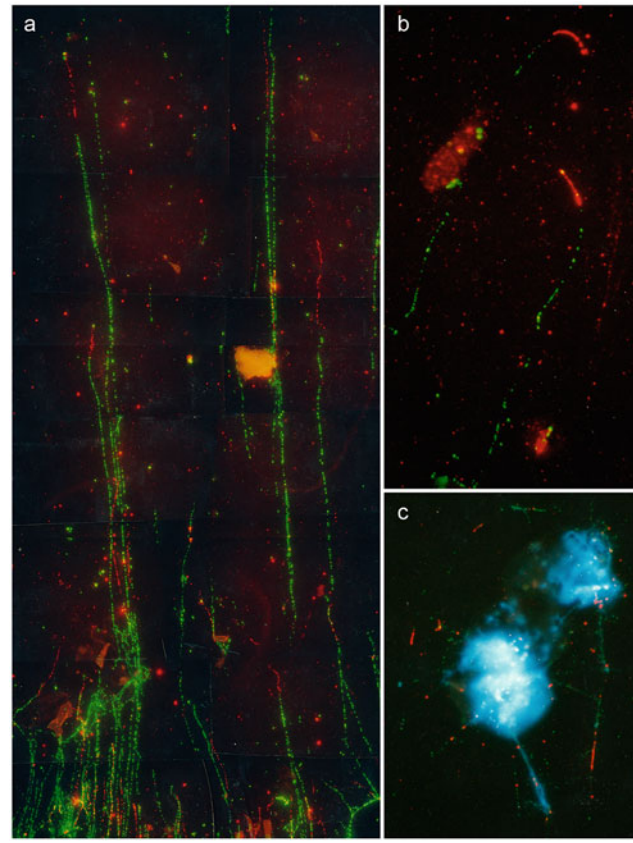

Fig. 1 Extended DNA fibers from young tomato leaves visualized by FISH. (**a**) FISH to tomato DNA fibers with a telomeric probe (red) and a subtelomeric repeat TGR1 (green). Note the long parallel stretches of fluorescent TGR1 signals that can span up to 2 Mb. The red telomeres denote the end of chromosomal DNA molecule. (**b**) The red-green fluorescent signals of telomere and TGR1 have the same pattern, suggesting they are the ends of a pair of chromatids of the same chromosome. (**c**) If nuclei are not sufficiently lysed, the nucleus (blue) is still visible while few short fibers are visible

produce nuclear halos [8, 9], which consist of extended DNA loops surrounding the remaining nuclear matrix. In the early nineties, various high-resolution mapping papers appeared showing FISH to extended DNA fibers or chromatin fibers (Table 1). The protocol as we discuss here consists of two main steps to prepare DNA or chromatin fibers: (1) lysis of purified nuclei and (2) spreading of the released DNA or chromatin fibers. Since there are many ways to disrupt nuclei and spread DNA or chromatin, several different protocols have been published (Table 1).

The protocol for nuclei isolation starts with separation of nuclei from cell walls and cytoplasm. Although some contamination with cytoplasm debris is inevitable, the presence of too many organelles, in particular chloroplasts and amyloplasts, may hamper proper

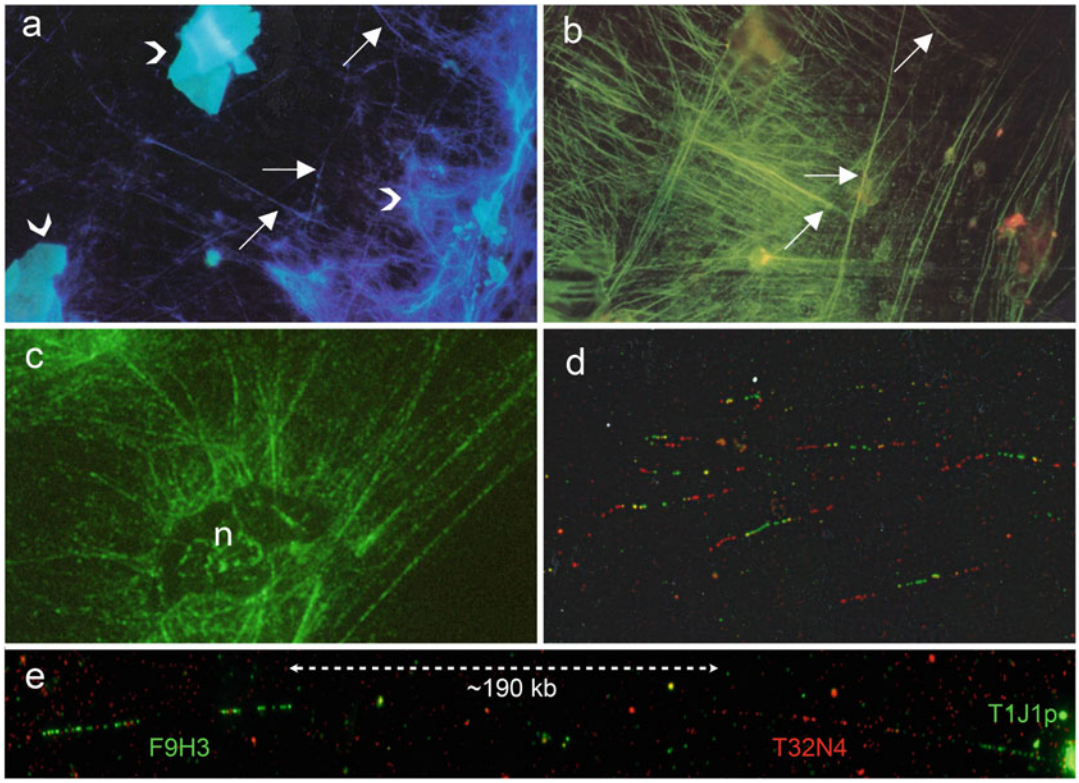

Fig. 2 Extended DNA fibers from tomato and Arabidopsis visualized by FISH, DAPI, YOYO, and in situ labeling. Tomato DNA fiber preparation stained simultaneously with DAPI (**a**) and YOYO (**b**). DAPI also stains other cell components (arrowheads), while with YOYO many more DNA fibers are visible. Arrows indicate similar DNA fibers in **a** and **b**. (**c**) In situ labeling with FITC-dUTP reveals a crisscross pattern of many DNA fibers near the disrupted nucleus (n). Note that the DNA of the extended DNA fiber, although attached to the glass slide, is well accessible for the DNA polymerase to synthesize fluorescent DNA strands. (**d**) A characteristic image of DNA fiber-FISH with unique DNA sequences from Arabidopsis. The partially overlapping red-green-red cosmids span 30–35 kb each. (**e**) FISH to Arabidopsis accession C24 with three BACs each spanning approximately 90–100 kb. The distance between the regions F9H3 (green) and T32N4 (red) is estimated at ±190 kb and spans part of an inversion. This distance was unknown for this accession, of which the genomic sequence was aligned against the reference accession Col-0, where the regions F9H3 and T32N4 are separated by almost 1 Mb. Hence, the region with the inversion was not properly mapped in C24 by sequencing, while fiber-FISH reveals the precise map positions

interpretation of the fluorescent image. A simple pre-treatment with a mild detergent such as Triton X-100 can help dissolve membranes, thereby degrading most of the organelles. While nuclear envelopes are also damaged by the detergent, nuclei with their compact chromatin organization remain sufficiently intact during the treatment. Although in most studies unfixed tissue is used, ethanol/acetic acid fixed nuclei were also found suitable for obtaining extended DNA fibers [4, 15, 23].

The next step involves disruption of isolated nuclei and release of chromatin or DNA fibers. Chromosomal DNA is complexed

Table 1
Overview of DNA fiber preparation methods

Stretched fiber	Lysis buffer	Stretching method	Cell type	Reference
Chromatin fiber	0.5 M NaCl + triton + 0.2 M urea	Slide retrieval from solution	Cultured human cell	[10, 11]
Chromatin fiber	0.5 M NaCl + triton + 0.2 M urea	Parafilm dragging	Maize callus	[12]
Chromatin fiber	Alkaline (NaOH)	None	Cultured human cell	[4]
Chromatin fiber	Alkaline (NaOH)	Coverslip sliding	Cultured human cell	[13]
Chromatin fiber	Formamide	Coverslip sliding	Cultured human cell	[13]
Nuclear halo	2 M NaCl	None	Cultured human cell	[14]
Nuclear halo	3,5-diiodosalicylic acid + EDTA	None	Cultured human cell	[14]
Chromosome stretching	60% acetic acid + triton	Squash with rolling thumb	Maize	[15]
Chromosome stretching	Proteinase K	Removal of lysis buffer by ethanol/acetic acid	Isolated plant chromosomes	[16]
DNA fiber	Alkaline (NaOH)	Tilting of slide	Isolated plant nuclei	[6]
DNA fiber	None	Coverslip drying (molecular combing)	DNA clones, *E. coli* DNA	[17, 18]
DNA fiber	None	Coverslip drying (molecular combing)	Lambda DNA	[7]
DNA fiber	None	Coverslip drying (molecular combing)	BAC DNA	[19]
DNA fiber	Nonidet/EDTA	Gentle removal of lysis buffer	Cultured human cell	[20]
DNA fiber	SDS/EDTA	Tilting of slide	Cultured human cell	[21]
DNA fiber	SDS/EDTA	Tilting of slide	Isolated plant nuclei	[6]
DNA fiber	SDS/EDTA	Coverslip sliding	Isolated plant nuclei	[19]
DNA fiber	SDS/EDTA	Tilting of slide with coverslip sliding	Young plant tissue	[22, 23]
DNA fiber	SDS/EDTA and proteinase K	Tilting of slide	Isolated plant nuclei	[24]

with histones, non-histone proteins, and RNA molecules forming a nucleosome polymer. Quite a few chemical treatments are able to dismantle chromosome structure and separate DNA from proteins, of which high salt or strong detergent in combination with EDTA is most often applied. Mild treatment (e.g., 0.5 M NaCl) leads to relatively intact chromatin fibers, whereas stronger disruption of the chromatin (e.g., SDS/EDTA) results in release of naked DNA fibers.

Lysis of purified nuclei is accompanied by unfolding of DNA in every direction in the aqueous droplet, and this may impede FISH mapping of DNA sequences. Hence, a stretching force is needed to generate extended DNA fibers that are visible as long arrays under the microscope. It is inevitable that the stretching method will break the megabase pair long DNA fibers of the chromosomes. However, stretching with a mild force will limit the breakage of the DNA fibers. Another problem that may occur is the crisscross pattern of DNA fibers without a directional flow of fibers. This may occur if the disruption or lysis of the nuclei is too strong, which may occur by high concentration of a strong detergent and/or EDTA [6]. In case of chromatin fibers and halo-FISH, chromatin is partially intact, but chromosomal structure got lost to a great extent. Several proteins are still complexed with the DNA. Hence, both FISH and immunostaining can be applied [12, 25]. DNA combing is based on the stretching of isolated high molecular weight DNA molecules from cloned fragments or from viral/phage DNA. Stretching of the DNA molecules occurs by the receding meniscus when the droplet with DNA molecules is dried on a slide (reviewed by [26]). Similar results with isolated DNA clones can be obtained via sliding the DNA solution across the slide using a coverslip [19]. With this method, even circular DNA molecules from BAC clones were maintained as circular structures.

Here, we present and discuss a simple and efficient method to prepare extended DNA fibers from any plant tissue. In addition, we describe a general FISH method to detect target sequences and add alternative steps to circumvent some problems that often occur. Several conditions for the preparation of DNA fibers and FISH detection have been described but are in principle not necessary. A successful DNA fiber-FISH experiment relies on high-quality DNA fibers and DNA probes, appropriate reference targets, and positive control preparations. Therefore, we recommend the following actions when DNA fiber-FISH is applied:

- Since the morphological reference is absent, it is strongly recommended to use appropriate reference probes to check the mapping. Fiber-FISH experiments with at least two probes in different colors are required to reliably map the DNA sequence of interest (Figs. 1a, b and 2d, e).

- Good quality FISH probes are essential for a successful experiment. Therefore, check the quality of your labeled probes.
- It is recommended to check the presence of accessible DNA fibers prior to the fiber-FISH method. This can be done by DAPI staining or YOYO staining (Fig. 2a, b). Even more sensitive is to apply in situ labeling with FITC-dUTP of the test preparation (Fig. 2c). It would be wasteful if you apply FISH to slides with low-quality fibers.
- The use of control probes (e.g., rDNA or other repeat) is recommended to test whether the FISH procedure has been correctly established. If your probe of interest gives no signal, but the control probe did, then something may be wrong with your probe (or its target), but not with the FISH procedure.

2 Materials

Sterilization of the material or the solutions is not necessary because DNase is not active in most solutions of this protocol.

2.1 Isolation of Nuclei

1. 0.5 g plant material (*see* **Note 1**).
2. Nuclei isolation buffer (NIB): 10 mM Tris–HCl, pH 9.5, 10 mM EDTA, 100 mM KCl, 0.5 M sucrose, 4 mM spermidine, 1.0 mM spermine, 0.1% (v/v) 2-mercaptoethanol. Keep the NIB on ice (*see* **Note 2**).
3. 10% Triton X-100 in NIB.
4. DAPI: 1 μg/mL 4,6-diamidino-2-phenylindole in Vectashield antifade mounting medium.
5. Razor blade.
6. Petri dishes.
7. Eppendorf tubes.
8. Wide-bore tips.
9. Nylon mesh (pore size 125 μm) filter.
10. Microscopic slides.
11. Coverslips (22 × 22 mm^2).

2.2 DNA Fiber Preparation

1. STE buffer: 0.5% (w/v) SDS, 5 mM EDTA, 100 mM Tris–HCl pH 7.0.
2. Ethanol/acetic acid (3:1).
3. DAPI: 1 μg/mL 4,6-diamidino-2-phenylindole (DAPI) in Vectashield antifade mounting medium.
4. 0.1 μM YOYO in Vectashield antifade mounting medium.

5. Microscopic slides.
6. Coverslips (22 × 50 mm^2).

2.3 Probe Labeling

Probes can be labeled by nick translation with fluorescent dye conjugates (Cy3, Cy5, fluorescein, rhodamine, AMCA, DEAC, etc.), or non-fluorescent haptens (biotin, digoxygenin). Nick translation kits with various labeled dUTP can be purchased from several companies (Thermo Fisher Scientific, Jena Bioscience, Sigma-Aldrich, or Perkin Elmer, etc.). We generally use Cy3-dUTP (Jena Bioscience) and Alexa-Fluor-488-dUTP (Thermo Fisher Scientific). Non-fluorescently labeled probes need to be visualized by fluorescent antibodies, for which there are several protocols available [23, 27, 28]. The advantage of direct labeling is the shorter procedure. The advantage of indirect detection is that it is more sensitive. However, the background can be higher due to non-specific binding of the antibodies.

For the labeling of the probes, please follow the instructions from the manufacturer. Store the labeled probes in the fridge or in the freezer.

2.4 In Situ Hybridization

1. Labeled probe(s) typically 50 ng/μL (*see* Subheading 2.3).
2. Hybridization buffer (HB50), containing 50% deionized formamide, 2× SSC (1× SSC = 0.15 M NaCl/0.015 M Na_3 citrate) and 50 mM sodium phosphate (pH 7.0), 10% dextran sulfate in HB50.
3. 3 M sodium acetate (pH 5.2).
4. Ice-cold 96% ethanol.
5. 70% ethanol.
6. In case of separate denaturation (*see* Subheading 3.3.2), the individual components of the hybridization buffer are needed:
   (a) Labeled probe(s) typically 50 ng/μL (*see* Subheading 2.3).
   (b) Warm (37 °C) 50% dextran sulfate.
   (c) Wide-bore tip to pipet dextran sulfate.
   (d) HB50 (2× SSC, 50 mM sodium phosphate pH 7.0, 50% deionized formamide).
   (e) Ice-cold 2× SSC.
   (f) Ice-cold 70% ethanol.
7. Coplin jars.
8. Vectashield antifade mounting medium (Vector Laboratories).
9. Ethanol series (70%, 90%, and 100%).
10. Coverslips (22 × 22 mm^2, 22 × 36 mm^2, 24 × 50 mm^2).

11. Moist chamber (plastic box with wet paper at the bottom and platform to place the slides).
12. Incubator (37 °C).
13. Hot plate.

3 Methods

3.1 Isolation of Nuclei

1. Place tissue and pipet 0.1–0.5 mL NIB into Petri dish on ice (*see* **Note 3**).
2. Chop the tissue with a razor blade to a fine suspension. Slightly tilt the dish allowing the suspension to move to one side, while large tissue fragments remain in the middle of the dish. Keep chopping until only small (<0.5 mm) tissue fragments are left and the suspension becomes green (in case of leaf tissue). This process may take few minutes.
3. Filter as much of the suspension through a 120 μm mesh filter into an Eppendorf tube. Use wide-bore tips. Add 0.5 mL NIB to the Petri dish to save the remaining nuclei and pipet this also through a 120 μm mesh filter into an Eppendorf tube (*see* **Note 4**).
4. Add 1/20 volume of 10% Triton X-100 in NIB to remove chloroplasts.
5. Spin down the filtrate at 1000 g for 5 min, at 4 °C (*see* **Notes 5** and **6**).
6. Remove the supernatant and resuspend the (white) pellet with nuclei in ~25 μL NIB.
7. Inspect the nuclei by mixing 2 μL of suspension with 5 μL of 1 μg/mL DAPI in Vectashield on a microscope slide. Examine under fluorescence microscope (40× objective) (*see* **Notes 7** and **8**).
8. If necessary, adjust the concentration of nuclei. Either dilute the concentration by adding extra NIB or concentrate the suspension by centrifugation (1000 g for 5 min) and removal of part of the supernatant.
9. The suspension can be used immediately for DNA fiber preparation. Alternatively, mix the suspension with an equal volume of glycerol and store at −20 °C until use. The suspension can be stored for several weeks (*see* **Note 9**).

3.2 Preparation of DNA Fibers

1. Pipet 1–2 μL of the suspension onto one end of a clean microscope slide. Spread the suspension across a 1 cm^2 area of the slide to prevent accumulation of the nuclei in the center of the droplet. Air-dry for 2–3 min at RT.

2. Lyse the nuclei by adding 30 µL of STE and incubate for 4 min at RT (*see* **Note 10**).
3. Tilt the slide at an angle of ~45° to let the buffer float slowly downward (*see* **Note 11**).
4. Alternatively: Pipet two 50 µL droplets of STE onto a long coverslip (22 × 50 mm^2) at 30 mm distance. Pick up the coverslip with the microscope slide by touching the slide to the droplets and allowing the STE to fill the space between the slide and the coverslip via capillary forces. Keep the slide horizontal. Incubate for 30 s. Carefully remove the coverslip by slowly tilting the microscope slide, allowing the coverslip to slip off.
5. Air-dry the slides at RT and fix the DNA fibers in a Coplin jar with ethanol/acetic acid (3:1) for 2 min.
6. Air-dry the slides at RT and store the preparations in a dry box until use.
7. Check the quality of the fibers, by taking one slide, add 10 µL of DAPI or YOYO, and cover with a 22 × 50 mm coverslip. Examine the fibers under the microscope (*see* **Note 12**).

3.3 FISH to DNA Fibers

3.3.1 FISH to DNA Fibers (Standard)

1. Bake the slides at 60 °C for 20 min on a hotplate.
2. Purify the labeled probe (*see* Subheading 2.3) by adding 1/10 volume of 3 M sodium acetate and 2.5 volumes ice-cold ethanol. Mix gently and keep on ice for 30 min.
3. Centrifuge at 10,000 g for 30 min at 4 °C.
4. Wash the pellet with 70% ethanol and centrifuge at 10,000 g for 10 min.
5. Air-dry the pellet and resuspend in HB50.
6. Mix 50–250 ng of each labeled probe (*see* **Note 13**) with HB50 to a final volume of 20–40 µL.
7. Add the hybridization mixture to the slide preparation and cover with a 22 × 22 m^2 coverslip.
8. Denature the extended DNA fibers and the probe DNA on a hotplate at 80 °C for 2 min.
9. Put the slides in a moist chamber (*see* Subheading 2.4, **item 11**) and incubate overnight at 37 °C. In case highly repetitive regions are to be detected, a few hours (2–3) incubation is sufficient.

3.3.2 FISH to DNA Fibers Using Separate Denaturation of Probe and Target DNA

In general, the labeled probes are precipitated, dried, and dissolved in HB50 hybridization buffer. These steps take much time and, in some cases (if the probe contains long fragments, e.g., from large DNA clones and is not properly dissolved), can lead to a high

background. Therefore, we often use the procedure of *separate denaturation of probe and target DNA*.

1. Bake the slides at 60 °C for 20 min on a hotplate.
2. Take 50–250 ng of each labeled probe (*see* Subheading 2.3) and add HB50 to a final volume of 20–30 μL (*see* **Note 13**).
3. Denature the probes at 95 °C for 5 min and cool down on ice.
4. Denature the extended DNA fibers by adding a droplet of 30 μL of HB50 to the slide, cover with a coverslip (22 × 36 m^2) and heat for 2 min on a hotplate at 80 °C.
5. After denaturation, immediately rinse the slide in ice-cold 2× SSC for 2 min followed by 2 min rinsing in 70% ice-cold ethanol, 2 min in 90% ethanol, and 2 min in 100% ethanol.
6. Air-dry the slide preparation at RT.
7. Add one-fifth volume of warm 50% dextran sulfate to the probe mixture and mix well (*see* **Note 14**).
8. Add the probe mixture to the slide preparation and cover with a coverslip (22 × 22 mm^2 or 22 × 36 mm^2 depending on the volume of the probe mixture).
9. Put the slides in a moist chamber (*see* Subheading 2.4, **item 11**) and incubate overnight at 37 °C. In case highly repetitive regions are to be detected, a few hours (2–3) incubation is sufficient.

3.4 Post-hybridization Wash

1. Remove the coverslip by washing in 2× SSC at RT.
2. Remove the liquid as much as possible, but do not let the slide become dry.
3. Pipet 50 μL of SF50 to the slide and cover with 22 × 50 mm^2 coverslip (*see* **Note 15**).
4. Incubate at 42 °C for 20–30 min.
5. Remove the coverslip by washing in 2× SSC.
6. Dehydrate via 2 min washes in ethanol (70%, 90%, and 100%) and dry the slide *see* **Note 16**).
7. Add 12 μL of 1 μg/mL DAPI in Vectashield solution and cover with coverslip (22 × 50 mm^2).
8. The preparation is ready for microscopic inspection.

4 Notes

1. The amount of material depends on the tissue/organ type. For example, for young leaves, you need less material than for old leaves. The reason is that the density of cells (and therefore the

nuclear density) in young leaves is higher than in old leaves, where the cellular volume is much larger. In principle, one can start with 1–2 g of leaf tissue. Root tips and shoot apices contain many nuclei. A few (5–10) root tips or shoot apices should give sufficient nuclei for DNA fiber analysis. However, these organs are very small and difficult to handle for nuclei isolation if you have only few samples. For example, for Arabidopsis organs you will need more material than for the larger wheat organs. In this respect, the chopping method is often more efficient than the grinding method with liquid nitrogen, since it is more difficult to save all powder if small amounts of tissue (e.g., few root tips) are ground.

2. NIB without 2-mercaptoethanol can be prepared and stored at −20 °C. Add the 2-mercaptoethanol just before use.
3. The volume of buffer depends on the amount of tissue, since the tissue fragments will float in too much liquid, which impedes fragmentation by chopping with the razor blade.
4. A larger mesh filter (up to 250 μm) is also possible. Too small mesh size may lead to clogging of debris and nuclei. To obtain more nuclei the residue, which contains many nuclei that are stuck in the debris, can be transferred into a 0.5 mL NIB. This suspension can be filtrated through 120 μm mesh filter as described before.
5. The specific weight of nuclei is higher than other cell compartments or structures.
6. Instead of centrifugation, it is also possible to keep the tube right up on ice on the bench for some time.
7. The concentration of nuclei should be sufficient to easily find them without searching. However, too high concentration (overlapping nuclei) causes too high density of DNA fibers. A nuclei concentration of 5×10^5 per mL is often used as a directive.
8. Nuclei should be intact as much as possible. Long extended DAPI fluorescent threads indicate premature lysis of nuclei. This may affect the preparation of extended DNA fibers later in the protocol.
9. Remove the glycerol by centrifugation at 1000 g for 5 min and resuspending the pellet in an appropriate volume of NIB.
10. If the nuclei are insufficiently lysed, use STE with higher concentration (e.g., 50 mM EDTA) or use 2 M NaCl instead of STE.
11. This step enables the DNA fibers, which are partly attached to the glass surface, to move downward and gently stretch.

12. Fluorescent fibers should be visible over the entire slide. Nearby the site where the nuclei have been dropped the fibers show a crisscross pattern, whereas further away they show long parallel lines (Fig. 2a). Note that YOYO is more sensitive than DAPI and can make single DNA fibers visible (Fig. 2b, c).
13. For highly repetitive DNA elements, you need less labeled probe than for unique DNA sequences.
14. Use a wide-bore tip to pipet the dextran sulfate, which is less viscous at high temperature.
15. In many protocols, large volumes of the toxic formamide are used, which is not necessary. Therefore, we successfully tested the use of small quantities of formamide to remove non-specific binding of probes.
16. This step is not essential. After washing with 2× SSC, DAPI can be applied to the slide.

References

1. Pardue ML, Gall JG (1969) Molecular hybridization of radioactive DNA to the DNA of cytological preparations. Proc Natl Acad Sci 64:600–604
2. Volpi EV, Bridger JM (2008) FISH glossary: an overview of the fluorescence in situ hybridization technique. BioTechniques 45:385–409
3. Jiang J (2019) Fluorescence in situ hybridization in plants: recent developments and future applications. Chromosom Res 27:153–165
4. Heng HH, Squire J, Tsui LC (1992) High-resolution mapping of mammalian genes by in situ hybridization to free chromatin. Proc Natl Acad Sci 89:9509–9513
5. Wiegant J, Kalle W, Mullenders L, Brookes S, Hoovers JMN, Dauwerse JG, Van Ommen GJB, Raap AK (1992) High-resolution in situ hybridization using DNA halo preparations. Hum Mol Genet 1:587–591
6. Fransz PF, Alonso-Blanco C, Liharska TB, Peeters AJM, Zabel P, Jong JH (1996) High-resolution physical mapping in Arabidopsis thaliana and tomato by fluorescence in situ hybridization to extended DNA fibres. Plant J 9(3):421–430
7. de Jong JH, Fransz P, Zabel P (1999) High resolution FISH in plants – techniques and applications. Trends Plant Sci 4:258–263
8. Cook PR, Brazell IA, Jost E (1976) Characterization of nuclear structures containing superhelical DNA. J Cell Sci 22:303–324
9. Vogelstein B, Pardoll DM, Coffey DS (1980) Supercoiled loops and eucaryotic DNA replication. Cell 22:79–85
10. Cohen SM, Chastain PD, Cordeiro-Stone M, Kaufman DG (2009) DNA replication and the GINS complex: localization on extended chromatin fibers. Epigenetics Chromatin 2:6–6
11. Blower MD, Sullivan BA, Karpen GH (2002) Conserved organization of centromeric chromatin in flies and humans. Dev Cell 2:319–330
12. Jin W, Melo JR, Nagaki K, Talbert PB, Henikoff S, Dawe RK, Jiang J (2004) Maize centromeres: organization and functional adaptation in the genetic background of oat. Plant Cell Online 16:571–581
13. Fidlerová H, Senger G, Kost M, Sanseau P, Sheer D (1994) Two simple procedures for releasing chromatin from routinely fixed cells for fluorescence in situ hybridization. Cytogenet Genome Res 65:203–205
14. Gerdes MG, Carter KC, Moen PT, Lawrence JB (1994) Dynamic changes in the higher-level chromatin organization of specific sequences revealed by in situ hybridization to nuclear halos. J Cell Biol 126:289–304
15. Koo D, Jiang J (2009) Super-stretched pachytene chromosomes for fluorescence in situ hybridization mapping and immunodetection of DNA methylation. Plant J 59:509–516
16. Valárik M, Bartoš J, Kovářová P, Kubaláková M, De Jong JH, Doležel J (2004) High-resolution FISH on super-stretched flow-sorted plant chromosomes. Plant J 37: 940–950
17. Bensimon A, Simon A, Chiffaudel A, Croquette V, Heslot F, Bensimon D (1994)

Alignment and sensitive detection of DNA by a moving interface. Science 265:2096–2098

18. Weier H-UG, Wang L, Mullikin JC, Zhu Y, Cheng J-F, Greulich KM, Bensimon A, Gray JW (1995) Quantitative DNA fiber mapping. Hum Mol Genet 4:1903–1910
19. Jackson SA, Dong F, Jiang J (1999) Digital mapping of bacterial artificial chromosomes by fluorescence in situ hybridization. Plant J 17(5):581–587
20. Houseal TW, Dackowski WR, Landes GM, Klinger KW (1994) High resolution mapping of overlapping cosmids by fluorescence in situ hybridization. Cytometry 15:193–198
21. Parra I, Windle B (1993) High resolution visual mapping of stretched DNA by fluorescent hybridization. Nat Genet 5:17–21
22. Fransz P, de Jong H, Zabel P (1998) Plant molecular biology manual, pp 49–66. https://doi.org/10.1007/978-94-011-5242-6_4
23. Dechyeva D, Schmidt T (2016) Plant cytogenetics, methods and protocols. Methods Mol Biol 1429:23–33
24. Yang K, Zhang H, Converse R, Wang Y, Rong X, Wu Z, Luo B, Xue L, Jian L, Zhu L, Wang X (2011) Fluorescence in situ hybridization on plant extended chromatin DNA fibers for single-copy and repetitive DNA sequences. Plant Cell Rep 30:1779
25. Zhang W, Lee H-R, Koo D-H, Jiang J (2008) Epigenetic modification of centromeric chromatin: hypomethylation of DNA sequences in the CENH3-associated chromatin in Arabidopsis thaliana and maize. Plant Cell 20:25–34
26. Weier H-UG (2001) DNA fiber mapping techniques for the assembly of high-resolution physical maps. J Histochem Cytochem 49: 939–948
27. Lysak M, Fransz P, Schubert I (2006) Cytogenetic analyses of Arabidopsis. Methods Mol Biol 323:173–186. https://doi.org/10.1385/1-59745-003-0:173
28. Bey TD, Koini M, Fransz P (2017) Plant chromatin dynamics, methods and protocols. Methods Mol Biol 1675:467–480