Chapter 24

FISHing for Damage on Metaphase Chromosomes

P. Logan Schuck and Jason A. Stewart

Abstract

Fluorescence in situ hybridization (FISH) is used to examine chromosomal abnormalities and DNA damage. Developed in the early 1980s, this technique remains an important tool for understanding chromosome biology and diagnosing genetic disease and cancer. Use of FISH on metaphase chromosomes allows the visualization of chromosomal abnormalities at specific loci. Here, we describe methods for creating metaphase chromosome spreads and the use of telomere FISH probes to detect chromosome ends.

Key words FISH, Microscopy, Chromosome, Telomere, Metaphase spread

1 Introduction

In 1842, Carl Wilhelm von Nägeli first observed chromosomes in dividing plant cells [1]. While some herald this as the beginning of karyotyping, it was not until 1955 that the correct number of human chromosomes was determined by metaphase spread analysis, helping to usher in the field of chromosome biology [2]. This field was rapidly advanced in 1968 when the first in situ hybridization was successfully performed on *Xenopus* chromosomes [3]. This first approach used radiolabeled ribosomal RNA probes to detect stationary chromosomal DNA. The subsequent creation of biotin-labeled polynucleotides allowed for easy capture and fluorescein labeling of specific RNA/DNA sequences, making the technique more accessible [4].

Early fluorescent-labeled probes were large and relied on highly repetitive DNA sequences to obtain a detectable signal. However, this was a major improvement over autoradiographs, which required long exposure times and handling of radioactive material [3]. In the early 1980s, the quality and synthesis of probes continued to improve along with the detection of fluorescent dyes, leading to what we now know as fluorescence in situ hybridization (FISH) [5–8].

Lata Balakrishnan and Jason A. Stewart (eds.), *DNA Repair: Methods and Protocols*, Methods in Molecular Biology, vol. 1999, https://doi.org/10.1007/978-1-4939-9500-4_24,

Early FISH analysis was used to probe chromosome structure and stability, and is now employed to detect aneuploidy, gene deletions, gene duplications, translocations, and chromosome breakage. For example, this technique has been extensively employed in the mapping and analysis of common chromosomal fragile sites [9, 10]. FISH is also regularly used to study the replication and maintenance of repetitive DNA regions of the genome, such as centromeres, rDNA and telomeres [11–14]. More recently, a modified FISH technique was developed to more closely examine double-strand breaks at specific loci, using split-apart or breakaway probes. This technique relies on two colored FISH probes that flank the double-strand break [15, 16].

In the early 1990s, cytogeneticists realized the utility of this technique as a clinical diagnostic and began using FISH for karyotyping and the detection of chromosome abnormalities in diseases such as Fragile X and Down's syndrome [17–19]. The use of FISH as a clinical diagnostic continued to expand in intervening years becoming a powerful tool to detect and treat a number of genetic diseases and cancers. For example, FISH is used to identify whether breast cancers are HER2 positive or negative, which aids in the choice of drug treatment [20, 21]. It is also used in the treatment of leukemia [22]. In recent years, FISH has been combined with flow cytometry (Flow-FISH) for rapid detection of chromosome aberrations in clinical settings. Yet Flow-FISH is not capable of detecting chromosome fusions, chromosome fragility and structural aberrations [23]. With such limitations, traditional FISH remains a powerful tool for understanding basic chromosome biology and the molecular mechanisms of genetic disease and cancer.

Here, we describe the use of FISH to detect telomeric DNA on metaphase chromosomes. This technique can detect end-to-end chromosomal fusions, telomere fragility, and telomere loss [11, 13]. This protocol will describe the preparation of metaphase spreads and telomeric DNA with telomere FISH probes. However, this protocol can also be modified for use with FISH probes to other chromosomal loci [24, 25].

2 Materials

2.1 Cell Culturing

1. 6 cm sterile culturing dishes.
2. Cell growth media: specific to the cells being cultured (*see* **Note 1**).
3. Phosphate buffered saline (PBS): 137 mM sodium chloride, 2.7 mM potassium chloride, 10 mM disodium phosphate, 1.8 mM monopotassium phosphate pH 7.4.
4. Trypsin–EDTA Solution: 0.25% trypsin with 0.01% EDTA.

2.2 Cell Collection

1. 0.5 mg/mL colcemid in 100% ethanol. Solution should be stored at −20 °C. (*see* **Note 2**).
2. 15 mL conical tubes.
3. 0.075 M KCl.
4. 37 °C water bath.
5. Carnoy's fixative solution: three parts 100% methanol to 1 part glacial acetic acid (vol:vol), prepare fresh (*see* **Note 3**).

2.3 Metaphase Spreads

1. 1.5 mL microcentrifuge tubes.
2. Plain unfrosted slides stored in ultrapure water at 4 °C (*see* **Note 4**).
3. Heat block capable of reaching 70–80 °C.
4. Ultrapure water.
5. Paper towel or cloth.
6. Waste beaker.

2.4 FISH

1. Coplin jars or equivalent microscope slide staining rack/reservoir system.
2. PBS.
3. 4% formaldehyde in PBS.
4. 1 mg/mL pepsin in 10 mM glycine pH 2.0, prewarmed to 37 °C.
5. 70%, 90%, and 100% ethanol.
6. Telomere FISH probe (*see* **Note 5**).
7. 22 mm × 30 mm coverslip glass, No 1.5.
8. Hybridization oven or heat block capable of reaching 80 °C.
9. 10% blocking reagent: 10% Blocking reagent (Roche) (weight–volume) dissolved in 100 mM maleic acid, 150 mM NaCl. Adjust pH to 7.5 with NaOH. Autoclave and store at −20°C.
10. Hybridization buffer: 10 mM Tris–HCl, pH 7.2, 70% formamide, 1% blocking reagent. Make fresh and filter-sterilize.
11. Hybridization chamber: light blocking, humidified chamber (*see* **Note 6** and Fig. 3).
12. Wash solution #1: 10 mM Tris–HCl, pH 7.2, 70% formamide, 0.1% BSA. Dissolve the BSA prior to adding formamide.
13. Wash solution #2: 0.1 M Tris–HCl pH 7.2, 0.15 M NaCl, 0.08% Tween 20.
14. Mounting media containing 0.5 μg/mL DAPI.

3 Methods

3.1 Cell Collection

1. Grow cells overnight to 40–50% confluency in 6 cm culture dish. For HeLa cells, a good starting point is to plate ~5 × 10^5 cells the day prior to collection.
2. Add 4 μL of 0.5 mg/mL colcemid to each 6 cm dish and incubate for 2 h in a 37°C CO_2 incubator (*see* **Note 7**).
3. Collect the media from the 6 cm plate in a 15 mL tube.
4. Wash cells once with PBS. Add the PBS wash to the 15 mL tube with the media (*see* **Note 8**).
5. Add 0.5 mL trypsin–EDTA to the cells and incubate at 37 °C until cells detach from the plate (~3 min).
6. Add 3 mL of complete media to neutralize the trypsin and add the cells suspension to the tube with the media and PBS wash.
7. Spin down the cells in the 15 mL conical tube at 300 rcf (relative centrifugal force) for 4 min.
8. Wash the cells in PBS by removing the supernatant and resuspending the cell pellet in 5 mL of PBS (*see* **Note 9**).
9. Spin down the cells in the 15 mL conical tube at 300 rcf for 5 min and remove the supernatant.
10. Resuspend the cell pellet in the residual liquid and add 5 mL of prewarmed (37 °C) 0.075 M KCl dropwise to the cells, while gently vortexing the tube (*see* **Note 10**).
11. Immediately place the 15 mL conical with cells in a 37 °C water bath for 15–20 min. Ensure the cells are suspended by inverting the tubes occasionally (*see* **Note 11**).
12. Spin down the cells at 300 rcf for 5 min.
13. Remove the 0.075 M KCl. Resuspend the cell pellet in the residual liquid and then add 5 mL of Carnoy's fixative solution dropwise as in Subheading 3.1, **step 9** (*see* **Note 10**).
14. Place tubes at 4 °C at least overnight (*see* **Note 12**).

3.2 Slide Preparation

1. Ensure that the heat block is between 70 and 80 °C. Flip the heat block upside down so the flat surface is face up. Place a folded paper towel to fit over the heat block and dampen the paper towel with water so that it is wet throughout (*see* **Note 13** and Fig. 1).
2. Spin down the 15 mL conical tube containing the fixed cells at 800 rcf for 5 min.
3. Remove the supernatant and resuspend the cell pellet in 0.5–1 mL of fresh Carnoy's fixative solution (*see* **Note 14**).
4. Transfer the resuspended fixed cells to a 1.5 mL microcentrifuge tube and keep on ice or at 4 °C.

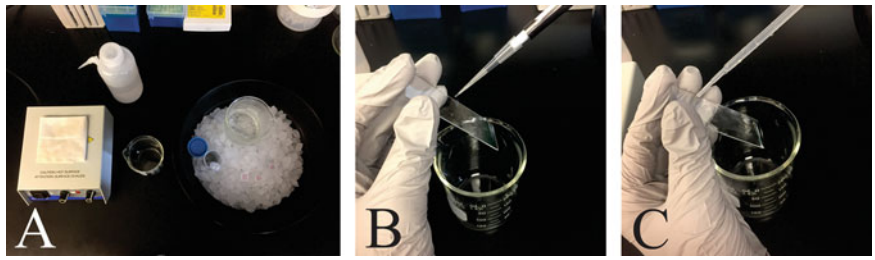

Fig. 1 (**a**) Setup for making metaphase spreads. The ice bucket contains samples in microcentrifuge tubes, fresh fixative with a transfer pipette and glass slides in a beaker of water. The heat block on the left has a paper towel folded over the top and is soaked with water regularly. The beaker in the middle of the image is a waste beaker to catch fresh fixative used to wash the slides. (**b**) Fixed cells are dropped onto a slide at an ~45° angle. (**c**) The slides are washed with fresh fixative before being placed onto the moist paper towel on the heat block

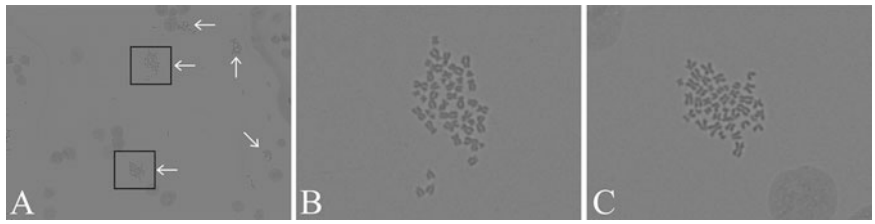

Fig. 2 (**a**) Chromosome spreads from HeLa cells under 10× magnification. Arrows indicate metaphase spreads. (**b**, **c**) Close-up of metaphase spreads seen in (**a**), denoted by boxes

5. Take a prechilled slide and hold it at an ~45° angle over the waste beaker (*see* **Note 15** and Fig. 1). From a few inches above the slide, gently drop 50 μL of the fixed cells across the middle of the slide so that the drops travel to the bottom of the slide (*see* **Note 16**).
6. Immediately rinse the slide with 1–2 mL of fresh Carnoy's fixative, running the solution down the slide.
7. Place the slide, with the cell side up, on the heat block with the wet paper towel for 30–60 s. Most or all of the fixative should be evaporated at this time (*see* **Note 17**).
8. Wipe the residual water off the back of the slide and let cool to room temperature.
9. Check the first slide of each sample under a light microscope to ensure that cell concentration and spreading are adequate (*see* Fig. 2) and then make additional slides, as needed.
10. Label and place the slides into a slide storage box or folder at least overnight before beginning the FISH protocol outlined below. The slides can also be stained with Giemsa or DAPI at this point to look for gross chromosomal abnormalities (*see* **Note 18**).

3.3 FISH Probe Application

Here, we will describe the procedure of telomere FISH (*see* **Note 5**). However, this method can be modified for use with other chromosome-specific probes (*see* **Note 19**).

1. Rehydrate the slides in PBS for 5 min by placing the slide in a Coplin jar, such that the fixed cells are submerged in the liquid (*see* **Note 20**). Ensure that enough liquid is used to completely submerge the region on the slide containing the cells. All subsequent steps are performed at room temperature, unless otherwise indicated.
2. Transfer the slides into a new Coplin jar with 4% formaldehyde in PBS for 2 min to fix the slides.
3. Wash the slides in Coplin jars with PBS for 5 min. Repeat twice with fresh PBS each time.
4. Treat slides with prewarmed pepsin at 37 °C for 5–8 min and no more than 10 min. It is recommended that the Coplin jar containing the pepsin solution be prewarmed to 37 °C (*see* **Note 21**).
5. Wash the slides in a PBS containing Coplin jar for 2 min. Repeat twice with fresh PBS each time.
6. Fix the spreads again in 4% formaldehyde as in Subheading 3.3, **step 2**.
7. Wash the slides in a PBS containing Coplin jar for 5 min. Repeat twice with fresh PBS each time.
8. Dehydrate the spreads by placing them in increasing ethanol concentrations for 1–2 min each. Start with 70% ethanol, move to 90% ethanol, and finally 100% ethanol.
9. Remove the slides from the Coplin jars and place them cell side up on paper towel. Allow the slides to air-dry. Ensure the slides are protected from dust and other debris as they dry.
10. Prepare the telomere FISH probe by diluting it to 10 nM in hybridization buffer (*see* **Note 22**). From this point on, minimize the amount of light exposure to the slides.
11. Prepare 35 μL of the probe + hybridization solution per slide. Place a small drop on the slide near the bottom where the cells are located. The remaining solution is spread in a line across a 22 mm × 30 mm glass coverslip. Take the slide and gently flip it over in your hand. Carefully fuse the solution on the slide with the that on the coverslip. The coverslip should then get pulled up onto the slide due to surface tension (*see* **Note 23**).
12. Incubate the slide, coverslip side up, at 80°C for 5 min (*see* **Note 24**).
13. Place the slide in a prewarmed hybridization chamber (37 °C) and allow the probe to hybridize with the DNA for at least two hours at room temperature. Slides can also be left to hybridize overnight (*see* **Note 25** and Fig. 3).

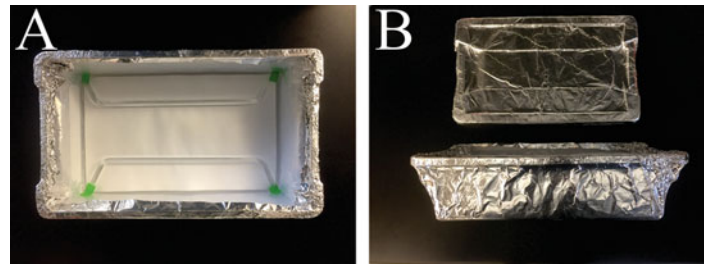

Fig. 3 Example of a homemade hybridization chamber. (**a**) A plastic storage container covered in aluminum foil with filter paper placed in the bottom of the container. Glass rods were bent to fit into the bottom of the container and hold microscope slides. (**b**) The lid and container are covered in aluminum foil to protect the samples from light

14. Place a small amount of the hybridization wash solution #1 in a petri plate or similar container. Gently dip the slides with the coverslip side down into the solution and allow the coverslips to slide off, when necessary gently help the coverslip off with a gloved finger or the side of the dish, being careful not to scrape the slide.
15. Wash slides in an aluminum foil covered, or other light protected, Coplin jar filled with wash solution #1 for 15 min. Repeat with a second wash step. All wash steps from this point on should be performed in light-protected Coplin jars.
16. Wash the slides with wash solution #2 for 5 min. Repeat twice (*see* **Notes 26** and **27**).
17. Dehydrate the slides as in Subheading 3.3, **step 8**.
18. Air-dry the slides in the dark.
19. Prepare 25 μL of mounting media with DAPI per slide. Place one drop on a 22 mm × 30 mm coverslip and apply the remaining mounting media on the slide, being careful not to scrape the pipette tip across the slide. Gently and slowly lay the coverslip down starting at one end of the mounting media on the slide and moving toward the other, avoiding the introduction of bubbles into the media.

3.4 Imaging FISH-Labeled Spreads

1. If necessary, allow the mounting media to harden overnight.
2. Image slides between 40 and 100× magnification, using the correct light filters. Ensure the proper immersion media is used, as microscopes objectives 40× and above typically require water or oil.
3. Take images of the FISH signals on metaphase chromosomes and overlay them with the DAPI. Save and export images (*see* Fig. 4 and **Note 28**).

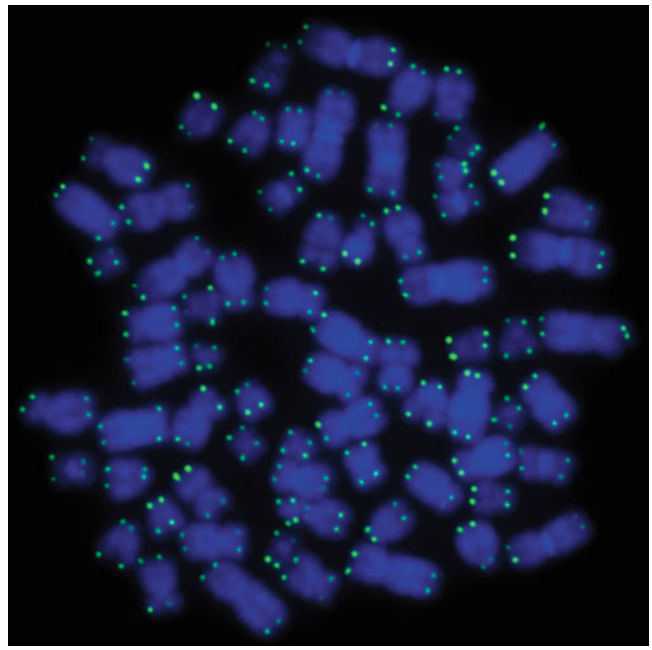

Fig. 4 Example image of telomere FISH on a metaphase spread. Blue, DAPI; Green, FITC-labeled telomere FISH probe

4 Notes

1. The protocol described is optimized for HeLa and HCT116 cells. Steps and conditions may need to be optimized for other cell lines.
2. Colcemid is a spindle poison that prevents the formation of microtubules, which causes the cells to arrest in prometaphase [26]. Other spindle poisons can be used in place of colcemid, such as colchicine and nocodazole. Nocodazole has the benefit of being reversible, whereas colcemid and colchicine are irreversible. The chemotherapeutic drug paclitaxel, sold under the name Taxol, also acts as a spindle poison.
3. The fixative solution should be kept cold (4 °C or on ice) and made fresh on the day of its use. Over time, methanol and acetic acid will form the ester, methyl acetate, which prevents chromosome spreading [27].
4. The slides should be stored in water at least overnight prior to their use. Each slide should be separated and individually submerged, which increases spreading efficiency during metaphase spread preparation.
5. Telomere FISH probes can target either the C-rich or G-rich strand and typically contain three telomere repeats (C-strand

probe: $[TTAGGG]_3$; G-strand $[CCCTAA]_3$) labeled with a fluorophore. A centromere probe can be combined with the telomere FISH protocol to help detect chromosomal fusions. Premade and custom probes are available for purchase from several companies and can be labeled with a variety of different fluorophores. The telomere probe used in this protocol has a peptide nucleic acid (PNA) backbone, which are more stable than DNA probes. Locked nucleic acid (LPA) and bridged nucleic acid (BNA) probes are also available, and they provide greater stability and binding than DNA probes.

6. A dark, humidified chamber is used to prevent the FISH probe solution from drying out during hybridization. Homemade chambers can be made out of a plastic box that has been blacked out using aluminum foil (*see* Fig. 3). In the bottom of the box, an absorbent material should be placed, along with a way to elevate slides. We find that parallel glass rods on top of filter paper works well. The filter paper is soaked with water to humidify the chamber.
7. Colcemid is used to enrich the mitotic cell population. Concentration and incubation time should be optimized for each cell line and to ensure no unintended side effects. Prolonged colcemid treatment has been shown to condense sister chromatids to the point they separate and attempt to reconstitute a nucleus [26].
8. Cells can also be collected by mitotic shake-off, which enriches the number of mitotic cells but yields lower total cell numbers. To collect cells by mitotic shake-off, tap the side of the dish to dislodge mitotic cells from the plate. Use of a screw-top, non-vented capped cell culture flask allows you to increase the strength at which you can tap the flask, increasing the number of mitotic cells obtained. Next, wash media across the cells several times and collect the media. Add PBS and repeat the steps above to dislodge and collect cells. Proceed to Subheading 3.1, **step 6**.
9. One way to minimize the loss and clumping of cells is to resuspend the cell pellet in the residual supernatant. This can be done by flicking the 15 mL conical tube several times or dragging it along a ridged surface several times such as a 1.5 mL centrifuge tube rack.
10. On a typical vortex, the speed should be set just below half full speed. The tube is held in one hand loosely to allow a sample to be gently vortexed, while the other hand is used to add the solution dropwise to the sample. Only the first 1–2 mL of the solution needs to be added to the sample dropwise.
11. This step is used to swell the cells. The 0.075 M KCl is a hypotonic solution and will cause the cells to take in water.

Incubation times may need to be adjusted for different cell types. There is a risk of having the cells burst if they are allowed to incubate in the 0.075 M KCl solution for too long or the solution is too hypotonic [28].

12. The fixed cells can be saved for at least several years.
13. Ensure that the paper towel on the heat block is wet each time before placing slides on it. When making multiple slides, water will need to be continually added to the paper towel at regular intervals. For safety, make sure that the heat block used is able to get wet.
14. The volume of Carnoy's fixative added will depend on the number of cells. For proper spreading, the cell density on the slide should be ~20–50% in most areas. To check this, make one metaphase slide and then check it under a light microscope. If the cell density is too high, then dilute the samples. If it is too low, then spin the cells down and resuspend them in a lower volume.
15. The slide should have a film of water on it when removed.
16. A common misconception in making metaphase spreads is that cells burst due to the force of being dropped from a long distance. However, the cells burst due to heating, humidity, and expansion of the volatile liquid inside, so the distance that you drop the solution does not need to be more than a few inches, which helps the solution spread across the slide.
17. This step influences the quality of the metaphase spreads. The spreads and interphase cells will be barely visible under the microscope. As you prolong the incubation time on the heat block, the cells will become more visible under the microscope, and, in our experience, the quality of the metaphase spreads decrease.
18. If you want to perform metaphase spreads analysis without FISH, the slides may be stained the next day using Giemsa stain or DAPI [28, 29]. This is useful for determining gross chromosomal abnormalities, such as chromosomal breaks or gaps, aneuploidy, or chromatid cohesion loss.
19. Chromosome- and locus-specific probes are available from a number of manufacturers. Many of these probes include their own hybridization buffer and instructions from the manufacturer, which should be used as a starting point for FISH analysis with the probe.
20. Between steps a lint-free tissue is used to blot off the excess liquid at the bottom of the slide.
21. Pepsin is used to remove protein from the chromosomes to provide better access for the probe. Other proteases such as trypsin can also be used.

22. The telomere probe concentration may need to be empirically determined. A range of 4–55 nM has been reported to work for telomere FISH. Telomere length, probe quality, fluorophore brightness, and hybridization conditions can affect signal detection.
23. This step must be done carefully to prevent bubbles from forming between the coverslip and slide. The volume can also be scaled for smaller or larger coverslips. The volume should be sufficient for the coverslip to slightly float in the liquid on the slide.
24. A hybridization oven is ideal, but a heat block or water bath may also be used for this step.
25. Ensure that an excess of water is on the filter paper to prevent the hybridization buffer drying out on the slide.
26. Instead of using mounting media containing DAPI in Subheading 3.3, **step 19**, DAPI (0.5 μg/mL final concentration) can be added to the second wash step and the wash extended to 10 min.
27. If the FISH probe signal is weak, the signal can be amplified with antibodies conjugated with biotin followed by an incubation with an avidin-conjugated fluorophore on the telomere FISH probe. Additional amplification steps can also be added.
28. Telomere FISH can be used to assess chromosome fusions, telomere fragility, telomere signal loss, and telomere length [30, 31]. There are several free software packages that are available for telomere length analysis, including TFL-Telo and telometer (an ImageJ plug-in) [32].

Acknowledgments

We would like to thank members of the Stewart lab for critical reading of the manuscript and helpful discussions. This work was supported by the National Institutes of Health [R00GM104409 to J.A.S.] and startup funds from the University of South Carolina.

References

1. Scott DH (1891) Carl Wilhelm von Nägeli. Nature 44:580. https://doi.org/10.1038/044580a0
2. Tjio JH (1978) The chromosome number of man. Am J Obstet Gynecol 130(6):723–724
3. Pardue ML, Gall JG (1969) Molecular hybridization of radioactive DNA to the DNA of cytological preparations. Proc Natl Acad Sci U S A 64(2):600–604
4. Langer PR, Waldrop AA, Ward DC (1981) Enzymatic synthesis of biotin-labeled polynucleotides: novel nucleic acid affinity probes. Proc Natl Acad Sci U S A 78 (11):6633–6637

5. Arndt-Jovin DJ, Robert-Nicoud M, Kaufman SJ, Jovin TM (1985) Fluorescence digital imaging microscopy in cell biology. Science 230(4723):247–256
6. Kricka LJ, Fortina P (2009) Analytical ancestry: "firsts" in fluorescent labeling of nucleosides, nucleotides, and nucleic acids. Clin Chem 55(4):670–683. https://doi.org/10.1373/clinchem.2008.116152
7. Bauman JG, Wiegant J, Borst P, van Duijn P (1980) A new method for fluorescence microscopical localization of specific DNA sequences by in situ hybridization of fluorochrome-labelled RNA. Exp Cell Res 128(2):485–490
8. Gall JG (2016) The origin of in situ hybridization – a personal history. Methods 98:4–9. https://doi.org/10.1016/j.ymeth.2015.11.026
9. Ma K, Qiu L, Mrasek K, Zhang J, Liehr T, Quintana LG, Li Z (2012) Common fragile sites: genomic hotspots of DNA damage and carcinogenesis. Int J Mol Sci 13 (9):11974–11999. https://doi.org/10.3390/ijms130911974
10. Mrasek K, Wilhelm K, Quintana LG, Theuss L, Liehr T, Leskovac A, Filipovic J, Joksic G, Joksic I, Weise A (2015) BAC-probes applied for characterization of fragile sites (FS). Methods Mol Biol 1227:289–298. https://doi.org/10.1007/978-1-4939-1652-8_15
11. Brault ME, Autexier C (2011) Telomeric recombination induced by dysfunctional telomeres. Mol Biol Cell 22(2):179–188. https://doi.org/10.1091/mbc.E10-02-0173
12. Amakawa G, Ikemoto K, Ito H, Furuya T, Sasaki K (2013) Quantitative analysis of centromeric FISH spots during the cell cycle by image cytometry. J Histochem Cytochem 61 (10):699–705. https://doi.org/10.1369/0022155413498754
13. Pinzaru AM, Hom RA, Beal A, Phillips AF, Ni E, Cardozo T, Nair N, Choi J, Wuttke DS, Sfeir A, Denchi EL (2016) Telomere replication stress induced by POT1 inactivation accelerates tumorigenesis. Cell Rep 15 (10):2170–2184. https://doi.org/10.1016/j.celrep.2016.05.008
14. Koo DH, Zhao H, Jiang J (2016) Chromatin-associated transcripts of tandemly repetitive DNA sequences revealed by RNA-FISH. Chromosom Res 24(4):467–480. https://doi.org/10.1007/s10577-016-9537-5
15. Cuthbert G, Thompson K, Breese G, McCullough S, Bown N (2000) Sensitivity of FISH in detection of MLL translocations. Genes Chromosomes Cancer 29(2):180–185
16. Gascoyne RD, Lamant L, Martin-Subero JI, Lestou VS, Harris NL, Muller-Hermelink HK, Seymour JF, Campbell LJ, Horsman DE, Auvigne I, Espinos E, Siebert R, Delsol G (2003) ALK-positive diffuse large B-cell lymphoma is associated with Clathrin-ALK rearrangements: report of 6 cases. Blood 102 (7):2568–2573. https://doi.org/10.1182/blood-2003-03-0786
17. Piper J, Fantes J, Gosden J, Ji LA (1990) Automatic detection of fragile X chromosomes using an X centromere probe. Cytometry 11 (1):73–79. https://doi.org/10.1002/cyto.990110109
18. Ried T, Landes G, Dackowski W, Klinger K, Ward DC (1992) Multicolor fluorescence in situ hybridization for the simultaneous detection of probe sets for chromosomes 13, 18, 21, X and Y in uncultured amniotic fluid cells. Hum Mol Genet 1(5):307–313
19. Jalal SM, Law ME (1997) Detection of newborn aneuploidy by interphase fluorescence in situ hybridization. Mayo Clin Proc 72 (8):705–710
20. Petroni S, Caldarola L, Scamarcio R, Giotta F, Latorre A, Mangia A, Simone G (2016) FISH testing of HER2 immunohistochemistry 1+ invasive breast cancer with unfavorable characteristics. Oncol Lett 12(5):3115–3122. https://doi.org/10.3892/ol.2016.5125
21. Agersborg S, Mixon C, Nguyen T, Aithal S, Sudarsanam S, Blocker F, Weiss L, Gasparini R, Jiang S, Chen W, Hess G, Albitar M (2018) Immunohistochemistry and alternative FISH testing in breast cancer with HER2 equivocal amplification. Breast Cancer Res Treat 170(2):321–328. https://doi.org/10.1007/s10549-018-4755-5
22. van der Burg M, Poulsen TS, Hunger SP, Beverloo HB, Smit EM, Vang-Nielsen K, Langerak AW, van Dongen JJ (2004) Split-signal FISH for detection of chromosome aberrations in acute lymphoblastic leukemia. Leukemia 18 (5):895–908. https://doi.org/10.1038/sj.leu.2403340
23. Baerlocher GM, Vulto I, de Jong G, Lansdorp PM (2006) Flow cytometry and FISH to measure the average length of telomeres (flow FISH). Nat Protoc 1(5):2365–2376. https://doi.org/10.1038/nprot.2006.263
24. Kraker WJ, Borell TJ, Schad CR, Pennington MJ, Karnes PS, Dewald GW, Jenkins RB (1992) Fluorescent in situ hybridization: use of whole chromosome paint probes to identify unbalanced chromosome translocations. Mayo Clin Proc 67(7):658–662
25. Rives N, Wust S, David B, Duchesne V, Joly G, Mace B (1999) Fluorescence in situ

hybridization with chromosome paint probes: a novel approach to assess aneuploidy in human sperm nuclei. J Assist Reprod Genet 16 (1):46–51

26. Rieder CL, Palazzo RE (1992) Colcemid and the mitotic cycle. J Cell Sci 102(Pt 3):387–392
27. Baksi SM, Means JC (1988) Preparation of chromosomes from early stages of fish for cytogenetic analysis. J Fish Biol 32(3):321–325. https://doi.org/10.1111/j.1095-8649.1988.tb05369.x
28. Bayani J, Squire JA (2004) Preparation of cytogenetic specimens from tissue samples. Curr Protoc Cell Biol Chapter 22:Unit 22.2. https://doi.org/10.1002/0471143030.cb2202s23
29. Deng W, Tsao SW, Lucas JN, Leung CS, Cheung AL (2003) A new method for improving metaphase chromosome spreading. Cytometry A 51(1):46–51. https://doi.org/10.1002/cyto.a.10004
30. Hemann MT, Strong MA, Hao LY, Greider CW (2001) The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. Cell 107(1):67–77
31. Tanaka H, Abe S, Huda N, Tu L, Beam MJ, Grimes B, Gilley D (2012) Telomere fusions in early human breast carcinoma. Proc Natl Acad Sci U S A 109(35):14098–14103. https://doi.org/10.1073/pnas.1120062109
32. van Batenburg AA, Kazemier KM, Peeters T, van Oosterhout MFM, van der Vis JJ, Grutters JC, Goldschmeding R, van Moorsel CHM (2018) Cell type-specific quantification of telomere length and DNA double-strand breaks in individual lung cells by fluorescence in situ hybridization and fluorescent immunohistochemistry. J Histochem Cytochem 66 (7):485–495. https://doi.org/10.1369/0022155418761351