

A historical and modern perspective on plant cytogenetics

Debbie M. Figueroa and Hank W. Bass

Advance Access publication date 27 January 2010

Abstract

Plant cytogenetics has continued to flourish and make essential contributions to genomics projects by delineating marker order, defining contig gaps and revealing genome rearrangements. Here we review the field of plant cytogenetics from its conception through the eras of molecular biology and genomics. Significant advances in chromosome preparation, such as extended fiber-FISH, have greatly increased the axial resolution limits, while imaging and signal amplification technologies have improved our ability to detect small gene-sized probes. Combinations of traditional FISH technologies with chromatin immunocytochemistry serve to broaden the ability of plant cytogenetics to shed light on genome structure and organization. These advances are described, together with selected examples that illustrate the power of plant cytogenetics in guiding genome projects.

Keywords: FISH; cytology; chromosome; BAC; microscopy

CLASSICAL PLANT CYTOGENETICS

The field of plant cytogenetics was heavily influenced by Barbara McClintock's pioneering work on maize (*Zea mays*). Her method for unequivocal identification of individual chromosomes permitted major discoveries regarding the structure and dynamic behavior of the maize genome [1–6]. Using carmine-based chromatin staining procedures, McClintock showed that all of the individual chromosomes could be uniquely identified from a single meiotic nucleus with a combination of two metrics, the relative lengths and arm ratios of the chromosomes [2]. This approach proved useful for cytogenetic map development in other plant species, including rice (*Oryza sativa*) [7, 8], sorghum (*Sorghum propinquum*) [9], and tomato (*Lycopersicon esculentum*) [10, 11]. Plant species with similar-sized chromosomes, however, required the development of additional techniques for unmistakable cytogenetic resolution and karyotyping.

In 1968, Caspersson *et al.* used the fluorescent dye quinacrine to produce additional banding patterns,

Q-bands, on plant chromosomes [12]. In 1972, Vosa and Marchi compared Giemsa C-banding to Q-banding on the chromosomes of bean (*Vicia faba*), keeled garlic (*Allium carinatum*) and maize [13]. Development of chromosome-banding techniques greatly improved the usefulness of somatic chromosomes, which are significantly easier to acquire than meiotic chromosomes. Giemsa staining techniques permitted the identification of individual rice prometaphase chromosomes [14], as well as karyotype development for diploid rye (*Secale cereale*) [15] and Emir barley (*Hordeum vulgare*) [16]. In an effort to improve cytological techniques for plants, Schweizer demonstrated that cold pretreatments enhanced chromosome visualization for most of the species studied, except for *Vicia faba* [17]. Even under optimal staining conditions, the ability to distinguish all chromosomes clearly can be hampered by the inherent morphological similarity of chromosomes in certain plant species [18–20]. Over many decades, variations of the carmine-based and banding techniques were adapted and optimized for cytogenetic characterization of different plant species

Corresponding author. Hank W. Bass, Department of Biological Science, Florida State University, Tallahassee, FL 32306-4295, USA. Tel: +1-850-644-9711; Fax: +1-850-645-8447; E-mail: bass@bio.fsu.edu

Debbie M. Figueroa is a doctoral student working on the cytogenetic map of maize in the H.W. Bass laboratory.

Hank W. Bass is an Associate Professor at Florida State University, investigating the structure and function of maize chromosomes and telomeres using molecular biology and cytogenetics.

[14–17, 20, 21]. These classical approaches have proven invaluable for chromosome characterization, but the development of *in situ* hybridization, which allows for direct visualization of specific DNA sequences on chromosomes, produced a quantum leap forward by combining cytology with molecular biology [22, 23].

DEVELOPMENT AND APPLICATION OF MOLECULAR CYTOLOGY IN PLANTS

The development of *in situ* hybridization (ISH) techniques opened up opportunities for cytogenetic analysis of essentially any species, regardless of its inherent chromosome morphology [24–27]. In plants, the use of radioactive tracer or modified nucleotides (attached to biotin, digoxigenin, or fluorescent moieties) to make ISH probes permits microscopic visualization and localization of complementary sequences in cells and nuclei and on individual chromosomes [27–32]. Direct and indirect fluorescence *in situ* hybridization (FISH) has been broadly applied over the last 25 years, as recently reviewed by Jiang and Gill [33]. Although FISH is commonly used to map unique or low-copy-number sequences, it is also used to localize repetitive sequence to produce chromosome recognition cocktails or explore genome relations in polyploid or closely related plant species [34–37]. The broad applications of FISH in structural, comparative and functional genomics place plant cytogenetics in a unique position to

complement, accelerate, or guide plant-genome research [38–53].

SMALLER IS BETTER: STRATEGIES FOR IMPROVING THE DETECTION LIMIT WITH FISH

The power of cytogenetics is increasingly often focused on two related aspects of FISH, probe-size detection limit and axial-resolution limit. Here we use the phrase probe-size *detection limit* to mean the smallest FISH probe that can be clearly discerned and the phrase *resolution limit* to mean the smallest distance between two signals that can be resolved as separate and distinct in a microscopic image. Advances in microscopic sensitivity, signal increase and noise reduction have all contributed to improved detection limits, whereas advances in cytological resolution of closely linked loci (described below) are primarily derived from methods that lengthen the chromosome itself. Table 1 lists several key studies that highlight findings related to both of these issues.

Lowering the detection limit holds great promise for the common goal of being able to map cytogenetically any given single gene-sized DNA fragment such as a cDNA clone or an RFLP probe onto a chromosome. A key issue in detection limit centers on maximizing the signal-to-noise ratio, where the true FISH *signals* derive from the photons emitted by the hybridized probe molecules or their fluorescent ligands, and *noise* typically results from photons emitted by nonspecific or off-target fluorescence.

Table 1: Axial resolution and probe size detection limits in plant fluorescence *in situ* hybridization (FISH)

| Cell type | Cell stage | Target chromosome | | Axial resolution limit ^c (kb) | Probe size detection limit ^d (kb) | References ^e |
|-----------|----------------------------|--------------------------------|--------------------------|--|--|-------------------------|
| | | Chromatin ^a | Preparation ^b | | | |
| Somatic | Metaphase | Euchromatin Heterochromatin | Superstretched | 2000–10 000 | 2.27–10 | [33, 51, 52, 61] |
| | | | | 4000–5000 | > 100 | [61] |
| | | | | 5000–10 000 | 50–100 | [52, 61] |
| | Prometaphase Interphase | | Extended fiber | 70 | 1000–2000 | [65] |
| | | | | 2000 | | [60] |
| | | | | 100 | 10 | [33, 55] |
| Meiotic | Pachytene | Euchromatin Heterochromatin | Superstretched | 4.0 | 0.7 | [60, 71] |
| | | | | 100–40 | 3.1 | [33, 49, 52] |
| | | | | 120 | 50.0 | [61] |
| | | | | 1200 | | [61] |
| | | | | <50 | | [66] |

^aThe type of chromatin is indicated for studies where it was specified.

^bIf other than conventional spread or squash technique.

^cThe smallest reported probe size resulting in detection of a FISH signal in a given study.

^dThe minimum reported distance between two FISH signals ordered and resolved along the chromosome axis.

^ePrimary or review paper describing the resolution- or detection-limit values.

Because of the sensitivity of high-resolution digital CCD cameras, detection limit is restricted not by probe size but rather by the signal that can be detected above the background noise. Routine detection of probes smaller than 1 kb remains difficult, although significant improvements in detection limits have been reported by many laboratories over the last 25 years [54–58].

Larger DNA segments, such as those from bacterial artificial chromosomes (BACs), are useful for producing stronger FISH signals, but these often contain repetitive sequences that may complicate the detection of specific target loci. Transgenomic FISH and pooled-BAC-PCR methods have been developed to improve specificity of detectable

BAC FISH signals [42, 48, 50–52]. Figure 1 illustrates a modern application of FISH that builds on genomics resources to develop a cytogenetic map of maize. In this case, the genetic marker being FISH mapped, maize Core Bin Marker 1.05, CBM1.05 (*csu3*), is only 1.06 kb in size, near the practical detection limit for pachytene chromosomes. A maize marker-selected sorghum BAC clone is used as a surrogate probe to identify the cytological location of CBM1.05 (*csu3*) already linkage mapped to the short arm of chromosome 1. This example shows a set of image data with several types of FISH probes including a transgenomic BAC (arrow, Figure 1C and F), whole chromosome paint (Figure 1B), and repetitive centromeric DNA

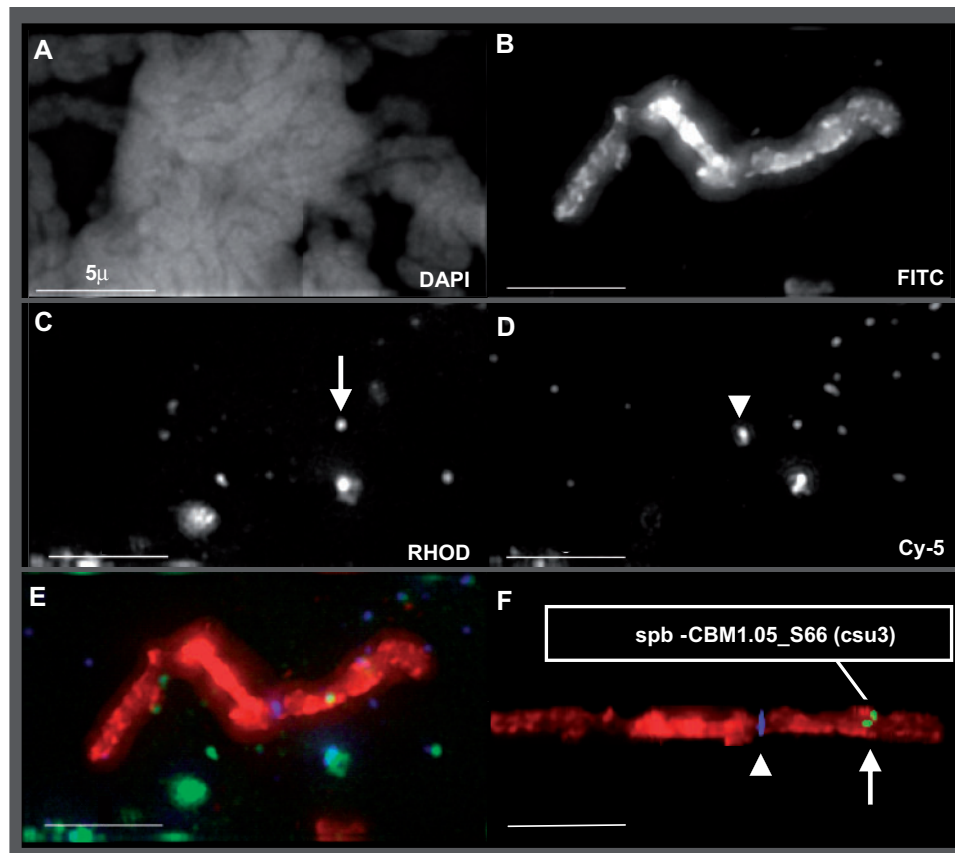


Figure 1: Fluorescence *in situ* hybridization (FISH) of a *Sorghum propinquum* BAC onto maize chromosome I in OMAdl.36, a maize-chromosome-addition line of oat. FISH mapping was carried out as previously described [48, 50]. **(A)** 4',6-diamidino-2-phenylindole (DAPI) image of DAPI-stained pachytene chromosome spread from OMAdl.36. **(B)** Fluorescein isothiocyanate (FITC) image showing maize chromosome I hybridized with direct-labeled Alexa-488-dUTP total maize DNA. **(C)** Rhodamine (RHOD) image from direct-labeled BAC FISH signal (arrow) with the maize RFLP-selected *S. propinquum* BAC a0026E17. **(D)** Cy-5 image of centromere FISH signal (arrowhead) with direct-labeled CentC [103]. **(E)** Three-color overlay of the FITC (red), RHOD (green), and Cy-5 (blue) images. **(F)** Straightened projection of the maize chromosome with the three-color overlay scheme of panel E. The locations of the centromere (arrowhead) and CBM1.05 BAC FISH signals (arrow) are indicated, together with the resulting cytogenetic locus name (boxed). All scale bars are 5 μ m.

(arrowhead, Figure 1D and F). The cytogenetic position of this maize marker is hereby determined to be at cytological position 1S.66 (Chromosome 1, Short arm, at 66% of the distance from centromere to telomere).

LOCATION, LOCATION, LOCATION: SPATIAL RESOLUTION IN PLANT CYTOGENETICS

Another common goal in plant cytogenetics is to resolve the location and arrangement of two or more DNA sequences in relation to each other along the axis of the target chromosome. Axial resolution typically refers to the ability to distinguish the relative positions of two loci as either *proximal* or *distal* to each other relative to the centromere. The axial resolution limit is usually reported in either base pairs or spatial units, such as the centiMcClintock, cMC [59]. These limits have been reported from various studies that used different probes and chromosome preparations, giving rise to apparent discrepancies, ranging from <0.7 kb in fiber-FISH to 10 000 kb in some metaphase(M)-FISH (Table 1 and references therein). These differences are attributed to variation in the axial compaction of DNA within the chromosomes and in the spacing of the probes used in a given study. Clearly the choice of chromosome type (e.g. somatic or meiotic) and how it is prepared (e.g. squashes or stretching) are primary determinants of resolution limit in a given experiment.

M-FISH based on plant meristem tissues, such as root tip, provides readily available material but can produce variable axial resolution depending on whether the probes are in euchromatic or heterochromatic regions (see Table 1 and references therein). Prometaphase chromosomes further improve axial resolving power [54, 60], whereas interphase-FISH (I-FISH) provides a reported resolution of ~50–100 kb [61, 62]. Despite having the poorest axial resolution, M-FISH remains a crucial method in plant cytogenetics for rapid assignment of cloned sequences to chromosomes and for ordering loci separated by at least several mega base pairs [33, 51, 52, 61].

Meiotic chromosome preparations, on the other hand, offer additional cytological landmarks and better axial resolution than do those from somatic cells [2, 10, 11]. Pachytene chromosomes are

longer than their somatic counterparts by a factor of 6–25, depending on the species and method of chromosome preparation [11, 49, 63, 64]. Although meiotic chromosomes are not as readily available as somatic chromosomes, they are typically abundant and synchronized when isolated from pollen mother cells.

Methods specifically designed to unravel or stretch chromosomes before FISH have further increased axial resolution and allowed for finer-scale mapping of sequences than does conventional mitotic or meiotic FISH. Spreading interphase chromosomes for fiber-FISH, protease treatments of flow-sorted metaphase chromosomes, and superstretching of pachytene chromosomes are all methods that have enhanced our cytological view of chromosomal structures and sequence arrangements [65–67]. Fiber-FISH offers particularly high resolution (Table 1) and is used to characterize complex genomic arrangements in plant nuclei or plastids [67–70]. Given the average stretching degree of plant DNA fibers (3.12 kb/μm) [60, 67, 71] and the optical resolution limit of conventional epifluorescence microscopy (200–300 nm), we can predict an axial resolution limit of ~600–700 bp.

CYTOGENETICS, IN THE POSTGENOMICS ERA

Plant cytogeneticists were among the earliest of genome researchers. They were visualizing genomes decades before the structure of DNA was discovered, 50 years before DNA cloning, and nearly a century before the first plant genome was sequenced [72]. The fundamental and classical cytological techniques remain excellent starting points for plant cytogenetic studies. Building on this foundation, FISH has extended our ability to identify specific chromosomes for almost any plant species with morphologically indistinguishable chromosomes [73, 74]. FISH permits rapid cytogenetic characterization and chromosome identification by means of a variety of probes such as those from repetitive DNAs, large-fragment clones, or closely related species. The recent development of cytogenetic maps of loblolly pine (*Pinus taeda*) [75] and Chinese bitter orange (*Poncirus trifoliata*) [76] nicely demonstrate this point. These maps were rapidly developed

from a combination of traditional chromosome preparations with FISH that used probes from BACs or conserved repetitive sequences from *Arabidopsis*.

Cytogenetic maps, which are based on the ultimate contigs, provide a unique conceptual framework for structural and functional genomics research. Molecular cytology offers an efficient means for sequence localization, validation of contig order and gap size, as well as characterization of complicated regions such as centromeres. For example, cytogenetic analysis of genetically mapped markers from the pericentromeric heterochromatin regions of maize chromosome 9 [52], tomato chromosomes 2 and 6 [77–79], and potato (*Solanum tuberosum*) chromosome 6 [79, 80] has helped resolved complex underlying genomic structures. Similarly, FISH has served to define heterochromatin–euchromatin boundaries in tomato [77, 78, 81], to localize centromeres relative to genetically mapped markers in both tomato [80] and maize [82], to produce high-resolution mapping within a contig in *Arabidopsis* [71], and to estimate the physical sizes of gaps between BACs in rice [60, 83, 84] and tomato [77, 78].

The use of cytogenetics to guide genome-sequencing efforts in tomato and potato also serves to illustrate the synergy between plant genomics and cytogenetics. Pachytene- and fiber-FISH were used to identify seed BACs for sequencing of tomato chromosomes 2 and 6 [77, 78]. FISH in tomato was used to investigate previously reported line-specific inversions and other discrepancies in genetically mapped marker arrangements [77, 78, 85]. More recently, similar FISH-guided strategies are being used to guide the BAC selection process as part of the potato genome-sequencing project [86].

FISH is also a powerful tool for comparative genomics, as beautifully demonstrated for members of the Brassicaceae [35, 87, 88], Solanaceae [79, 80] and Poaceae [42, 50, 89]. Fiber-FISH confirmed that *Arabidopsis thaliana* and *Brassica rapa* divergence was associated with chromosomal duplications [87]. In addition, comparative chromosome painting with pooled BAC probes was used to investigate ancestral relationships among species that diverged within the Brassicaceae [34, 35, 88, 90]. Collectively, these studies reveal the methods associated with plant cytogenetics to be uniquely informative and beneficial for genome analysis.

COMBINED TECHNIQUES AND THE FUTURE FOR PLANT CYTOGENETICS

The advances in microscopy, chromosome preparation techniques, and reagents for visualizing chromatin show great promise for plant cytogenetics, especially when used together. This concept is well illustrated by recent plant centromere studies in rice [91] and maize. For example, Zhong *et al.* [92] combined FISH, chromatin immunoprecipitation and immunocytochemistry to characterize the maize centromere-specific histone H3 variant, CENH3. They showed that CENH3 was associated with the kinetochore protein CENPC and characterized its propensity to colocalize with maize centromere-associated repetitive DNA elements CentC, CRM, and CentA. Jin *et al.* [68] used FISH and immunostaining to demonstrate that CentC and CRM sequences are interspersed at maize centromeres but that only a subset of these sequences were closely associated with CenH3. Zhang *et al.* [93] used extended chromatin fiber preparations to demonstrate that the CENH3-associated CentC sequences were relatively hypomethylated, whereas Koo and Jiang [66] developed and used a pachytene-chromosome superstretching technique to document the uneven distribution of this CentC hypomethylation at high resolution. Using combinations of methods such as molecular and chromatin cytology with new chromosome preparations and high-resolution imaging adds new insights and models for understanding chromosome organization at multiple scales.

In summary, we have described how plant cytogenetics plays a vital role in a wide range of modern research disciplines, from structural and functional genomics to comparative evolutionary biology. Emerging fields such as plant chromosome engineering also rely heavily on molecular cytological analysis [94–96]. New breakthroughs in imaging technologies, such as 3D structured illumination and stochastic optical reconstruction microscopy [97–102], offer even more hope for bridging the shrinking gap between the molecular and cytological views of the chromosomes and genomes of plants. Witnessing the integration of plant cytogenetics with rapidly advancing fields such as high-resolution imaging, epigenetics and genomics is exciting. Although grounded in techniques pioneered nearly a century ago, plant cytogenetics is still evolving, providing

crucial and integrative tools for genetic and genomic analysis of plant chromosomes and genomes.

Key Points

- Cytogenetics was built in part on historical studies of plant genome structure.
- Cytogenetics provides a conceptual foundation for modern genomics.
- Molecular cytology uses clones sequences for fluorescence *in situ* hybridization to integrate molecular biology and genetics with cytogenetics.
- Plant cytogenetics is an indispensable tool for modern genome projects, providing rapid discovery or validation of physical maps and guiding efficient choice of bacterial artificial chromosomes for sequencing.

FUNDING

National Science Foundation (DBI-0321639).

References

1. Creighton HB, McClintock B. A correlation of cytological and genetical crossing-over in *Zea mays*. *Proc Natl Acad Sci USA* 1931;**17**:492–7.
2. McClintock B. Chromosome morphology in *Zea mays*. *Science* 1929;**69**:629.
3. McClintock B. The stability of broken ends of chromosomes in *Zea mays*. *Genetics* 1932;**26**:234–82.
4. McClintock B. The production of homozygous deficient tissues with mutant characteristics by means of the aberrant mitotic behavior of ring-shaped chromosomes. *Genetics* 1938;**23**:315–76.
5. McClintock B. The stability of broken ends of chromosomes in *Zea mays*. *Genetics* 1941;**26**:234–82.
6. McClintock B. The significance of responses of the genome to challenge. *Science* 1984;**226**:792–801.
7. Shastri SVS, Ranga Rao DR, Misra RN. Pachytene analysis in *Oryza*. I. Chromosome morphology in *Oryza sativa*. *Indian J Genet & Plant Breed* 1960;**20**:15–21.
8. Misra RN, Shastri SVS. Pachytene analysis in *Oryza*. VIII. Chromosome morphology and karyotypic variation in *O. sativa*. *Indian J Genet & Plant Breed* 1967;**27**:349–68.
9. Magoon ML, Shambulingappa KG. Karyomorphology of *Sorghum propinquum* and its bearing on origin of 40-chromosome sorghum. *Chromosoma* 1961;**12**:460–5.
10. Barton DW. Pachytene morphology of the tomato chromosome complement. *Am J Bot* 1950;**37**:639–43.
11. Ramanna MS, Parkken P. Structure of and homology between pachytene and somatic metaphase chromosomes of tomato. *Genetica* 1967;**38**:115–33.
12. Caspersson T, Farber S, Foley GE, *et al*. Chemical differentiation along metaphase chromosomes. *Exp Cell Res* 1968;**49**:219–22.
13. Vosa CG, Marchi P. Quinacrine fluorescence and Giemsa staining in plants. *Nat New Biol* 1972;**237**:191–2.
14. Kurata N, Omura T. Karyotype analysis in rice I. A new method for identifying all chromosome pairs. *Jpn J Genet* 1978;**54**:251–5.
15. Gill BS, Kimber G. The Giemsa C-banded karyotype of rye. *Proc Natl Acad Sci USA* 1974;**71**:1247–9.
16. Linde-Laursen I. Giemsa C-banding of the chromosomes of "Emir" barley. *Hereditas* 1975;**81**:285–9.
17. Schweizer D. Differential staining of plant chromosomes. *Chromosoma* 1973;**40**:307–20.
18. Mok DWS, Mok MC. A modified Giemsa technique for identifying bean chromosomes. *J Hered* 1976;**67**:187–8.
19. Pijnacker LP, Ferwerda MA. Giemsa C-banding of potato chromosomes. *Can J Genet Cytol* 1984;**26**:415–9.
20. Yu H, Liang GH, Kofoid KD. Analysis of C-banding chromosome patterns of *Sorghum*. *Crop Sci* 1991;**31**:1524–7.
21. Lavania UC. Differential staining and plant chromosomes—progress in cytogenetics. *Curr Sci* 1978;**47**:255–60.
22. Gill BS, Friebe B. Plant cytogenetics at the dawn of the 21st century. *Curr Opin Plant Biol* 1998;**1**:109–15.
23. Harper LC, Cande WZ. Mapping a new frontier; development of integrated cytogenetic maps in plants. *Funct Integr Genomics* 2000;**1**:89–98.
24. Gall JG, Pardue ML. Formation and detection of RNA-DNA hybrid molecules in cytological preparations. *Proc Natl Acad Sci USA* 1969;**63**:378–83.
25. Pardue ML, Gall JG. Molecular hybridization of radioactive DNA to the DNA of cytological preparations. *Proc Natl Acad Sci USA* 1969;**64**:600–4.
26. Pardue ML, Gall JG. Nucleic acid hybridization to the DNA of cytological preparations. *Methods Cell Biol* 1975;**10**:1–16.
27. John HA, Birnstiel ML, Jones KW. RNA-DNA hybrids at the cytological level. *Nature* 1969;**223**:582–7.
28. Ambros PF, Matzke MA, Matzke AJ. Detection of a 17 kb unique sequence (T-DNA) in plant chromosomes by *in situ* hybridization. *Chromosoma* 1986;**94**:11–8.
29. Fransz PF, Stam M, Montijn BM, *et al*. Detection of single-copy genes and chromosome rearrangements in *Petunia hybrida* by fluorescence *in situ* hybridization. *Plant J* 1996;**9**:767–74.
30. Peacock WJ, Dennis ES, Rhoades MM, *et al*. Highly repeated DNA sequence limited to knob heterochromatin in maize. *Proc Natl Acad Sci USA* 1981;**78**:4490–4.
31. Shen DL, Wang ZF, Wu M. Gene mapping on maize pachytene chromosomes by *in situ* hybridization. *Chromosoma* 1987;**95**:311–4.
32. Xu J, Earle ED. Direct and sensitive fluorescence *in situ* hybridization of 45S rDNA on tomato chromosomes. *Genome* 1994;**37**:1062–5.
33. Jiang J, Gill BS. Current status and the future of fluorescence *in situ* hybridization (FISH) in plant genome research. *Genome* 2006;**49**:1057–68.
34. Lysak MA, Fransz PF, Ali HBM, *et al*. Chromosome painting in *Arabidopsis thaliana*. *Plant J* 2001;**28**:689–97.
35. Lysak MA, Pecinka A, Schubert I. Recent progress in chromosome painting of *Arabidopsis* and related species. *Chromosome Res* 2003;**11**:195–204.
36. Kato A, Lamb JC, Birchler JA. Chromosome painting using repetitive DNA sequences as probes for somatic

- chromosome identification in maize. *Proc Natl Acad Sci USA* 2004;**101**:13554–9.
37. Lamb JC, Birchler JA. Retroelement genome painting: cytological visualization of retroelement expansions in the genera *Zea* and *Tripsacum*. *Genetics* 2006;**173**:1007–21.
 38. Suzuki HY, Futsuhara F, Takaiwa F, *et al.* Localization of glutelin gene in rice chromosome by in situ hybridization. *Jpn J Genet* 1991;**66**:305–12.
 39. Gustafson JP, Dille JE. Chromosome location of *Oryza sativa* recombination linkage groups. *Proc Natl Acad Sci USA* 1992;**89**:8646–50.
 40. Woo SS, Jiang JM, Gill BS, *et al.* Construction and characterization of a bacterial artificial chromosome library of *Sorghum bicolor*. *Nucleic Acids Res* 1994;**22**:4922–31.
 41. Song YC, Gustafson JP. The physical location of 14 RFLP markers in rice (*Oryza sativa* L.). *Theor Appl Genet* 1995;**90**:113–9.
 42. Zwick MS, Islam-Faridi MN, Czeschin DG, Jr, *et al.* Physical mapping of the liguleless linkage group in *Sorghum bicolor* using rice RFLP-selected sorghum BACs. *Genetics* 1998;**148**:1983–92.
 43. Reamon-Buttner SM, Schmidt T, Jung C. AFLPs represent highly repetitive sequences in *Asparagus officinalis* L. *Chromosome Res* 1999;**7**:297–304.
 44. Huang XQ, Zeller FJ, Hsam SLK, *et al.* Chromosomal location of AFLP markers in common wheat utilizing nullitetrasonic stocks. *Genome* 2000;**43**:298–305.
 45. Sadler MT, Ponelies N, Born U, *et al.* Physical localization of single-copy sequences on pachytene chromosomes in maize (*Zea mays* L.) by chromosome in situ suppression hybridization. *Genome* 2000;**43**:1081–3.
 46. Peters JL, Constandt H, Neyt P, *et al.* A physical amplified fragment-length polymorphism map of *Arabidopsis*. *Plant Physiol* 2001;**127**:1579–89.
 47. Islam-Faridi MN, Childs KL, Klein PE, *et al.* A molecular cytogenetic map of sorghum chromosome 1. Fluorescence in situ hybridization analysis with mapped bacterial artificial chromosomes. *Genetics* 2002;**161**:345–53.
 48. Koumbaris GL, Bass HW. A new single-locus cytogenetic mapping system for maize (*Zea mays* L.): overcoming FISH detection limits with marker-selected sorghum (*S. propinquum* L.) BAC clones. *Plant J* 2003;**35**:647–59.
 49. Wang CJ, Harper L, Cande WZ. High-resolution single-copy gene fluorescence in situ hybridization and its use in the construction of a cytogenetic map of maize chromosome 9. *Plant Cell* 2006;**18**:529–44.
 50. Amarillo FI, Bass HW. A transgenomic cytogenetic sorghum (*Sorghum propinquum*) bacterial artificial chromosome fluorescence in situ hybridization map of maize (*Zea mays* L.) pachytene chromosome 9, evidence for regions of genome hyperexpansion. *Genetics* 2007;**177**:1509–26.
 51. Lamb JC, Danilova T, Bauer MJ, *et al.* Single-gene detection and karyotyping using small-target fluorescence in situ hybridization on maize somatic chromosomes. *Genetics* 2007;**175**:1047–58.
 52. Danilova TV, Birchler JA. Integrated cytogenetic map of mitotic metaphase chromosome 9 of maize: resolution, sensitivity, and banding paint development. *Chromosoma* 2008;**117**:345–56.
 53. Albini S. A karyotype of the *Arabidopsis thaliana* genome derived from synaptonemal complex analysis at prophase I of meiosis. *Plant J* 1994;**5**:665–72.
 54. Desel C, Jung C, Cai D, *et al.* High-resolution mapping of YACs and the single-copy gene Hs1(pro-1) on *Beta vulgaris* chromosomes by multi-colour fluorescence in situ hybridization. *Plant Mol Biol* 2001;**45**:113–22.
 55. Jiang J, Hulbert SH, Gill BS, *et al.* Interphase fluorescence in situ hybridization mapping: a physical mapping strategy for plant species with large complex genomes. *Mol Gen Genet* 1996;**252**:497–502.
 56. Khrustaleva LI, Kik C. Localization of single-copy T-DNA insertion in transgenic shallots (*Allium cepa*) by using ultra-sensitive FISH with tyramide signal amplification. *Plant J* 2001;**25**:699–707.
 57. Stephens JL, Brown SE, Lapitan NL, *et al.* Physical mapping of barley genes using an ultrasensitive fluorescence in situ hybridization technique. *Genome* 2004;**47**:179–89.
 58. Zhong XB, de Jong JH, Zabel P. Preparation of tomato meiotic pachytene and mitotic metaphase chromosomes suitable for fluorescence in situ hybridization (FISH). *Chromosome Res* 1996;**4**:24–8.
 59. Lawrence CJ, Seigfried TE, Bass HW, *et al.* Predicting chromosomal locations of genetically mapped loci in maize using the Morgan2McClintock Translator. *Genetics* 2006;**172**:2007–9.
 60. Cheng Z, Buell CR, Wing RA, *et al.* Resolution of fluorescence in-situ hybridization mapping on rice mitotic pro-metaphase chromosomes, meiotic pachytene chromosomes and extended DNA fibers. *Chromosome Res* 2002;**10**:379–87.
 61. de Jong JH, Fransz P, Zabel P. High resolution FISH in plants—techniques and applications. *Trends Plant Sci* 1999;**4**:258–63.
 62. Jiang J, Gill BS, Wang GL, *et al.* Metaphase and interphase fluorescence in situ hybridization mapping of the rice genome with bacterial artificial chromosomes. *Proc Natl Acad Sci USA* 1995;**92**:4487–91.
 63. Fransz PF, Armstrong S, de Jong JH, *et al.* Integrated cytogenetic map of chromosome arm 4S of *A. thaliana*: structural organization of heterochromatic knob and centromere region. *Cell* 2000;**100**:367–76.
 64. Zoller JF, Herrmann RG, Wanner G. Chromosome condensation in mitosis and meiosis of rye (*Secale cereale* L.). *Cytogenet Genome Res* 2004;**105**:134–44.
 65. Valarik M, Bartos J, Kovarova P, *et al.* High-resolution FISH on super-stretched flow-sorted plant chromosomes. *Plant J* 2004;**37**:940–50.
 66. Koo DH, Jiang J. Super-stretched pachytene chromosomes for fluorescence in situ hybridization mapping and immunodetection of DNA methylation. *Plant J* 2009;**59**:509–16.
 67. Jackson SA, Wang ML, Goodman HM, *et al.* Application of fiber-FISH in physical mapping of *Arabidopsis thaliana*. *Genome* 1998;**41**:566–72.
 68. Jin WW, Melo JR, Nagaki K, *et al.* Maize centromeres: organization and functional adaptation in the genetic background of oat. *Plant Cell* 2004;**16**:571–81.
 69. Lilly JW, Havey MJ, Jackson SA, *et al.* Cytogenomic analyses reveal the structural plasticity of the chloroplast genome in higher plants. *Plant Cell* 2001;**13**:245–54.
 70. Nagaki K, Cheng Z, Ouyang S, *et al.* Sequencing of a rice centromere uncovers active genes. *Nat Genet* 2004;**36**:138–45.
 71. Fransz PF, Alonso-Blanco C, Liharska TB, *et al.* High-resolution physical mapping in *Arabidopsis thaliana* and

- tomato by fluorescence in situ hybridization to extended DNA fibres. *Plant J* 1996;**9**:421–30.
72. The Arabidopsis Genome Initiative. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* 2000;**408**:796–815.
 73. Dong F, Song J, Naess SK, *et al.* Development and application of a set of chromosome-specific cytogenetic DNA markers in potato. *Theor Appl Genet* 2000;**101**:1001–7.
 74. Fransz P, Armstrong S, Alonso-Blanco C, *et al.* Cytogenetics for the model system *Arabidopsis thaliana*. *Plant J* 1998;**13**: 867–76.
 75. Islam-Faridi MN, Nelson CD, Kubisiak TL. Reference karyotype and cytomolecular map for loblolly pine (*Pinus taeda* L.). *Genome* 2007;**50**:241–51.
 76. Moraes AP, Mirkov TE, Guerra M. Mapping the chromosomes of *Poncirus trifoliata* Raf. by BAC-FISH. *Cytogenet Genome Res* 2008;**121**:277–81.
 77. Koo DH, Jo SH, Bang JW, *et al.* Integration of cytogenetic and genetic linkage maps unveils the physical architecture of tomato chromosome 2. *Genetics* 2008;**179**:1211–20.
 78. Szinay D, Chang SB, Khrustaleva L, *et al.* High-resolution chromosome mapping of BACs using multi-colour FISH and pooled-BAC FISH as a backbone for sequencing tomato chromosome 6. *Plant J* 2008;**56**:627–37.
 79. Tang XM, Szinay D, Lang C, *et al.* Cross-species bacterial artificial chromosome-fluorescence in situ hybridization painting of the tomato and potato chromosome 6 reveals undescribed chromosomal rearrangements. *Genetics* 2008;**180**:1319–28.
 80. Iovene M, Wielgus SM, Simon PW, *et al.* Chromatin structure and physical mapping of chromosome 6 of potato and comparative analyses with tomato. *Genetics* 2008;**180**: 1307–17.
 81. Stack SM, Royer SM, Shearer LA, *et al.* Role of fluorescence in situ hybridization in sequencing the tomato genome. *Cytogenet Genome Res* 2009;**124**:339–50.
 82. Okagaki RJ, Jacobs MS, Stec AO, *et al.* Maize centromere mapping: a comparison of physical and genetic strategies. *J Hered* 2008;**99**:85–93.
 83. Feng Q, Zhang Y, Hao P, *et al.* Sequence and analysis of rice chromosome 4. *Nature* 2002;**420**:316–20.
 84. Sasaki T, Matsumoto T, Yamamoto K, *et al.* The genome sequence and structure of rice chromosome 1. *Nature* 2002;**420**:312–6.
 85. Peterson DG, Lapitan NL, Stack SM. Localization of single- and low-copy sequences on tomato synaptonemal complex spreads using fluorescence in situ hybridization (FISH). *Genetics* 1999;**152**:427–39.
 86. Visser RGF, Bachem CWB, de Boer JM, *et al.* Sequencing the potato genome: outline and first results to come from the elucidation of the sequence of the world's third most important food crop. *Am J Potato Res* 2009;**86**:417–29.
 87. Jackson SA, Cheng ZK, Wang ML, *et al.* Comparative fluorescence in situ hybridization mapping of a 431-kb *Arabidopsis thaliana* bacterial artificial chromosome contig reveals the role of chromosomal duplications in the expansion of the *Brassica rapa* genome. *Genetics* 2000;**156**:833–8.
 88. Mandakova T, Lysak MA. Chromosomal phylogeny and karyotype evolution in x=7 crucifer species (Brassicaceae). *Plant Cell* 2008;**20**:2559–70.
 89. Draye X, Lin YR, Qian XY, *et al.* Toward integration of comparative genetic, physical, diversity, and cytomolecular maps for grasses and grains, using the sorghum genome as a foundation. *Plant Physiol* 2001;**125**:1325–41.
 90. Lysak MA, Koch MA, Pecinka A, *et al.* Chromosome triplication found across the tribe Brassiceae. *Genome Res* 2005;**15**:516–25.
 91. Yan H, Ito H, Nobuta K, *et al.* Genomic and genetic characterization of rice Cen3 reveals extensive transcription and evolutionary implications of a complex centromere. *Plant Cell* 2006;**18**:2123–33.
 92. Zhong CX, Marshall JB, Topp C, *et al.* Centromeric retroelements and satellites interact with maize kinetochore protein CENH3. *Plant Cell* 2002;**14**:2825–36.
 93. Zhang WL, Lee HR, Koo DH, *et al.* Epigenetic modification of centromeric chromatin: hypomethylation of DNA sequences in the CENH3-associated chromatin in *Arabidopsis thaliana* and maize. *Plant Cell* 2008;**20**:25–34.
 94. Carlson SR, Rudgers GW, Zieler H, *et al.* Meiotic transmission of an in vitro-assembled autonomous maize minichromosome. *PLoS Genet* 2007;**3**:1965–74.
 95. Yu W, Han F, Gao Z, *et al.* Construction and behavior of engineered minichromosomes in maize. *Proc Natl Acad Sci USA* 2007;**104**:8924–9.
 96. Ananiev EV, Wu C, Chamberlin MA, *et al.* Artificial chromosome formation in maize (*Zea mays* L.). *Chromosoma* 2009;**118**:157–177.
 97. Huang B, Jones SA, Brandenburg B, *et al.* Whole-cell 3D STORM reveals interactions between cellular structures with nanometer-scale resolution. *Nat Methods* 2008;**5**: 1047–52.
 98. Huang B, Wang W, Bates M, *et al.* Three-dimensional super-resolution imaging by stochastic optical reconstruction microscopy. *Science* 2008;**319**:810–3.
 99. Rust MJ, Bates M, Zhuang X. Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). *Nat Methods* 2006;**3**:793–5.
 100. Carlton PM. Three-dimensional structured illumination microscopy and its application to chromosome structure. *Chromosome Res* 2008;**16**:351–65.
 101. Wang CJ, Carlton PM, Golubovskaya IN, *et al.* Interlock formation and coiling of meiotic chromosome axes during synapsis. *Genetics* 2009;**183**:905–15.
 102. Gustafsson MG, Shao L, Carlton PM, *et al.* Three-dimensional resolution doubling in wide-field fluorescence microscopy by structured illumination. *Biophys J* 2008;**94**: 4957–70.
 103. Ananiev EV, Phillips RL, Rines HW. Chromosome-specific molecular organization of maize (*Zea mays* L.) centromeric regions. *Proc Natl Acad Sci USA* 1998;**95**:13073–8.