Chromosome Identification by Fluorescent *in situ* Hybridization with myTags® DNA Probes

BRINGING COLOR TO CHROMOSOME IDENTIFICATION

The use of microscopy for identification of specific chromosomes can be a challenge when working with organisms for which only limited cytogenetic tools, if any, exist. Target-specific DNA Fluorescent *in situ* Hybridization (DNA FISH) is a powerful technique for the detection of specific chromosomal loci. The myTags® product line from Arbor Biosciences provides services for the design and manufacturing of synthetic FISH probes that delivers computer-designed custom probe sets specific to their target sequences. With these techniques, it is possible to produce a highly specific collection of probes targeting one or more loci on each chromosome. By carefully selecting the number of loci to detect per chromosome, identifying their locations on each arm, and using more than one labeling color, it is possible to uniquely encode every single chromosome of a given species. Each condensed chromosome on a metaphase spread can be clearly identified by its unique pattern of colored spots, in a technique known as **"chromosome indexing"**.

Here, we present several published applications of chromosome indexing in plants, and outline major points of consideration when undertaking new chromosome indexing projects for different species. This easy-to-use technique can be applied to address many critical genomic research questions in plants, animals, insects, or any other species.

APPLICATIONS OF FLUORESCENT *IN SITU* HYBRIDIZATION IN CHROMOSOME INDEXING

Chromosome Identification

Figure 1 (Liu *et al.* , 2019) shows the use of just two unique probe sets for complete chromosome sorting. One probe set was labeled with a green fluorescent tag and the second with a red fluorescent tag. The characteristic red and green pattern allowed the researcher to easily sort the 12 pairs of chromosomes of two different rice species (Zhongxian 3037 and Nipponbare).

Figure 1. *FISH analysis of Zhongxian 3037 (a) and Nipponbare (b) rice on mitotic metaphase chromosomes using FAM (green) and digoxigenin (red) probes on somatic cell chromosomes in mitotic metaphase. (c) Chromosomal arrangement of 12 pairs of chromosomes in Zhongxian 3037 (c) and Nipponbare (d) somatic cells from subpanel (a) and (b), respectively. Scale bar = 5 μm.*
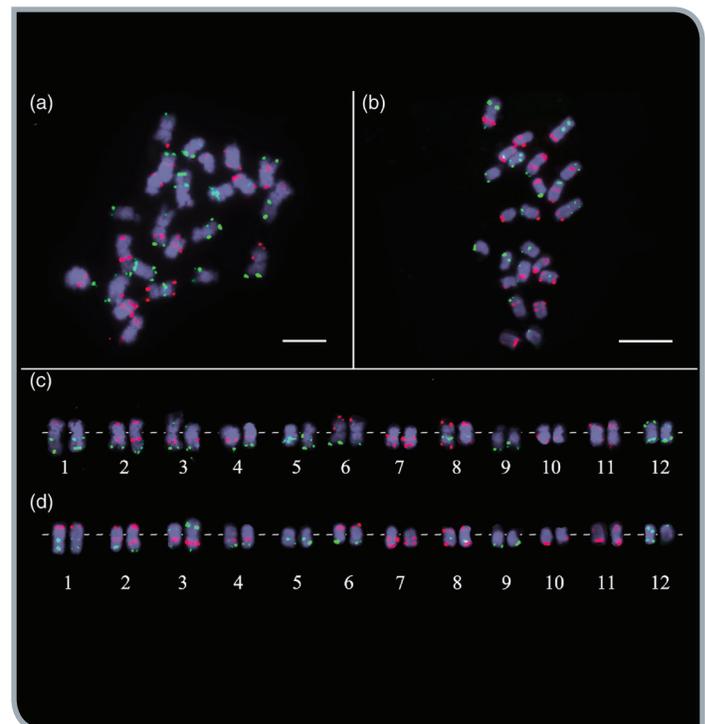
Ploidy Determination with Indexed Chromosomes

Indexing chromosomes with DNA FISH can provide insights into ploidy levels. The number of copies of each chromosome can be easily counted from a metaphase spread as shown in Figure 2 (Meng *et al.*, 2019). This example is in polyploid sugarcane *S. spontaneum*. The top portion of the figure shows all of the chromosomes of the complete metaphase cell while the bottom portion shows chromosomes sorted and indexed based on the pattern of green and red DNA FISH signals. The result is 8 groups of 8 identical chromosomes.
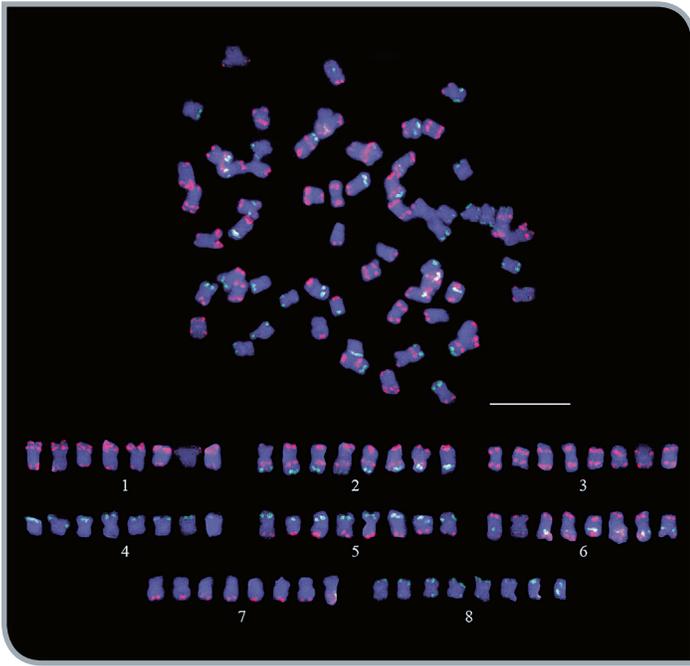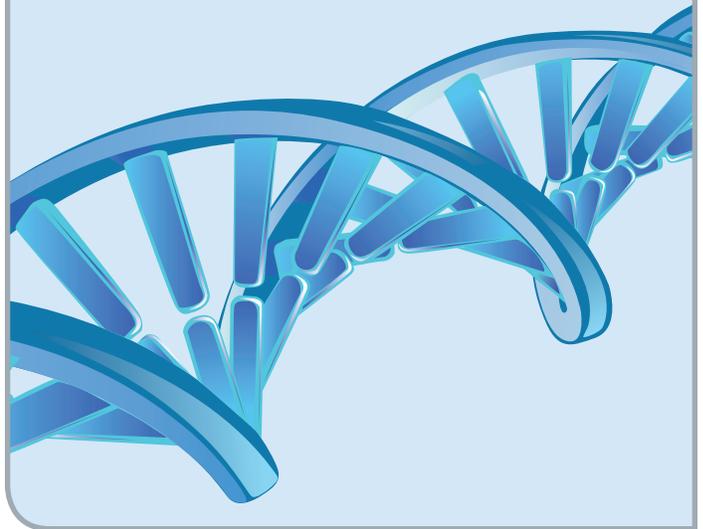

Figure 2. *Homologous chromosome grouping in polyploid sugarcane* S. spontaneum. *Scale bar = 10 µm.*

Tracking Chromosomes Through Evolution

Chromosome indexing has proven to be a powerful tool to identify chromosome relationship between related species. Probe sets derived from one or more species with sequenced genomes can be applied to identify chromosomes in unsequenced related species as demonstrated for the Solanum genus. In Figure 3, the indexing strategy identified a level of chromosome rearrangements in particular species. This type of rearrangement can also be found in cultivars of plants of agronomical interest.

Figure 3. *Two probe sets derived from the potato genome and cross-selected against the tomato genome were used to index chromosomes from 6 members of the Solanum genus. Arrows point to chromosome rearrangements compared to potato. See Braz* et al., *2018 for details.*

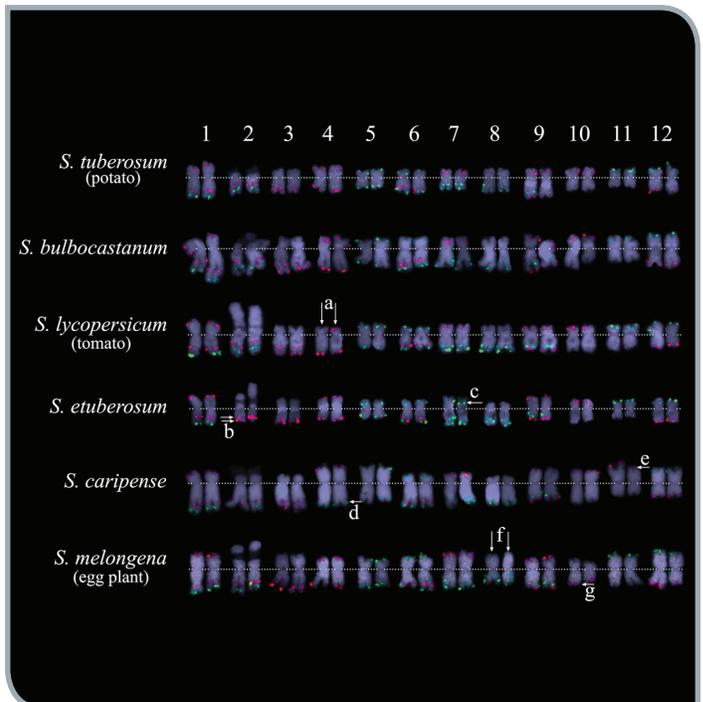## ADVANTAGES OF myTAGS® INDEXING WITH DNA FISH

- **DNA FISH chromosome indexing allows for unambiguous karyotype generation.**

- **Computer-aided design generates highly target-specific probes vs. BAC-derived probes.**

- **Fully custom synthetic oligonucleotide probes can be labeled with any dye.**

- **Probes can be designed for use across species for comparative analysis.**

DESIGN STRATEGIES FOR SUCCESSFUL CHROMOSOME INDEXING PROJECTS

There are several important principles to be utilized when designing a chromosome indexing experiment. Access to a sequenced reference genome that has been assembled into chromosomes is required. This is used to designate specific loci on the chromosomes that will be localized by the fluorescent probes. These specific fluorescent "spots" generate a unique pattern for each chromosome. Each spot must be easily resolved as a distinct and uniformly bright fluorescent signal. The brightness of the signal is controlled by the number of labeled probes per spot. The spacing of the spots on each chromosome arm determines the ability to resolve them as separate fluorescent signals. The Arbor Biosciences team of expert scientists will assist you in implementing these key design principles for any chromosomal indexing project.

1. Number of colors

A typical design would utilize two colors in order to have sufficient indexing signal patterns for each chromosome, but it is possible to use a single color for organisms with a very small number of chromosomes. Species with large numbers of chromosomes may benefit from using a third color.

2. Number of probes per spot

Arbor Biosciences generally recommends a minimum of 1,800 probes per fluorescent spot, with higher numbers giving a brighter signal. Our standard 27,000 probe library can provide up to 15 spots in one color. Typically 1,800 probes will cover a region of a few hundred kilobases. It is more important to maintain uniform signal by keeping the number of probes constant within each spot rather than having a constant target size for each spot.

3. Number of spots

We recommend using the minimum number of spots to maximize the number of probes per spot. For species with acrocentric chromosomes, chromosomes of significantly different sizes or where the centromere position can be easily recognized on stained metaphase chromosomes, it is possible to use the arm length as another level of identification. For example, a chromosomes with a green spot on a short arm would be different from a chromosome with a green spot on a long arm.

4. Spot location on chromosome arms

For uniform hybridization parameters and optimal design, spots should be located as far as possible from the centromere. For this reason, very short chromosome arms may not be a good target for spot location.

5. Distance between adjacent spots

To easily resolve more than one spot per arm, it is ideal to separate them by at least 5 to 15 Mb. This is a general guideline and may not apply to all organisms. The Arbor Biosciences team can provide assistance in refining this part of the design.

6. Multi-species hybridization

Probes can be optimized to hybridize to more than one species. Access to the sequenced genomes for the other species of interest is helpful. The brightness of the hybridization signal may be reduced as the sequence homology between species decreases.

7. Special cases: Plan for known translocations

Chromosome indexing can be used to track major translocation events (like the translocation of the end of a chromosome to another chromosome) or chromosome synteny across species or cultivars. The indexing strategy can be planned around the expected patterns in all samples so that the new pattern created by the translocation is unique and does not match other chromosome patterns.
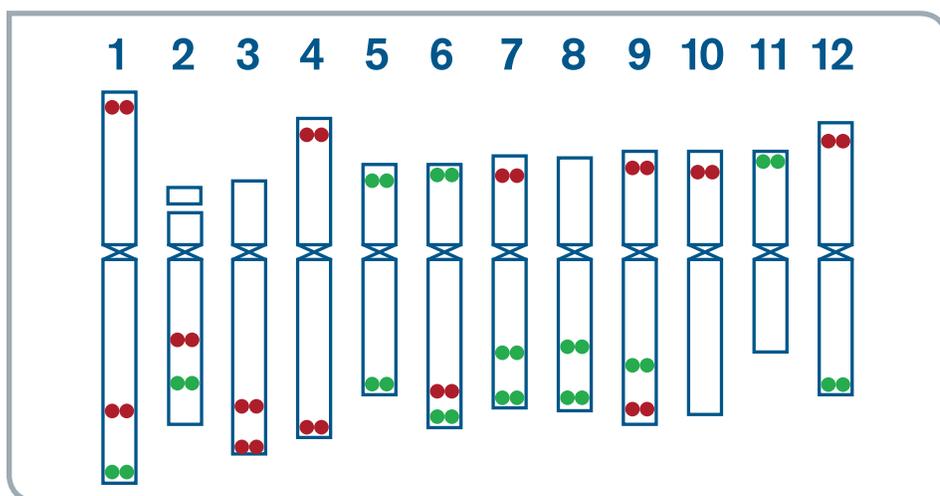

Figure 4. *Two-color chromosome indexing strategy for positive identification of each chromosome. Spots are presented as pairs corresponding to the two chromatids that will be labeled at the same time and as separate spots. (Braz et al. , 2018).*

What does Arbor Biosciences need for your design?

• Assembled genomic sequence of the species (one or more) to be studied

• Indexing strategy in the form of a diagram, similar to Figure 4

• The approximate coordinates of the regions to be targeted by each spot

• Optionally, you can perform the design yourself for Arbor Biosciences to manufacture

What will Arbor Biosciences provide you?

• Complimentary probe design service (optional)

• Set of custom designed synthetic indexing probes

• Optional labeling services

• Detailed probe design contents required for study publication
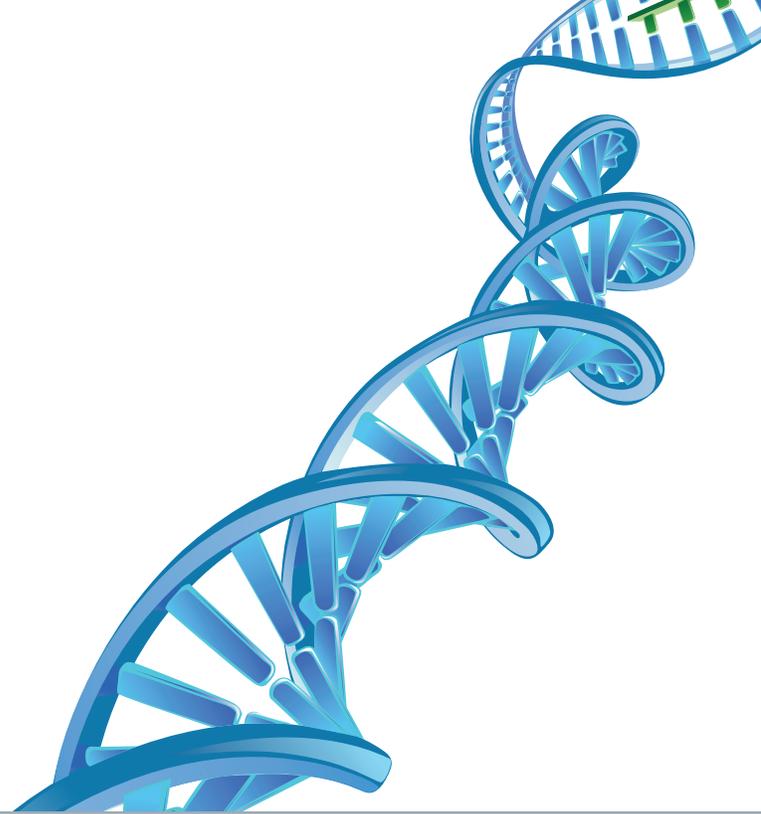
CONCLUSION

Chromosome indexing is a powerful and easy-to-use technique for rapid identification and tracking of chromosomes using target-specific DNA Fluorescent *In Situ* Hybridization (FISH). This powerful tool can be used to identify major chromosomal structural changes and rapidly compare them between samples. With Arbor Biosciences' complimentary probe design services and affordable manufacturing of customized myTags® FISH probes, we will help make your next chromosomal indexing project a success. Contact our team of dedicated scientists today to get started.

REFERENCES

Liu *et al.* (2019) **Dual-color Oligo-FISH Can Reveal Chromosomal Variations and Evolution in Oryza Species.** *The Plant Journal.*

Meng* *et al.* (2019) **Characterization of a Saccharum spontaneum with a Basic Chromosome Number of x = 10 Provides New Insights on Genome Evolution in Genus Saccharum.** *Theoretical and Applied Genetics.*

Braz *et al.* (2018) **Comparative Oligo-FISH Mapping: An Efficient and Powerful Methodology to Reveal Karyotypic and Chromosomal Evolution.** *Genetics.*

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