myTags® – Synthetic FISH Probes Design Schemes for Plant and Animal Molecular Cytogenetics

Recombination, Translocation, Ploidy and Karyotyping Applications

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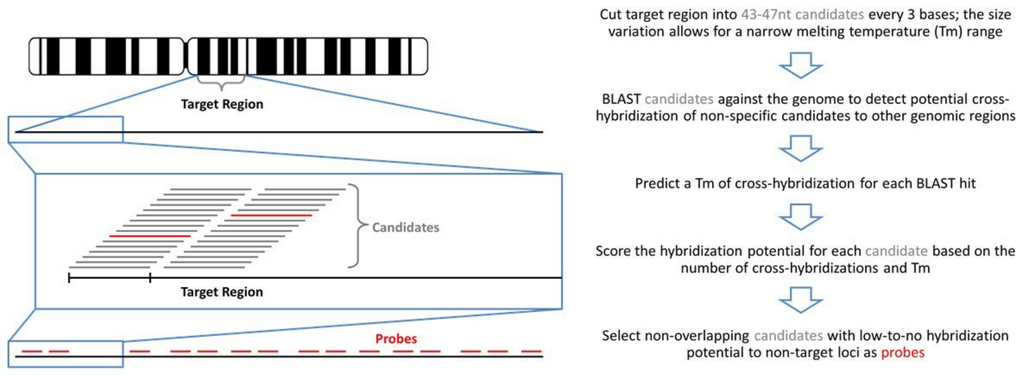
Developing the karyotype of most non-model plant and animal species relies on identification of individual chromosomes, which has been a major challenge. We have developed a technology to design, synthesize and label custom target-specific synthetic oligonucleotide fluorescent probes (myTags®) for fluorescent *in situ* hybridization (FISH). Through multiple collaborations we demonstrated these probes can be used to uniquely index chromosomes for rapid identification, from both diploid and polyploid species, paint whole chromosome to identify rearrangements such as translocations, or follow haplotypes through meiotic crossovers over multiple generations. These techniques were proven in multiple plant species but could also be applied to animal species. Probes designed from the potato genome were successfully used to identify the 12 homeologous chromosomes among distantly related Solanum species, including tomato and eggplant. In maize, whole chromosome painting demonstrated ability to identify translocations between chromosomes, while haplotype-specific chromosome painting permitted to follow parental and recombinant chromosomes in F1 hybrids and F2 progenies. We believe these techniques can be applied to a wide range of plant and animal species for analyzing recombination, rearrangements, karyotypes and chromosomal relationships for fundamental research and breeding purpose.

Probe Design Strategy

myTags® are computer-designed probes highly specific to their targets.

Our proprietary algorithm searches for any potential cross-hybridizations of a probe candidate across the entire genome. Unlike BAC random-labeling, *in silico* design permits excluding non-specific probes before synthesis and labeling. It also offers full flexibility in terms of placing target region(s) along the chromosome(s). Best signals are achieved for target greater than 10-20 Kb. A signal amplification method is currently under development to detect smaller targets. Other software such as Chorus[1] and OligoMiner[4] are available.



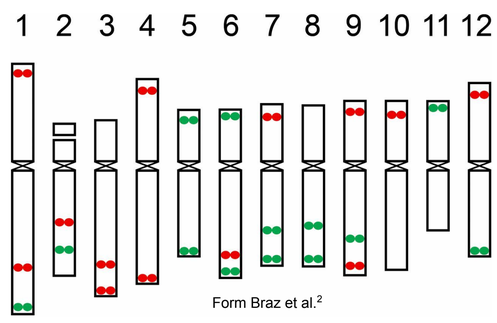
Probe Library Formats

Immortal vs. Labeled Probe Libraries

myTags® can be obtained as immortal template DNA libraries to be labeled on demand using a protocol that can generate an almost infinite amount of labeled probes. We have found that an *in-vitro* transcription followed by a reverse transcription primed with a fluorescently labeled tag is the most efficient way to generate a large amount of single-stranded labeled DNA probes. The fluorescent primer can be synthesized with one to several fluorophore molecules, and allows for consistent and adjustable labeling intensity. We recommend the use of 3 fluorophores per primer for small loci and/or a low number of probes.

Chromosome Indexing Strategy

To accurately identify chromosomes on a metaphase spread, it is possible to index them by targeting small regions with probes. By carefully choosing the number of targeted regions, their relative positions and colors, each chromosome is uniquely indexed and can be positively identified under the microscope. All probes to be labeled with the same dye are synthesized as a single library. Here, only two libraries are needed, a "green" one and a "red" one to index 12 chromosomes.

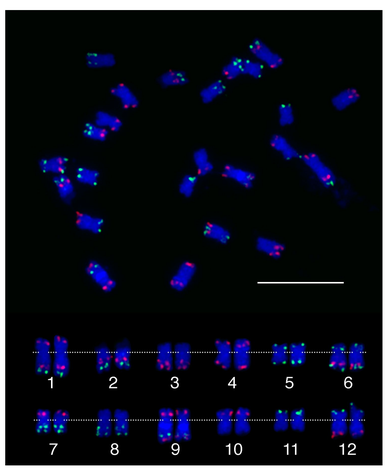


It is also possible to design libraries to paint whole chromosomes to more precisely map rearrangements such as translocation as shown on the right, or discriminate between parental allele (bottom right)

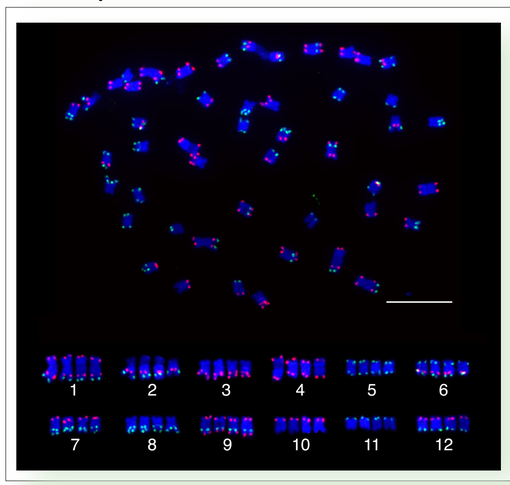
Chromosome Indexing with Two Labeled Probe Libraries

FISH mapping of potato chromosomes using two myTags® probe libraries[2]. The top panel show a complete metaphase potato cell. Homologous chromosomes in the bottom panel were digitally excised from the same cell and paired. The centromeres of the chromosomes are aligned by a dotted line. Chromosomes were indexed using the scheme presented on the left.

Signals correspond to about 2000 - 2250 probes designed with the Chorus software[1]. Each probe molecule is labeled with a single dye. Targeted region lengths vary from 266 Kb (bottom of chr 5) to 706 Kb (top of chr 6). Adjacent regions are spaced by 6.8 Mb (chr 6) to 14.7 Mb (chr 1).
Bar is 10 µm. Image from Braz et al.[2]



Ploidy Determination with Indexed Chromosomes

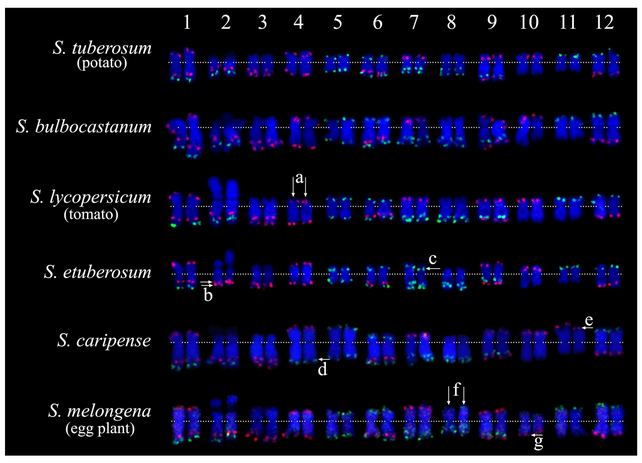


Chromosome identification in polyploid potato cultivar Katahdin[2]. The top panel shows a complete metaphase cell hybridized with the same two myTags® probe libraries as used in lower left section of this poster. The bottom panel shows the 4 homologous chromosomes of each of the 12 potato chromosomes digitally excised from the same cell.

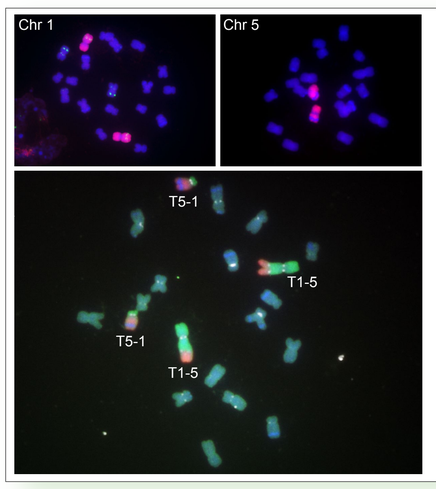
Bar is 10 µm. Image from Braz et al.[2]

Comparative Karyotyping Between Species (or Cultivars)

Carefully designed probes can be used to survey across taxa. Here is a comparative karyotyping of six diploid *Solanum* species[2]. Chromosomes 1–12 from each species are arranged from left to right. Karyotypes of potato was developed from the same metaphase cells as above. Karyotypes of the remaining five species are developed from individual metaphase cells. Arrows point to chromosome rearrangements compared to potato. See Fig. 4 from Braz et al.[2] for details.



Whole Chromosome Painting and Translocation Studies

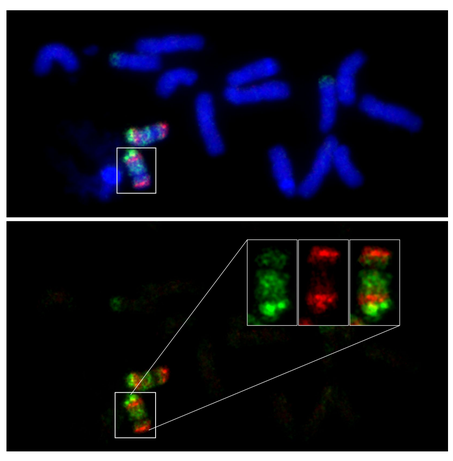


Top: Individual oligonucleotide paints for maize chromosomes 1 and 5[3]. Each library was labeled with ATTO-550 (red) and probed onto root tip metaphase spreads of inbred line KYS. Chr 1 is counterprobed with TAG microsatellite (green). Chr 5 is defined by being near metacentric with a large heterochromatic knob in the long arm (gap in painting).
Bottom: Use of whole-chromosome paints to characterize chromosomal aberrations[3]. Labeled myTags® libraries of chromosomes 1 (green) and 5 (red) were applied to homozygous material of reciprocal translocation T1-5 (8041). The centromere satellite, CentC, is labeled in white. The reciprocally exchanged chromosomes are labeled T1-5 and T5-1.
From Albert et al.[3]

Localizing Crossovers on Recombinant Inbred Lines

Oligo-FISH characterization of an intermated B73 x Mo17 recombinant inbred line (4 generations following F2). Green and red probe sets are specific to B73 and Mo17 chromosome 10 alleles, respectively.

Signals from one copy of chromosome 10 are exemplified. Two Mo17 blocks (green), including the centromere and two B73 blocks (red) are identified on chromosome 10 (adapted from do Vale Martins, L., et al.).



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SPECIAL THANKS

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