Synthetic Oligonucleotide *in situ* Hybridization Probes Provide Flexible, High-Fidelity Target Detection

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The use of *in situ* hybridization is a long-standing application used to visualize a wide range of DNA or RNA sequences in chromosomes, cells, tissue sections or whole tissue samples. There are several technical challenges and limitations associated with traditional probe sources, such as BACs and other biologically-derived material. Many of these problems are overcome by using oligonucleotides synthesized in a pool as a probe source. myTags *in situ* hybridization probes from Daicel Arbor Biosciences are bioinformatically-designed, fully customizable labeled or immortalized oligo pools ready for *in situ* hybridization in virtually any species or research model organism. Highlighted are several published and ongoing applications using myTags, including in visualization of genome organization and chromatin topology, chromosome identification, scaffold assembly, haplotyping, detection of large-scale structural variation, target gene mapping and expression detection. The design, synthesis, and downstream treatment processes have been streamlined to maximize specificity and flexibility, while minimizing cost.

Key Applications

Visualizing target sequences on chromosomes, in cells and nuclei by fluorescence *in situ* hybridization (FISH) is a fundamental tool in genomics and cell biology. Recently, *in situ* hybridization applications have benefited tremendously from synthetic oligo pools carefully designed to maximize specificity and sensitivity in a variety of hybridization experiments. myTags *in situ* probes are a highly flexible and cost-effective toolkit deploying this transformative technology. Highlighted here are numerous examples of their successful use in the literature:

Applications	Description	Featured Publications
3D Visualization and Analysis of Genome Organization	Coupling oligo-FISH with 3D microscopy and image reconstruction to study spatial organization of the nuclear genome, chromatin topology and interactions between chromosomes/chromatin.	Giorgetti, L. *et al.* (2016), *Nature*, doi:10.1038/nature18589. Zheng, M. *et al.* (2019), *Nature*,doi:10.1038/s41586-019-0949-1. Boyle, S. *et al.* (2020), *Genes & Development*, doi:10.1101/gad.336487.120.
Super-resolution Imaging of Nuclear Organization and Structures	Oligo-FISH in combination with super-resolution imaging techniques allows for analyzing the subcellular localization and spatial arrangement of targeted DNA sequences and RNA transcripts at the single cell level.	Beliveau, B.J. *et al.* (2015), *Nature Communications*, doi:10.1038/ncomms8147. Wang, P. *et al.* (2020), *Genome Biology*, 21(1), doi:10.1186/s13059-020-02030-2. Kubalová, I. *et al.* (2021) , Cell Biology, doi:10.1101/2021.09.16.460607.
Chromosome Identification or Indexing, Karyotyping and Chromosome Painting	Development of chromosome identification/indexing system with oligo-FISH allows for karyotyping based on individually identified chromosomes and studies of chromosome-scale genetic adaptation and evolution. Chromosome painting using chromosome or haplotype-specific oligo probes for studies of chromosomal evolution, monitoring chromosome pairing and transmission and assessing genomic sequence assembly.	do Vale Martins, L. *et al.* (2019), *Nature Communications*, doi:10.1038/s41467-019-12646-z. Agrawal, N. *et al.* (2020), *Frontiers in Plant Science*, doi:10.3389/fpls.2020.598039. de Oliveira Bustamante, F. *et al.* (2021), *Theoretical and Applied Genetics*, doi:10.1007/s00122-021-03921-z.
Detection of Chromosome Structure and Rearrangement	FISH-based assays to reveal chromosome structures and rearrangements including translocations, segmental duplications/deletions, and presence-absence variations, which are essential for studying recombination between chromosomes, chromosome synteny and evolution, massive gene duplication and amplification events, etc.	Albert, P.S. *et al.* (2019), *PNAS*, doi:10.1073/pnas.1813957116. Piperidis, N. and D'Hont, A. (2020),, *The Plant Journal*, doi:10.1111/tpj.14881 Wang, K. *et al.* (2022), *Chromosome Research*, doi:10.1007/s10577-021-09680-3.
Evaluation of Gene Expression and Regulation	Visualization of chromatin interactions and spatial arrangement/localization of target genes, nascent or mRNA transcripts. Using oligo-FISH allows for investigation of transcriptional and epigenetic regulation activities including the detection of SARS-CoV-2 RNA.	Wang, P. *et al.* (2020), *Genome Biology*, doi:10.1186/s13059-020-02030-2. Markaki, Yolanda and Plath, Kathrin (2021), doi:10.1016/j.cell.2021.10.022. Mirabelli, C. *et al.* (2021), *PNAS*, doi:10.1073/pnas.2105815118.

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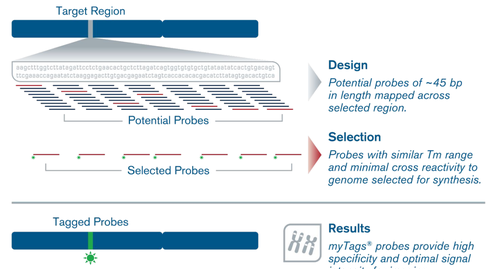
Custom Probe Design

Our experts apply an advanced proprietary probe design algorithm to craft a custom solution for your targeting needs. We have decades of experience designing custom probes for a wide variety of applications.



Single or Indexed Oligo Synthesis

For standalone, complex, and/or large probe designs, our Single Synthesis option provides maximum value. For smaller or multiple designs, our new Indexed Synthesis option maximizes flexibility and cost-effectiveness. All probesets are delivered individually and include composition verification via next-generation sequencing (NGS).

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Select from a variety of common fluorescent or non-fluorescent options to enhance your signal, in both standard and high-sensitivity configurations. Our flexible label options support multiplex ISH for colocalization and co-expression studies. Combine with our flexible oligo pool formats for a fully customized solution.

Label Options	
DY415	ROX
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ALEXA-488*	ATTO-633*
MAX	ATTO-647N*
ATTO-532	ATTO-655
ATTO-550*	ATTO-665
TAMRA	BIOTIN
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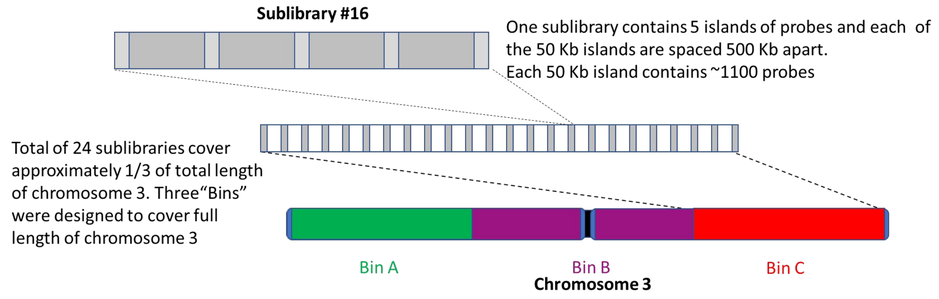
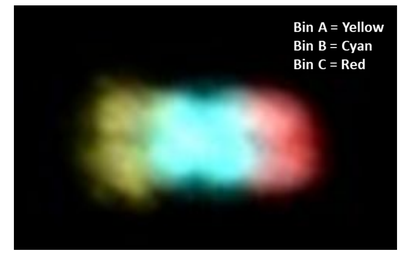
Design Strategy for Indexed Probes "Painting" Human Chromosome 3

The full length of human chromosome 3 was divided into 3 "Bins". Each bin contains 24 different indexed sublibraries. Each sublibrary covers a length of about 500 Kb and contains 5 islands each with about 1100 unique probes. These individual indexed sublibraries are deconvoluted by PCR. The composition of the deconvoluted pools is confirmed by NGS. All 24 sublibraries from each of the 3 bins can be combined to cover the full length of the chromosome. Alternatively, each sublibrary can be hybridized separately. This demonstrates the flexibility of Daicel Arbor Bioscience's new Indexed Synthesis technology.



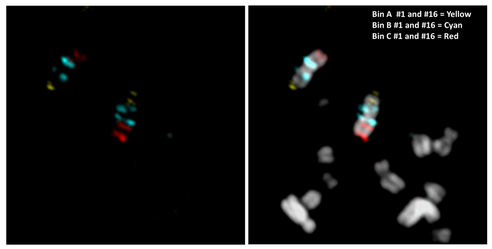
"Painting" Human Chromosome 3

Metaphase preparations were obtained from HT 1080 human cell lines. The three different bins of probes were labeled and hybridized to the preparations.



Labeling Human Chromosome 3 with Indexed Sublibraries

From each of the three bins, two different sublibraries (#1 and #16) were selected and hybridized to the preparations.



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Application Note - Chromosome Identification

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