# Target-specific synthetic oligonucleotide libraries for use in Fluorescent In Situ Hybridization

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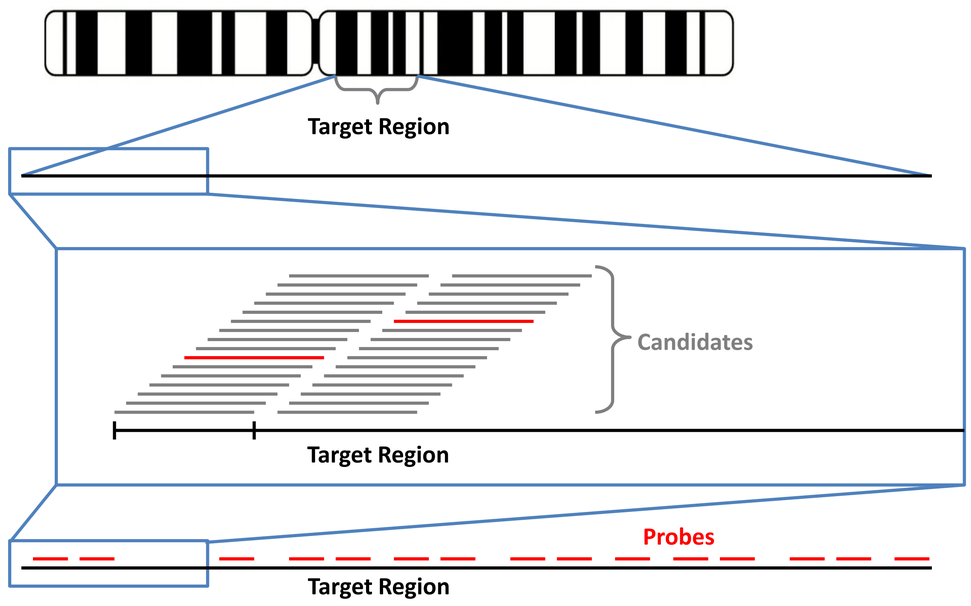
## Introduction

Human cytogenetic applications rely on detecting the presence, position, and location of specific chromosomal regions within the nucleus as well as chromosomal abnormalities, mainly through the use of Fluorescent In Situ Hybridization (FISH). Conventionally, FISH probes have been generated from PCR amplification of genomic regions or BACs followed by nick translation to incorporate fluorophore(s), a method that often incorporates repeated elements into the probes, reducing the specificity to the target region. We have developed a method to produce large quantities of customizable FISH probes (MYtags™) from synthetic oligonucleotides using in vitro and reverse transcription. MYtags™ show improved specificity and coverage over traditional genome-derived probes.

## Design




Cut target region into 43-47nt candidates every 3 bases; the size variation allows for a narrow melting temperature (Tm) range

BLAST candidates against the genome to detect potential cross-hybridization of non-specific candidates to other genomic regions

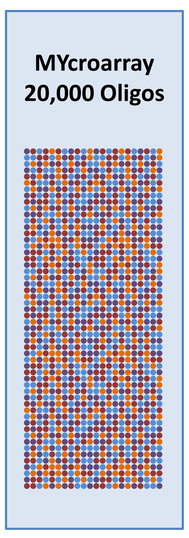
Predict a Tm of cross-hybridization for each BLAST hit

Score the hybridization potential for each candidate based on the number of cross-hybridizations and Tm

Select non-overlapping candidates with low-to-no hybridization potential to non-target loci as probes

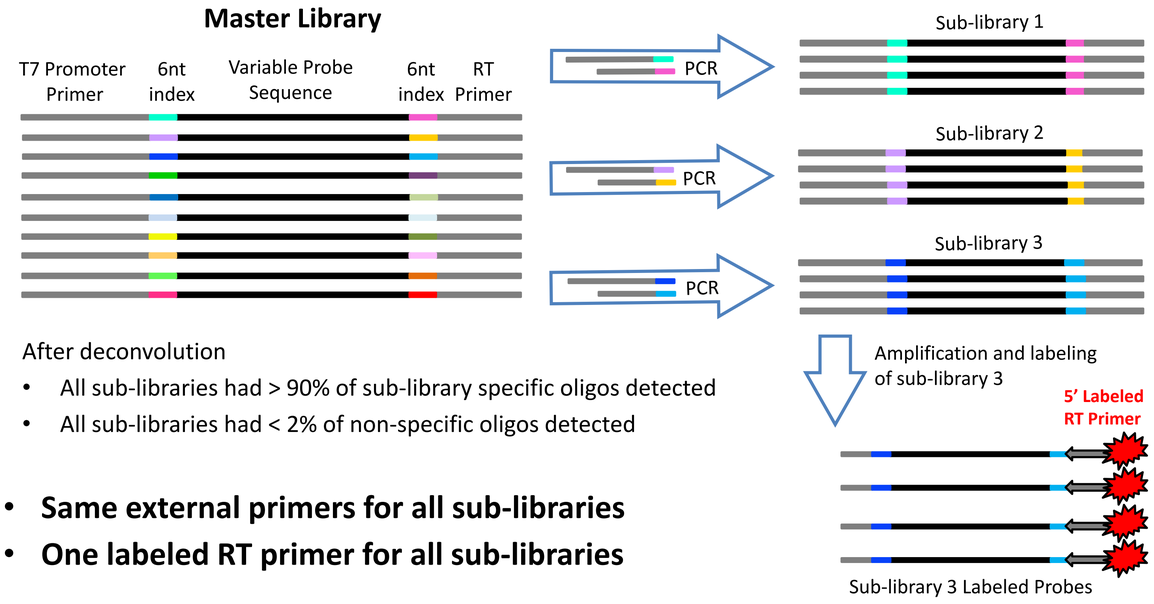
## Synthesis

- Up to 20,000 unique probes per slide
- Cleaved into a pool




## Deconvolved Sub-Libraries

**10 sub-libraries with unique combinations of indexed primer sets were synthesized as a master library and then deconvolved through PCR and detected on arrays**




After deconvolution

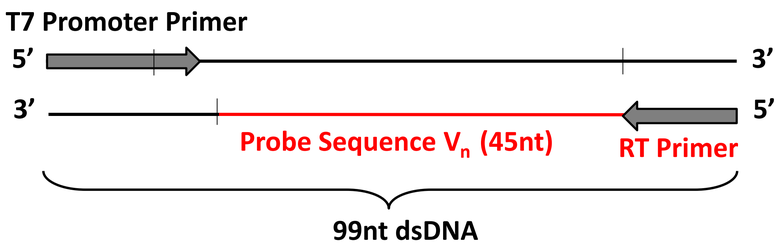
- All sub-libraries had > 90% of sub-library specific oligos detected
- All sub-libraries had < 2% of non-specific oligos detected

- **Same external primers for all sub-libraries**
- **One labeled RT primer for all sub-libraries**

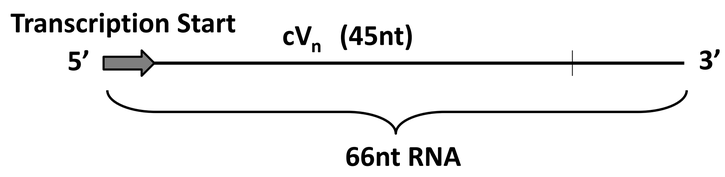
## Amplification & Labeling

### MYtags™ Immortal Library→ MYtags™ Labeled Probes

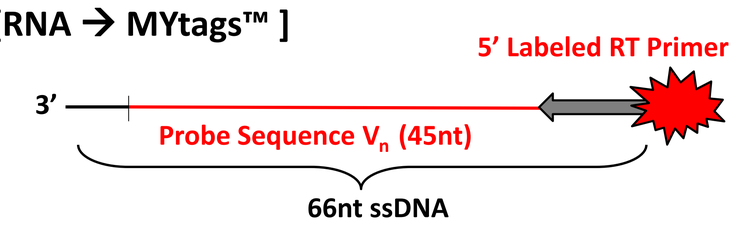
**1. PCR**




**2. In Vitro Transcription [dsDNA → RNA]**




**3. Reverse Transcription [RNA → MYtags™ ]**




### MYtags™ Immortal Library

- Produce labeled probes according to project needs
- Flexibility to use different labels


### MYtags™ Labeled Library

- Ready-to-use FISH probes
- Single or triple-labeled probes


### Yield

0.175ng Immortal Library

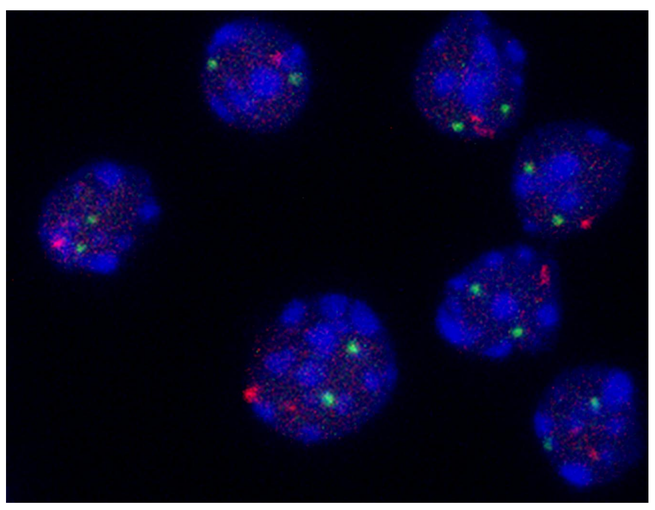
960ng dsDNA

170µg RNA

70µg Labeled Library (ssDNA probes)

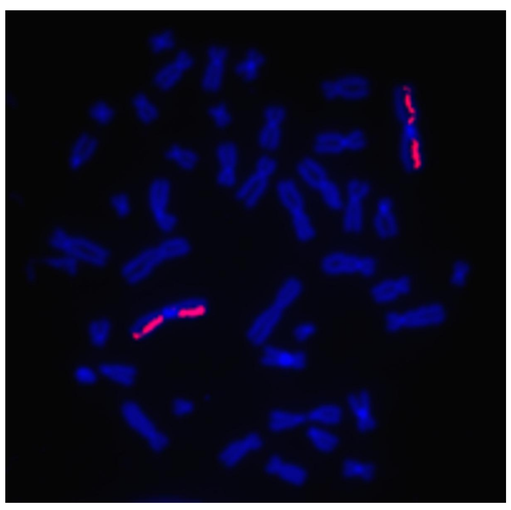
MY tags

## Applications



23pmol (0.5ug) input MYcroarray designed and IVT-RT labeled probes libraries in ATTO-594 (green) and ATTO-550 (red). Photo by Bingtao Hao, Jane Skok Lab, NYU

| Target Size | # Unique Probes | Probe Density (probes/kb) | Dye |
|---|---|---|---|
| 2.75Mb | 14402 | 5.23 | ATTO-594 (green) |
| 3.17Mb | 13684 | 4.32 | ATTO-550 (red) |



Directional Genomic Hybridization™ (dGH) using IVT-RT labeled probes library in ATTO-550 (red) on human chromosome 1. Photo by Erin Robinson, KromaTiD Inc.

**MYtags™ have been used in 3D-DNA FISH, Cryo-FISH, and RNA-FISH for mammalian, insect, and plant cells**

## MYtags™ - Advantages of highly customizable FISH probe libraries

- Custom probe design increases flexibility in FISH applications
- Target-specific complex probe libraries
- Eliminates repetitive elements from probe libraries
- Custom probes designed for minimal non-specific and cross-hybridization
- Probes designed with normalized Tm for single hybridization profile
- High resolution mapping – order of few kb
- Vary probe densities from 1-20 probes per kb
- More efficient penetration of probes

MYtags™ labeling protocol adapted from:
Murgha et al., in prep, Combined in vitro transcription and reverse transcription method to amplify and label complex synthetic oligonucleotide probe libraries
Murgha YE, Rouillard J-M, Gulari E (2014) Methods for the Preparation of Large Quantities of Complex Single-Stranded Oligonucleotide Libraries. PLoS ONE 9(4): e94752.