Fluorescence in situ hybridization (FISH) utilizes fluorescent probes to bind portions of DNA that have a high degree of sequence complementarity. This allows researchers to detect and localize specific DNA sequences on chromosomes or RNA targets in various cell and tissue types to determine the spatial-temporal patterns of gene expression within. In medicine, FISH can be used to diagnose or evaluate the progression of a disease, such as cancer, to identify a particular species or to perform various types of karyotyping.

Developing a FISH assay requires the use of oligonucleotide probe sets, like oligopaint probes, which are fluorescently labeled, single-stranded DNA oligonucleotides that can be used to visualize genomic regions ranging in size from tens of kilobases to many megabases. LC Sciences’ OligoMix offers a unique solution for researchers looking to generate oligopaint probes, as users are able create fully designed libraries of tens of thousands of specific, single-stranded oligonucleotide sequences for binding particular genomic regions. Several researchers have demonstrated the effectiveness of OligoMix in their FISH-assays and have provided model strategies for generating oligopaint probes through their work. The strategies they present are important because they provide an experimental model other individuals can emulate and apply to new areas of fluorescence hybridization.

In work conducted by Beliveau et al. (2014), researchers describe protocols for FISH, 3D-FISH, and oligopaint probe preparation and demonstrate their effectiveness in targeting several different genomic regions. The first set of probes they generated contained 20,020 oligos and was designed to target a 2.5-Mb region on human chromosome X (Xq13.1) in diploid (XY) MRC-5 cells. Researchers produced this probe set by following a protocol established in earlier work and carried out hybridization using a protocol optimized for labeling chromosomal DNA in fixed tissue culture cells. The basic protocol for generation of the oligopaint FISH probes begins with a complex ssDNA library of thousands to hundreds of thousands of unique oligos; like the OligoMix libraries which are quickly and inexpensively generated using LC Sciences’ microfluidic array.

Their oligos were designed with a pair of primer sequences (a forward primer and a reverse compliment of the reverse primer) that flanked a genomic sequence bound on either side by sites for nicking endonucleases, Nb.BsmI and Nb.BsrDI. Incorporating two nicking endonuclease sites allowed for the production of strand specific probes. Amplification with a labeled F primer and digestion with Nb.BsrDI yields a probe targeting the reverse complement of the genomic sequence, whereas amplification with a labeled R primer and digestion with Nb.BsmI yields a probe targeting the genomic sequence. Researchers note that a successful Oligopaint probe preparation typically converted 20% to 30% of the labeled primer into purified ssDNA FISH probe.

In addition to the probes designed for targeting human chromosome X, investigators applied the same probe preparation and hybridization strategies to perform two-color FISH on a field of tetraploid Drosophila Kc167 cells and a three-color FISH on Drosophila salivary polytene chromosomes. The results of all three FISH experiments are depicted below:

OligoMix® Microarray Synthesized Oligos for FISH Applications

Though NGS is capable of very high levels of coverage even on complex genomes, it is still advantageous to reduce the complexity of samples and sequence smaller targeted regions – in particular, when sample numbers are very high and the goal is detection of less prevalent or extremely rare mutations.
OligoMix was also incorporated in a separate three color FISH experiment carried out by Schmidt et al. from the Dana-Farber Cancer Institute. They designed a multiplexed library which targeted a centromere-proximal portion of the right arm of Drosophila chromosome 3 and subsequently performed three-color FISH in S2R+ cells. One probe set consisted of a 679 oligonucleotides targeting a 56-kilobase region at 82A1, while another consisted of 719 oligonucleotides targeting a 50-kilobase region at 82D2-82D5 and a third, single Cy5-labelled oligonucleotide targeted the highly repetitive dodeca percentromeric satellite sequence.

The probe generation strategy implemented by Schmidt et al. is unique, as researchers used circle-to-circle amplification (c2ca), instead of PCR, to generate their oligopaint probes. In circle-to-circle amplification, targeted template strands are cyclized via ligation into circular template strands, which are subsequently synthesized into chain-like repeated copies of the circular template by an enzyme with high processivity and strand displacement capacity. This amplification method is unique, because it overcomes some of the drawbacks of PCR like sequence-dependent amplification bias. In addition to this, c2ca is an isothermal process and therefore does not require quick temperature changes which impede the scalability of PCR reactions. Because c2ca amplified oligos do not carry a direct label, researchers used technology that calls for a common binding site for fluorophore-labelled ‘secondary’ oligonucleotide.

When applied to their three-color FISH assay, researchers observed crisp, clean signals with very low background from the c2ca generated oligopaint probes. They reported staining efficiency in the table below:

<table>
<thead>
<tr>
<th>Probe Set</th>
<th>Span kb</th>
<th>Complexity</th>
<th>Chr.</th>
<th>Start</th>
<th>Stop</th>
<th>n</th>
<th>0 Foci</th>
<th>1 Focus</th>
<th>2 Foci</th>
<th>&gt;2 Foci</th>
<th>% Labeling</th>
</tr>
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<tbody>
<tr>
<td>82A1</td>
<td>56</td>
<td>679</td>
<td>3R</td>
<td>60,018</td>
<td>116,183</td>
<td>105</td>
<td>3</td>
<td>70</td>
<td>29</td>
<td>3</td>
<td>97.1</td>
</tr>
<tr>
<td>82D2-82D5</td>
<td>50</td>
<td>719</td>
<td>3R</td>
<td>559,697</td>
<td>609,617</td>
<td>105</td>
<td>6</td>
<td>79</td>
<td>19</td>
<td>1</td>
<td>94.3</td>
</tr>
<tr>
<td>dodeca</td>
<td>Unknown</td>
<td>1</td>
<td>3R</td>
<td>N/A</td>
<td>N/A</td>
<td>105</td>
<td>0</td>
<td>51</td>
<td>43</td>
<td>11</td>
<td>100</td>
</tr>
</tbody>
</table>

Highly efficient Oligopaint FISH with probe sets made by barcoded c2ca (a) A cartoon that illustrates the structure of c2ca-amplified Oligopaint probes and the secondary oligonucleotide strategy used to recruit fluorescent label (b). Two confocal images of three-color FISH performed in Drosophila S2R+ cells with a probe set of 679 oligonucleotides targeting 56 kb at 82A1 (green), a probe set of 719 oligonucleotides targeting 50 kb at 82D2-82D5 (red) and a single oligonucleotide targeting the highly repetitive dodeca percentromeric satellite sequence (white). The Oligopaint probes were visualized by the addition of fluorophore-labelled secondary oligonucleotides complementary to binding sequences encoded in the probe molecules (left panel: 82A1—ATT0565, 82D2-82D5—ATT0488; right panel: 82A1—6-FAM, 82D2-82D5—TYE563), while the dodeca probe carried a Cy5 direct label. Note that Drosophila pairs its homologous chromosomes in somatic cells; therefore, most cells are expected to have only one focus despite that fact that S2R+ cells are tetraploid for chromosome 3. Images are maximum Z projections. Scale bars, 5 μm.