Oligo FISH Probe Customization

Shatter BAC FISH technology limitations with true customization

True customization

FISH customization service providers in the market primarily rely on BAC technology, a limited library of bacteria clones to produce FISH probes. Previously clone availability had dictated which probes can be made in what region. Leveraging the Agilent’s oligo library flexible manufacturing capability, customers may now design and order via SureDesign (a web-based application) any probes targeting any region with base pair level precision.

Improve probe specificity with precise targeting

Oligo FISH customization eliminates the mismatch between the targeted region of interest and probe coverage region. Oligo FISH probes are designed on the computer (via SureDesign) which allows precise targeting of the region of interest, therefore significantly improving probe specificity with no partial coverage and no “overhangs” outside the region.

Eliminate probe size limitation

With BAC technology, custom FISH probes are limited to sizes of approximately 150 kb or larger. Oligo FISH customization eliminates that size limitation. Robust signals can be seen on 50 kb sized probes, and success with probe sizes down to 20 kb have been reported.

With greater adoption of array and NGS technology, clinical researchers are finding a greater quantity of, as well as finding smaller, genetic aberrations. Oligo FISH customization enables findings at higher resolution for rapid confirmation and targeted screening needs.

Shown here is a 50 kb custom design for IKZF1 deletion detection in Leukemia. Previously this small deletion was only detected through PCR technology, due to the BAC FISH probe size limitation.

Figure 1. SureFISH Probes are designed in silico to allow precise targeting of the sequence of interest.

Figure 2. A 12 kb custom probe shows a clear signal (red) in metaphase spread. The green signal is a cohyb control.

Figure 3. A 50 kb custom IKZF1 probe shows great hybridization signal (red).

Figure 4. IKZF1 deletion diagram shown is from below publication: “Refinement of IKZF1 recombination hotspots in pediatric BCP-ALL patients”. Am J Blood Res. 2013; 3(2): 165–173.

Read more about IKZF1 deletion in the publication: “Identification and molecular characterization of recurrent genomic deletions on 7p12 in the IKZF1 gene in a large cohort of BCR-ABL1-positive acute lymphoblastic leukemia patients; on behalf of Gruppo Italiano Malattie Ematologiche dell’Adul to Acute Leukemia Working Party (Gimema AL WP)”. 2009 114: 2159-2167. Prepublished online July 9, 2009. doi:10.1182/blood-2008-08-173963.
Expanded utility to non-standard or non-human sequences

Oligo FISH technology enables FISH probes for non-standard sequences. For the first time within the industry, it will be possible to order custom probes for non-human applications.

Shown: three-color custom probes for a canine cancer application

Probe quality without compromise

Previously custom FISH probes have had known quality issues. BAC libraries used for custom FISH had been developed for the human genome project and not specifically for FISH applications. In order to achieve high quality, BAC catalog probes typically require long development cycles to fine tune probe signal and to block non-specific staining. Due to limited demand and turn around time expectations, custom BAC probes do not go through the same development cycle. As a result, custom BAC probes are lower quality and frequently fail. Often multiple probes are ordered for the same region, to compensate for performance risks.

All approved Agilent oligo FISH custom probes must pass in-house FISH QC before shipment. Confidence in quality is derived from an in silico design process which eliminates all repetitive sequences including junk DNA and potential segment duplications, and thus eliminates concerns of non-specific stains. Additionally, in silico QC translates target sequences to oligo probe characteristics and thus enables predictable probe performance.

Figure 5. Three-color custom probes for canine cancer application, using Agilent oligo customization.

Figure 6. Three-color custom probes for canine cancer application, using BAC technology.

Figure 7. Repeat-free probes lead to minimal cross hybridization and low background.

Figure 8. PAX3 BA – custom probes with catalog quality. A) Agilent custom probes B) competitor catalog probes

Figure 9. CIC BA – high quality custom. A) Agilent custom probes. B) Competitor custom probes.
Design and order your custom probe through SureDesign today:

1. Choose copy number, break-apart, or dual fusion probes
2. Enter target region as genes or chromosome coordinates
3. Receive proposed oligo probe design
4. Choose dye color and place order
5. Probe ships out in 2–3 weeks after placement of orders

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Trusted Answers. Together.