Designing Custom Oligo FISH Probes for the Detection of Chromosomal Rearrangements in FFPE Tissues

Anne Bergstrom Lucas[1], *Mike Ruvolo*[1], Julia Barboza[3], Vinayak Kulkarni[1], Siyuan Chen[1], Becky Mullinax[2], Jennifer Venneri[2], Stephanie Fulmer-Smentek[1], Scott Happe[3], and Maithreyan Srinivasan[1]

Diagnostics and Genomics Group, Agilent Technologies, [1]Santa Clara and [2]La Jolla, California and [3]Cedar Creek, Texas

Agilent Technologies

ASHG2013-3217F

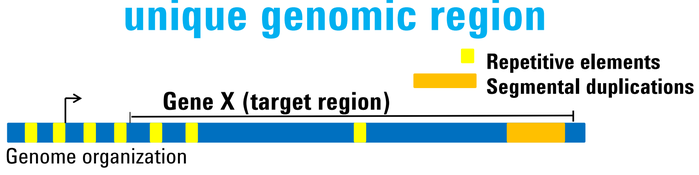
Introduction

Cancer cells frequently contain chromosomal rearrangements that result in oncogene activation, and the genes involved in these rearrangements are increasingly being identified using molecular technologies. We have developed a new generation of fluorescently labeled in situ hybridization (SureFISH) probes for the detection of these rearrangements. SureFISH probes are comprised of thousands of unique long oligonucleotides that are tiled across the targeted chromosomal region avoiding non-unique portions of the genome. The oligonucleotides are synthesized using Agilent's Oligonucleotide Library Synthesis (OLS) technology. Using knowledge of translocation breakpoints, SureFISH probes are designed to detect the translocated sequences using both break-apart and dual fusion strategies. The in silico design methodology and de novo synthesis of the SureFISH probes enables the optimization of design characteristics so that each probe provides balanced signals, facilitating the detection of chromosomal rearrangements. The flexibility afforded by the SureFISH design pipeline also enables rapid probe customization. Custom designs can be generated that target almost any genomic region, allowing for the production of probes that are not possible using other methods. We demonstrate the performance of both catalog/routine and custom probes on cytological samples and tissues that have been preserved in formalin and embedded in paraffin (FFPE).

Oligo-Based SureFISH Probe Design

Prior to the development of SureFISH probes, FISH was primarily performed using probes generated from bacterial artificial chromosomes (BACs). Because of their method of generation, BAC-based probes have limitations with regard to resolution and specificity.

SureFISH probes can target any unique genomic region



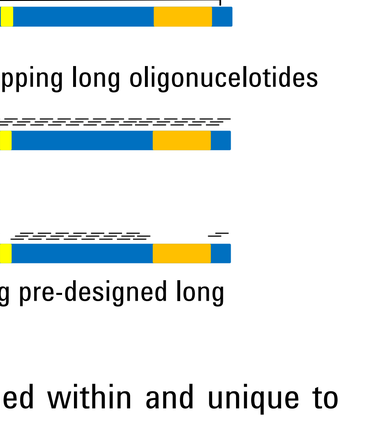
1) Tile region of interest with overlapping long oligonucelotides

2) Remove all non-unique oligos:

3) Manufacture labeled probes using pre-designed long oligonucleotides

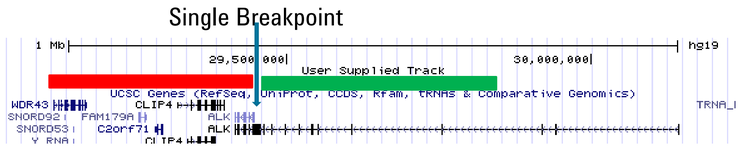
Only oligos that are contained within and unique to the targeted region are used in the probe, resulting in specific signals and eliminating the need for suppressive hybridization reagents such as Cot1 (Yamada, *et al.,* 2011).

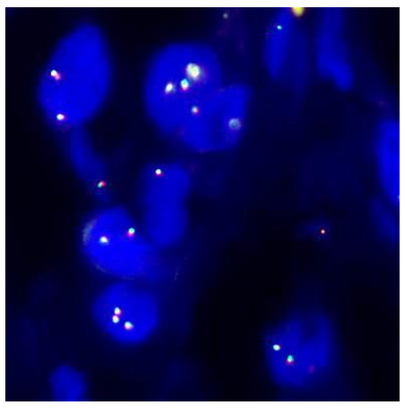
Break-apart probes for the detection of translocations

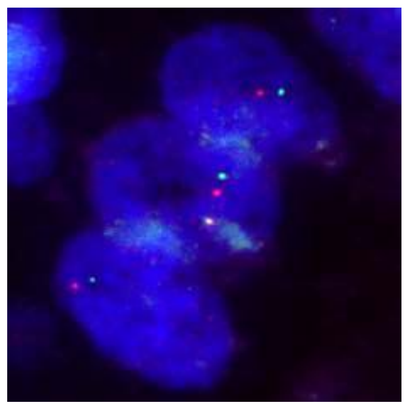


Analysis of FFPE Samples

ALK translocation probe*

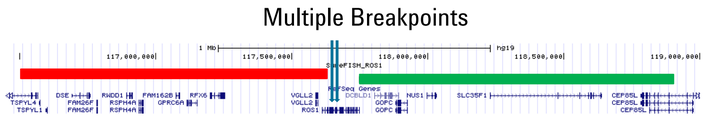


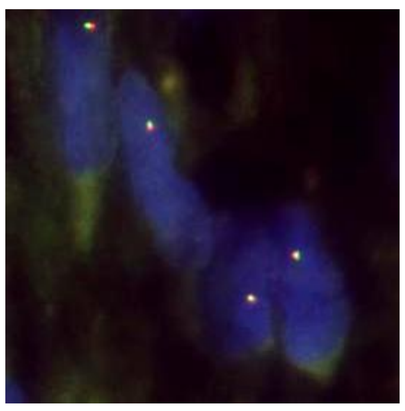


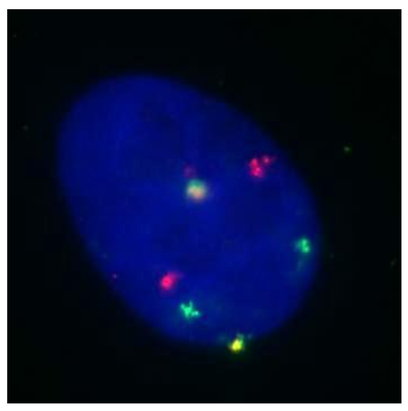


ALK translocation detection in a Lung Adenocarcinoma FFPE sample. Image showing co-localization of the red and green signals, consistent with intact ALK (Left). Distinct green and a distinct red foci indicating a rearrangement involving the ALK gene (Right). Probe was hybridized for 75min followed by visualization on epi-fluorescent microscope

ROS1 translocation probe*

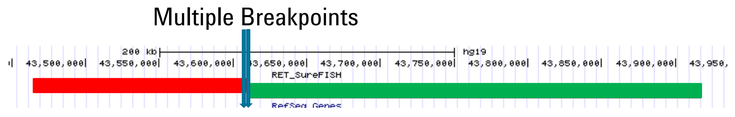


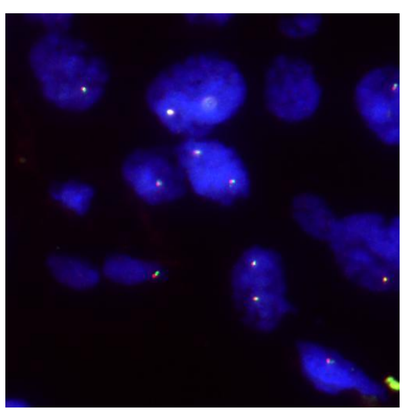


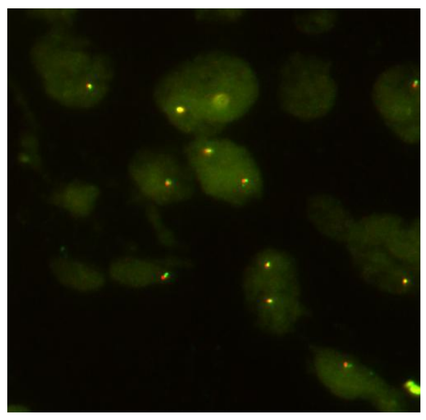


Lung Adenocarcinoma FFPE sample with co-localization of the red an green signals (Left). Cell line showing red and green signals, consistent with rearrangement of ROS1 (Right)

RET translocation probe*





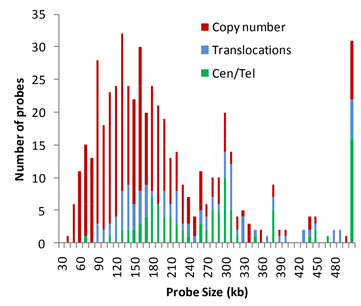


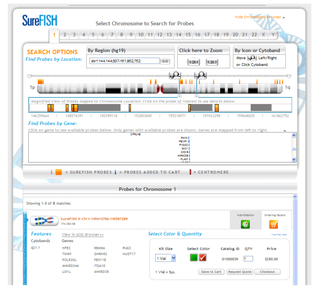
The bright signal and low background allow for the easy evaluation of the probe signal pattern. FFPE sample showing co-localization of the red and green signals. DAPI removed from right image

* Custom probe, not commercially available

Catalog probes for multiple applications

Over 400 probes available

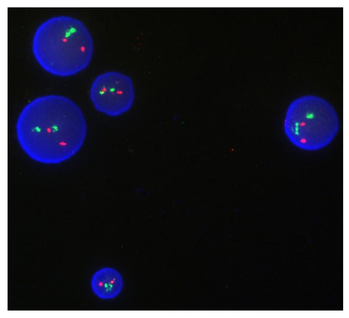


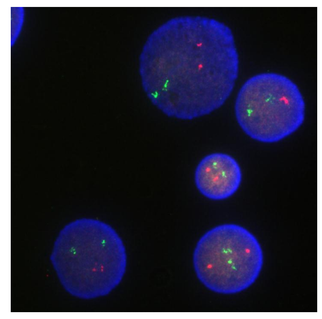


Probe size varies depending on the application, with resolution as low as 50kb. Probes are displayed on the SureFISH website by chromosomal position (www.agilent.com/genomics/SureFISH). Searching by location, target gene name, probe ID or keyword allows for rapid identification of probes of interest.

SureFISH technology provides high specificity

Lack of repetitive sequences results in specific signal and low background





SureFISH probes **BAC probes**

SureFISH and BAC-based probes targeting the BCR (green) and ABL (red) genes were hybridized to karyotypically normal cells. SureFISH probes showed lower background hybridization, demonstrating the enhanced specificity.

Conclusions

- *In silico* design coupled with Agilent's high fidelity oligo synthesis enable SureFISH probes to have maximum specificity and higher resolution than traditional FISH probes
- SureFISH probes are tailored for specific applications such as translocation detection utilizing Break-apart probe design strategy
- SureFISH probes perform well on FFPE samples with a workflow that is compatible with those for processing traditional FISH probes,

References

Yamada NA, Rector, LS, Tsang P, Carr E, Scheffer A, Sederberg MC, Aston ME, Ach RA, Tsalenko A, Sampas N, Peter B, Bruhn L, Brothman AR. (2011) *Cytogenet Genome Res*. 132(4):248-54.

Analyte Specific Reagent.
Analytical and performance characteristics are not established.