Novel four color FISH probe for simultaneous detection of RET break-apart and PDGFRα amplification

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Seamless Results.

INTRODUCTION

A number of critical genomic rearrangements are associated with therapeutics in non small cell lung carcinoma (NSCLC). There is frequently limited FFPE material available and in the interest of utilizing scarce clinical samples, we developed a four-color RET/PDGFRα/KIT/CC4 FISH probe. The probe provides coverage of the PDGFRα gene (4q12), the KIT gene (4q12), a copy control probe located at 4p12 on chromosome 4 and detection of RET gene rearrangements (10q11.2). This novel four color FISH probe has been validated for clinical use on 4 to 5 um thick FFPE sections.

CONCLUSION

An assay validation was performed using 23 negative samples consisting of de identified Human Bio specimens, xenografts and cell lines for genes of interest including 2 positive samples for PDGFRα amplification and 2 for RET rearrangement. The assay demonstrated 100% concordance with known standards and yielded clinically actionable cut-offs that comply with industry standards.

PROBE DESIGN

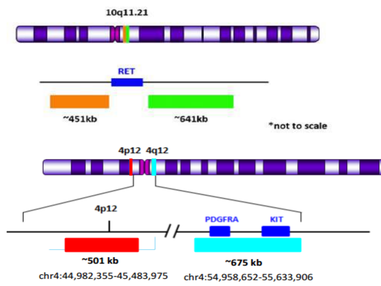

Figure 1 : Probe design for the RET/PDGFRα FISH probe manufactured by Biocare Medical LLC for RUO purposes only. The RET proto-oncogene encodes a receptor tyrosine kinase, and chromosomal rearrangements that generate a fusion gene resulting in the juxtaposition of the C-terminal region of the RET protein with an N-terminal portion of another protein, can also lead to constitutive activation of the RET kinase. The RET gene is localized to chromosome 10 (10q11.2) and has 21 exons. PDGFRα or the Platelet- derived growth factor receptor mutations are associated with idiopathic hypereosinophilic syndrome, somatic, familial gastrointestinal stromal tumors, and a variety of other cancers. The PDGFRα gene is localized to chromosome 4 (4q12) and has 28 exons. This gene plays a role in organ development, wound healing, and tumor progression.

PROBE LOCALIZATION

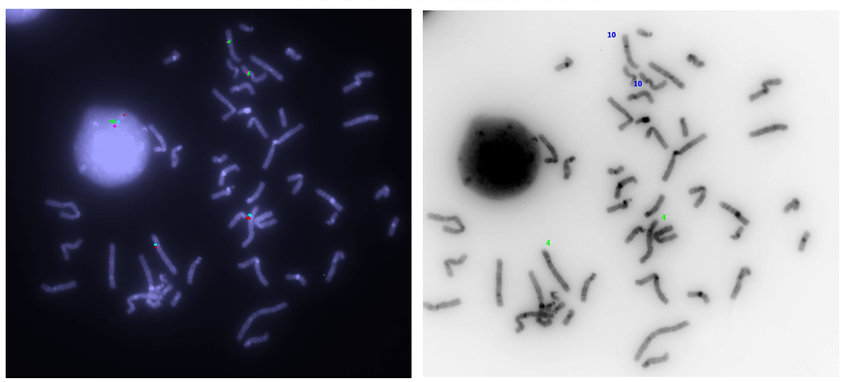

Figure 2: Two known normal male metaphase slides were prepared using standard techniques and hybridized using standard methodologies. Above is the fluorescent image seen under a Zeiss microscope compared to an inverted DAPI image that allows identification of chromosomes as a quality control measure. A total of 50 metaphases were analyzed, and five representative images were captured to confirm proper probe localization.

The PDGFRα/KIT/CC4/RET novel four color probe set displayed 100% probe sensitivity and 100% probe specificity and there was no evidence of background or cross hybridization detected.

ACCURACY

Sample ID/ Name	Results from Validation	
	Result by FISH	Result by NGS
NCIH 1693	5.62 copies PDGFRα Negative for RET rearrangement	4.6 copies PDGFRα RET mutation Not Detected
LC2Ad	Negative for PDGFRα amplification RET rearrangement >68%	PDGFRα amplification Not Detected CCD6-RET translocation Detected
GM19204 (Hapmap, WT)	Negative for PDGFRα amplification Negative for RET gene rearrangement	PDGFRα amplification Not Detected RET mutation Not Detected

Figure 3: Accuracy of FISH assay**:** all samples were assayed according to SOP approved by the Medical Director. For this validation, the normal cohort consisted of sectioned FFPE cell-line derived blocks and NSCLC specimens previously screened using an orthogonal technology at PacificDx. The abnormal cohort comprised of known positive NSCLC samples, sectioned FFPE cell-line derived blocks, and custom xenografts .

Assay acceptance criteria: >95% concordance in between Negative/ Positive calls between NGS and FISH methodologies.

IMAGING AND REPORTABLE RANGE

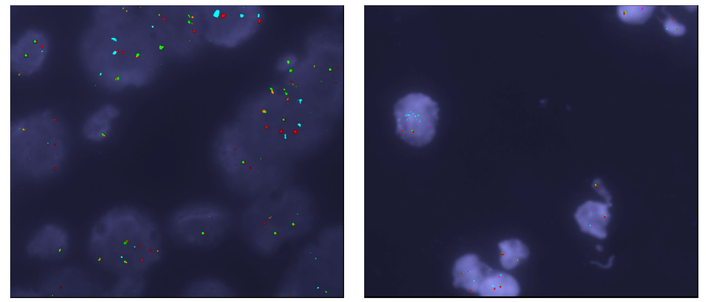

Figure 5: Representative images taken for validation samples. Image on right is from NCI-H827 cell line positive for 8x amplification of PDGFRα amplification and the image on the left is negative for both genes of interest.

Reportable ranges: A cohort of 23 known negative samples was used to calculate assay cut- off for PDGFRα copy number at 3.65 copies. Samples that have >6 PDGFRα average copy number or a test over reference ration of ≥2.0 are classified positive. Samples with a PDGFRα copy number between 4 and 6 are classified as equivocal /low positive if the ratio is <2.0.

RET gene rearrangements usually occur in high frequencies and the calculated cut off for classical 1R1G1F pattern was 4.0%. All other rearrangement patterns will be evaluated at greater than 5.0% based on significance.

REPEATABILITY AND REPRODUCIBILITY

Sample ID	RET Break-apart %/ Avg PDGFRα copy No. Repeat 1/ Day 1	RET Break-apart %/ Avg PDGFRα copy No. Repeat 2/ Day 1	RET Break-apart %/ Avg PDGFRα copy No. Repeat 3/ Day 1	RET Break-apart %/ Avg PDGFRα copy No. Repeat 4/ Day 2	RET Break-apart %/ Avg PDGFRα copy number Repeat 5/ Day 5	Standard deviation RET Break-apart/ Avg PDGFRα copy No.	% Confidence Interval
GM19204	2% / 2.26 copies	2%/ 2.30 copies	2%/ 2.35 copies	0% /2.20 copies	2% / 2.32 copies	0.014/ 0.06	0% / 2.54%
LC2Ad	68% / 3.16 copies	60% / 3.20 copies	62% / 3.24 copies	54% / 3.1 copies	60% / 3.5 copies	0.05/ 0.15	8.25%/ 4.76%
Sample I	2%/ 2.64 copies	2%/2.68 copies	2%/ 2.81 copies	2%/ 2.70 copies	2%/ 2.75 copies	0.07/ 0	0%/ 2.42%

Figure 4: Above are the results of the assay repeatability and reproducibility criteria. A set of three samples were selected from the validation dataset and repeated three times on the same day and twice more on different days. The results were combined and standard deviation calculated to derive the percent co- efficient of variation. The target criteria for assay acceptance is >95% concordance with reference results and percent co-efficent of variation of <25%. Both criteria have been met above successfully. The entire validation was performed on at least 20 normal/ negative samples from Non- Small Cell Lung Carcinoma cell lines or Human Bio specimens in order to derive cut- offs for PDGFRα amplification and RET gene rearrangement.

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