Visualizing KRAS point mutations in non-small cell lung cancer tumors using the BaseScope in situ hybridization assay

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Introduction

About 25% of non-small cell lung cancer (NSCLC) patients bear one or more KRAS mutations in their tumors, which is correlated with poor prognosis. The precise identification of somatic mutations in tumors is becoming increasingly important for studying tumor progression and developing targeted therapies. While sequencing technologies allow for mutation-profiling, they do not permit direct visualization and association of genetic alterations with cellular morphology. In addition, DNA mutational status does not predict expression of the mutant allele which may provide information connecting genotype to phenotype. Therefore, a technology for mutation detection at the transcript level directly in the tumor context is desirable.

To address this need we developed a specialized RNA *in situ* hybridization (ISH) method known as BaseScope. The BaseScop assay has a unique signal amplification system that allows for highly sensitive and specific detection of single nucleotide point mutations in tissues.

Methods

- **Objective:** To detect different KRAS single nucleotide point mutations in NSCLC tumors with tissue context
- **Sample used::** 48 core Human Non small-cell lung cancer FFPE tumor microarray
- **QC:** RNA quality and background signal threshold for each tumor core was determined by using *PPIB* (positive) and *dapB* (negative) control probes.
- **Assay:** BaseScope™ VS Red assay
- **Target probes:**

KRAS WT probe	KRAS point mutation probe
BA-Hs-KRAS-G12-34ntWT	BA-Hs-KRAS G12C
	BA-Hs-KRAS G12S
BA-Hs-KRAS-G12-35ntWT	BA-Hs-KRAS G12V
	BA-Hs-KRAS G12A

- **Quantification:**
  - Total number of cells per core were quantified using the HALO™ analysis software from Indica labs.
  - Positive signal was indicated by red punctate staining.
  - Number of cells with positive signal were counted manually.

BaseScope assays workflow for point mutation detection

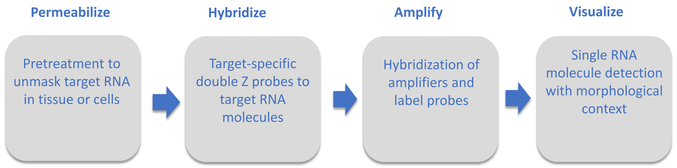

Figure 1: Step-wise depiction of the BaseScope assay workflow for spatial gene expression analysis.

Results

Detection of wild type KRAS in lung tumors using the BaseScope assay

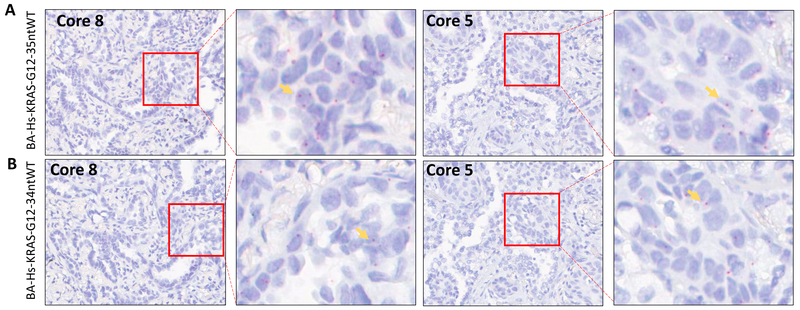

Figure 2: Visualization of wild type KRAS mRNA in NSCLC tumor TMA: Detection of WT KRAS using the **A**, BA-Hs-KRAS-G12-35ntWT BaseScope probe and **B**, BA-Hs-KRAS-G12-34ntWT BaseScope probe in tumor core 8 and core 5 of the NSCLC TMA. Positive signal is indicated by red punctate staining (Yellow arrows).

Frequently occurring KRAS mutations were identified using the BaseScope assay

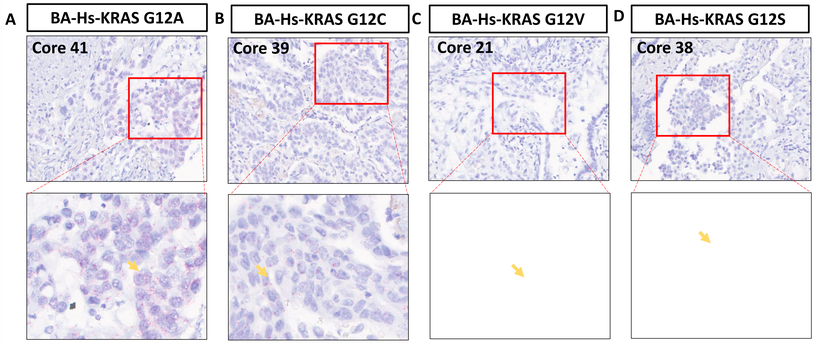

Figure 3: Detection of different KRAS mutations using specific BaseScope probes in NSCLC tumors: **A**, BA-Hs-KRAS G12A probe identified KRAS G12A positive cells in Core 41 of the TMA. B, BA-Hs-KRAS G12C probe identified KRAS G12C positive cells in Core 39 of the TMA. C, BA-Hs-KRAS G12V probe identified KRAS G12V positive cells in Core 21 of the TMA. D, BA-Hs-KRAS G12S probe identified KRAS G12S positive cells in Core 38 of the TMA. Positive signal is indicated by red punctate staining (Yellow arrows).

Performance assessment of the BaseScope assay revealed high sensitivity and specificity of detection for KRAS point mutations

A Analysis of KRAS mutation status by DNA sequencing

Tumor cores with only WT KRAS expression	Cores with KRAS point mutation	
1, 2, 3, 5, 7,8, 9, 10, 16, 17, 18, 22, 24, 26, 27, 29, 30, 33, 36, 44, 46,47,48 **(23)**	**Mutation**	**Cores with confirmed mutation**
	KRAS G12C	11, 12, 19, 25, 39, 45 **(6)**
	KRAS G12S	38 **(1)**
	KRAS G12V	9, 15, 21, 31, 37, 42 **(6)**
	KRAS G12A	34, 41, 43 **(3)**

***From the 48 cores of the Human NSCLC tumor microarray, 42 cores passed QC for RNA quality, out of which 39 were assessed for KRAS expression.**

B Analysis of KRAS mutation status using the BaseScope assay

Tumor cores with only WT KRAS expression	Cores with KRAS point mutation	
1, 2, 3, 5, 7,8,10,15,16, 17, 18, 22, 24, 26, 27, 29, 30, 33, 36, 44, 46, 47 **(22)**	**Mutation**	**Cores with confirmed mutation**
	KRAS G12C	11, 12, 19, 25, 39, 45 **(6)**
	KRAS G12S	38, 48 **(2)**
	KRAS G12V	9, 21, 31, 37, 42 **(5)**
	KRAS G12A	34, 41, 43, 9 **(4)**

C Performance characteristics of BaseScope KRAS assays

KRAS point mutations	No. of cores with specified mutation	No. of cores without specified mutation	BaseScope sensitivity	BaseScope specificity
G12C	6	33	100 (6/6)	100% (33/33)
G12A	3	36	100% (3/3)	97.22% (35/36)
G12V	6	33	83% (5/6)	100% (33/33)
G12S	1	38	100% (1/1)	97.3% (37/38)

Figure 4: Assessing the efficacy of the BaseScope assay in detecting KRAS mutations in NSCLC: **A,** DNA sequencing data indicating tumor cores with WT KRAS only or KRAS point mutations within the tumor TMA. **B,** BaseScope assay identified KRAS WT and KRAS mutation-positive cores using specific probes **C,** For *KRAS G12C*, the assay correctly identified all 6 sequencing-positive cores and identified the rest as negatives. For *KRAS G12V*, the assay detected 5 of 6 mutated cores with 100% specificity. Interestingly, for *KRAS G12S* and *KRAS G12A* mutations, the BaseScope assay demonstrated 100% sensitivity and 97% specificity

Summary

- Using the sequencing data as the gold standard, the BaseScope assay demonstrated 83-100% sensitivity and 97-100% specificity for various KRAS mutations .
- We demonstrate the development of an RNA ISH assay for point mutations detection with morphological context in FFPE tissues.

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