Low-cost, simple and rapid assay for single-molecule detection of gene fusions from RNA with ASPYRE

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Why detect gene fusions from RNA? Limitations in testing

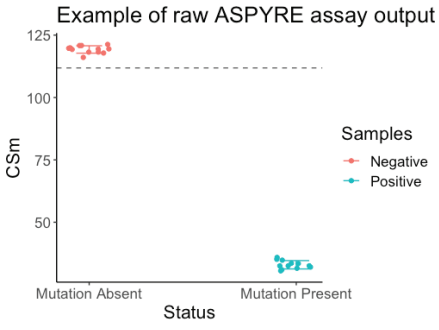
- In NSCLC, more than 10 effective therapies are available to target gene fusions, including alectinib (***ALK***), selpercatinib (***RET***), crizotinib (***ROS1***), and larotrectinib (***NTRK***)[1]
- RNA is an underused analyte, yet yields insight into gene expression, and is affected by mutations outside of exons
- Most NGS assays target DNA, requiring inference of gene rearrangements from DNA mutations and often resulting in poor coverage of fusions
- NGS is costly, slow, centralized and has large sample requirements
- PCR assays typically analyze only single genes, requiring sequential testing and often resulting in sample exhaustion

ASPYRE assay design – DNA & RNA combined

- ASPYRE-Lung panel detects genomic biomarkers across all NCCN-recommended genes in NSCLC from FFPE or blood, including:
  - deletions, insertions and *SNVs in EGFR, BRAF, KRAS, ERBB2* (from DNA)[2]
  - gene fusions: *ALK, RET, ROS1, MET, NTRK1, NTRK2, NTRK3* (from RNA)

ASPYRE assay output

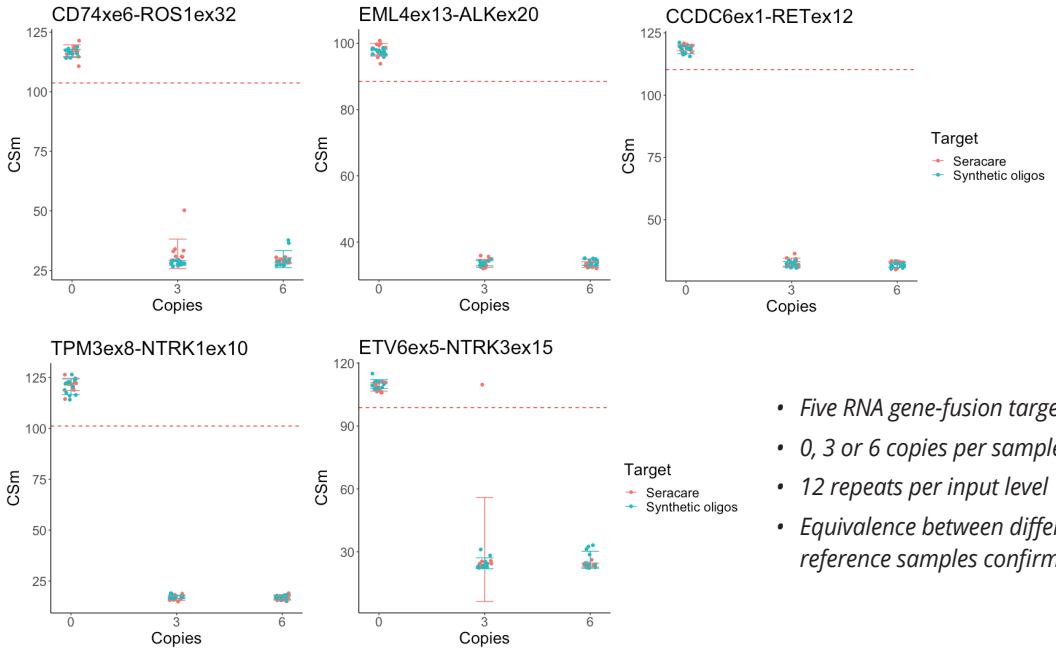
- Genomic variant detected based on fluorescence signal with onset time higher or lower than a control-based threshold
- Binary result: positive (mutation present) or negative (mutation absent) for target variant



The raw ASPYRE assay output for a single mutation – each point represents one assay. CSm: Cycle Sigmoid midpoint.

Equivalence of in-house & commercial reference standards

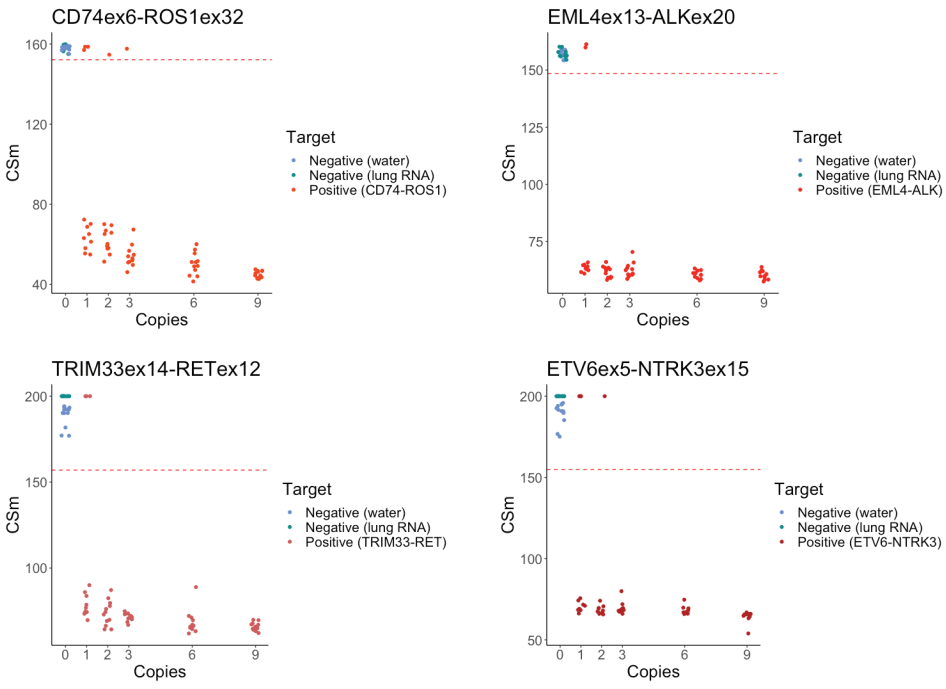
Commercial standard (Seraseq® Fusion RNA Mix v4) & in-house panel of synthetic oligos quantified by dPCR, tested using ASPYRE-lung RNA panel



- *Five RNA gene-fusion targets*
- *0, 3 or 6 copies per sample*
- *12 repeats per input level*
- *Equivalence between different reference samples confirmed*

Sensitivity – Detection of single-copies

Limiting dilution of sample input to estimate limit of detection



Detection of RNA gene fusion targets by ASPYRE-lung is consistent with single molecule detection limits when compared to expected Poisson distribution for input copy number

Theoretical number of copies in each reaction	Expected number of positive reactions (out of 12)	*ROS1* (*CD74-ROS1*) positive reactions	*ALK* (*EML4-ALK*) positive reaction	*RET* (*TRIM33-RET*) positive reactions	*NTRK* (*ETV6-NTRK3*) positive reactions
0	0	0	0	0	0
1	7.6 ± 1.7	9	10	9	8
2	10.4 ± 1.2	11	12	12	11
3	11.4 ± 0.8	11	12	12	12
6	12.0 ± 0.2	12	12	12	12
9	12.0 ± 0.03	12	12	12	12

ASPYRE-Lung assay with FFPE Patient Samples: Specific & Sensitive

Specificity of ASPYRE-Lung RNA panel using normal FFPE lung tissue

- Five FFPE lung tissue blocks from patients without diagnosed NSCLC
- Four curls taken per block, and RNA extracted
- 1 ng, 5 ng, and 10 ng RNA tested per curl

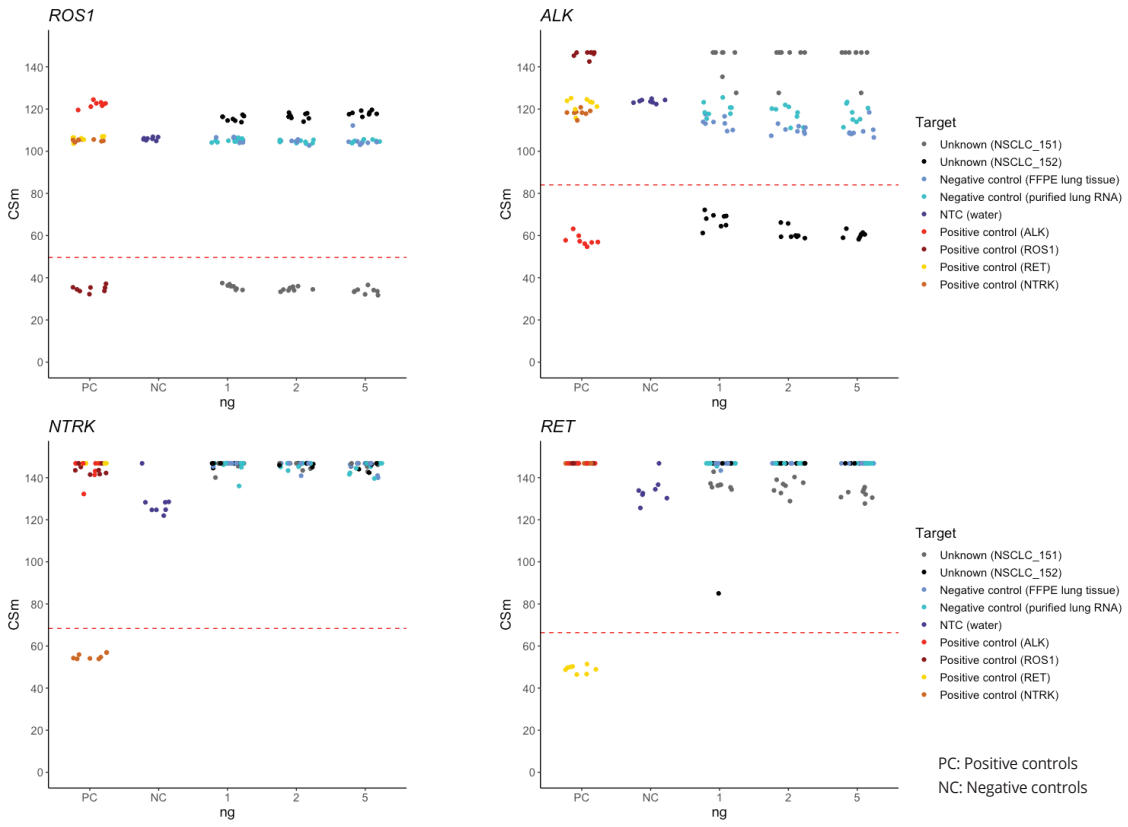
100% specificity for ALK, ROS1, RET, NTRK1, NTRK2 and NTRK3 fusions

Sensitivity of ASPYRE-Lung RNA panel using known fusion-positive FFPE lung tissue at sequential input quantities

- RNA extracted from two fusion-positive patient FFPE lung tissue samples
- Tested using ASPYRE-lung RNA panel (operator blind)
- 1, 2, 5, 10 ng input RNA
- 8 repeats per input sample level

One ALK & one ROS1 fusion-positive by orthogonal testing

ASPYRE-Lung results concordant for both samples at all input levels, including 1 ng

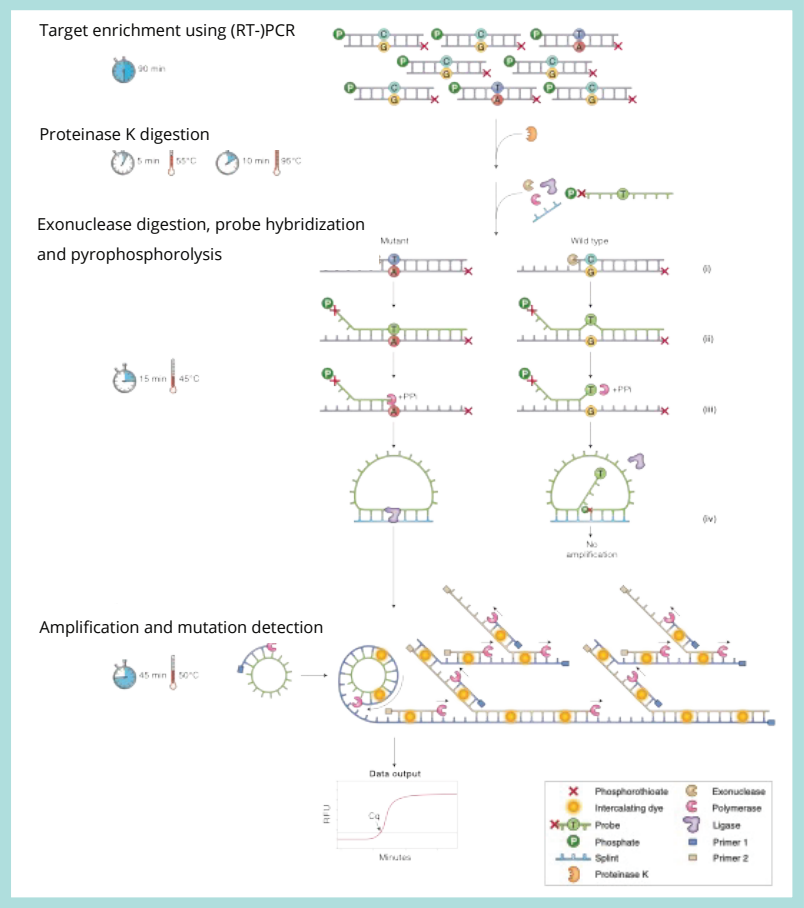


Sample	*ROS1* result (reactions)	*ALK* result (reactions)	RET result (reactions)	NTRK result (reactions
NTC	Negative (8/8)	Negative (8/8)	Negative (8/8)	Negative (8/8)
Human Lung RNA	Negative (8/8)	Negative (8/8)	Negative (8/8)	Negative (8/8)
ROS1 synthetic target	Positive (8/8)	Negative (8/8)	Negative (8/8)	Negative (8/8)
ALK synthetic target	Negative (8/8)	Positive (8/8)	Negative (8/8)	Negative (8/8)
RET synthetic target	Negative (8/8)	Negative (8/8	Positive (8/8)	Negative (8/8)
NTRK synthetic target	Negative (8/8)	Negative (8/8)	Negative (8/8)	Positive (8/8)
NSCLC_151: 1 ng, 5 ng, 10 ng	Positive (8/8) at 1 ng, 2 ng, 5 ng	Negative (8/8)	Negative (8/8)	Negative (8/8)
NSCLC_152: 1 ng, 5 ng, 10 ng	Negative (8/8)	Positive (8/8) at 1 ng, 2 ng, 5 ng	Negative (8/8)	Negative (8/8)

The ASPYRE assay

- **Simple, fast, low-cost**
- Simple:
  - runs on existing qPCR instruments
  - minimal user training
  - workflow only requires sample input & reagent transfer
- Fast: < 1 **day to result** after nucleic acid extraction
- **RNA & DNA** analysed **simultaneously** in one assay
- **36 gene fusions** detected
- 16 samples per qPCR run

Schematic overview of ASPYRE technology workflow



Conclusions

- ASPYRE-Lung RNA panel detects 36 most commonly found gene fusions from RNA including *ALK, ROS1, RET* and *NTRK* mutations
- Assay workflow takes < 1 day and is run concurrently with DNA sample
- Detection consistent with single molecule detection limits
- 100% sensitivity and specificity from clinical samples across all variants and input quantities
- ASPYRE-Lung provides low-cost, fast, local, actionable biomarker testing from tissue or blood, enabling all patients to benefit from targeted therapies

References

1. Tan AC, Tan DSW. Targeted therapies for Lung cancer patients with oncogenic driver molecular alterations. Journal of Clinical Oncology 2022 Feb 20;40(6): 611-625 doi: 10.1200/JCO.21.01626
2. Silva AL, Powalowska PK, Stolarek M, Gray ER, Palmer RN, Herman B, Frayling CA, Balmforth BW. Single-copy detection of somatic variants from solid and liquid biopsy. Scientific Reports 2021 Mar 16; 11(1): 6068 doi: 10.1038/s41598-021-85545-3

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