Ultra-sensitive molecular detection of gene fusions from RNA using ASPYRE

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ASPYRE

Why detect gene fusions from RNA?

- In NSCLC, effective therapies are available to target gene fusions include Alectinib (***ALK***), Selpercatinib (***RET***), Crizotinib (***ROS1***), and Larotrectinib (***NTRK***)[1]
- RNA is an underused analyte, which yields insight into gene expression, and reflects mutations outside of exons
- Single-target gene target assays can result in sample exhaustion due to sequential analysis to cover all targets
- NGS is an important discovery tool when first line screening yields no result … BUT has long turn-around time, high cost, high sample input requirement, and clinical implications are difficult to interpret

ASPYRE assay design – DNA & RNA combined

- ASPYRE-lung panel detects all NCCN-recommended biomarkers, including:
  - deletions, insertions and SNVs in *EGFR, BRAF, KRAS, ERBB2* (from DNA)[2]
  - gene fusions: *ALK, RET, ROS1, NTRK1, NTRK2, NTRK3* (from RNA)

DNA

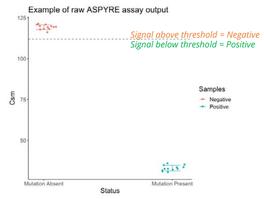
EGFR		BRAF		KRAS		ERBB2	
Exon 18	G719A	Exon 15	V600E	Exon 2	G12A	Exon 17	V659E
	G719S				G12C	Exon 20	Insertions
	G719C				G12D		
Exon 19	Deletions				G12F		
	Insertions				G12R		
Exon 20	T790M				G12S		
	S768I				G12V		
	C797S			Exon 3	Q61E		
	Insertion				Q61H		
Exon 21	L858R				Q61K		
	L861Q						

RNA

ALK	ROS1	RET	MET	NTRK1	NTRK2	NTRK3
Fusions	Fusions	Fusions	Exon 14 skipping	Fusions	Fusions	Fusions

ASPYRE assay output

- Binary result: positive (mutation present) or negative (mutation absent) for panel targets e.g. *ALK*-positive, *EGFR* T790M-positive
- Clinical result of mutation present or absent based on fluorescence signal that is higher or lower than a control-based threshold

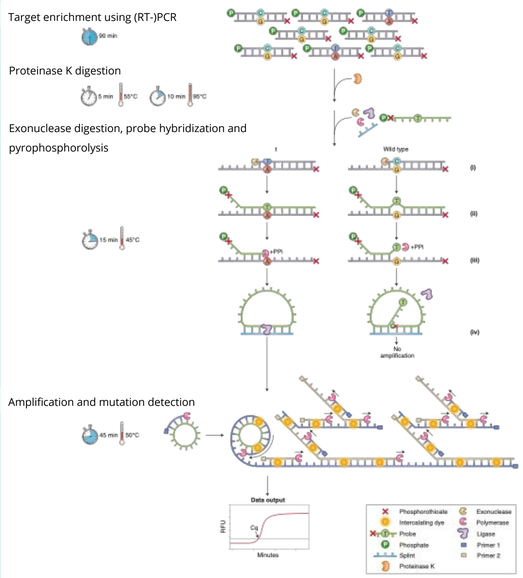


The raw ASPYRE assay output for a single mutation – interpreted for the user as negative or positive if above or below the separation threshold

The ASPYRE assay

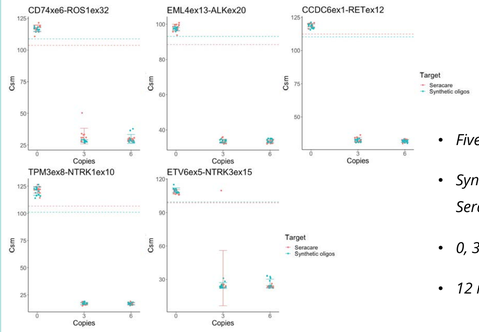
- **Simple, rapid, low-cost**
- Simple: pre-made mastermixes
- Simple: workflow requires sample input and reagent transfer
- Rapid: **< 1 day to result**
- **RNA & DNA** analysed **simultaneously** in one assay run
- **36 gene fusions** detected
- 22 DNA & 2 RNA PCR wells per patient
- 16 samples per qPCR run

Schematic overview of ASPYRE technology workflow



Equivalence to reference standards

In-house panel & Seraseq® Fusion RNA Mix v4, quantified by dPCR, tested using ASPYRE-lung RNA panel

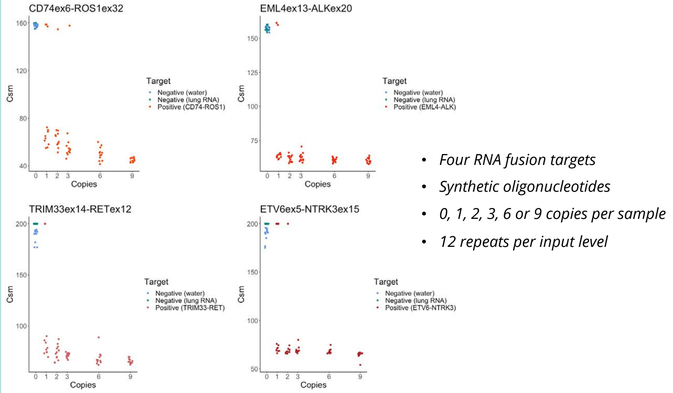


- *Five RNA gene-fusion targets*
- *Synthetic oligonucleotides or Seracare mix tested*
- *0, 3 or 6 copies per sample*
- *12 repeats per input level*

ASPYRE-lung assay can detect RNA gene fusions across different 3' driver gene families

Sensitivity – Detection of single-copies

Limiting dilution of sample input to estimate limit of detection



- *Four RNA fusion targets*
- *Synthetic oligonucleotides*
- *0, 1, 2, 3, 6 or 9 copies per sample*
- *12 repeats per input level*

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

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

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

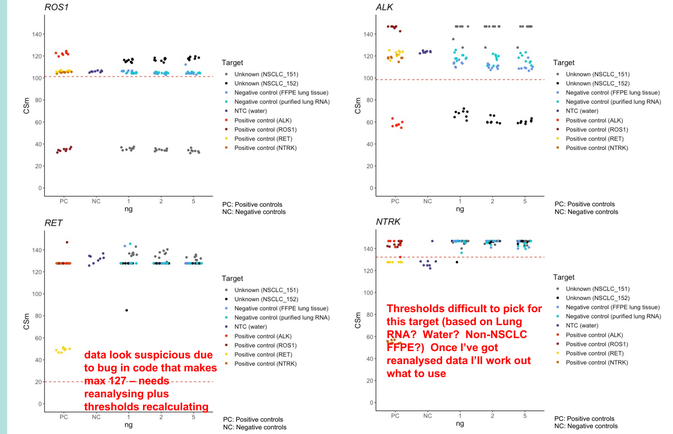
Specificity - test ASPYRE-lung RNA panel on five non-cancerous lung samples

- *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 - test ASPYRE-lung RNA panel on known fusion-positive FFPE tissue at different input levels

- *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*



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)

One sample was ALK fusion-positive, and one sample was ROS1 fusion-positive by orthogonal testing; ASPYRE-lung RNA results were concordant for both samples

Both samples yielded positive results at all sample input levels, including 1 ng

Conclusions

- ASPYRE-lung RNA panel detects 36 gene fusions from RNA
- Panel includes most common *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

References

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