# A Novel Comprehensive RNA-Merging Point Targeted Assay for Clinically Actionable RNA Fusions and Aberrant RNAs in Solid Tumors

P2238

NEO GENOMICS

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## Background

Gene fusions are major drivers of cancer and their accurate detection is key for supporting diagnosis and therapy selection. There are more than 65,000 annotated gene fusion events. Current fusions detection strategies are limited to amplicon panels querying only a small number of genes, or whole exome capture based assays, which reduces sensitivity. Hence, multiple small panels are required to capture all relevant fusions, which is prohibitive for small biopsies like fine needle aspirates. Hence, a clinical grade assay able to accurately detect a comprehensive set of most clinically relevant gene fusions for solid tumors is needed.

## Methods

We isolated total nucleic acid (TNA) from 141 FFPE tumor specimens or a control sample (Seraseq) and performed pair-ended, strand-specific hybridization-based RNA sequencing on Next-Seq 500 or Novaseq 6000 platforms. We selected 252 fusion genes from NCCN and WHO guidelines, published clinical studies, and the 120 most frequent curated fusions in solid tumors from COSMIC (v91). These fusions are clinically relevant to most frequent cancers, including breast, colorectal, lung, lymphoma, pancreatic, prostate, salivary gland, sarcomas, and thyroid cancers. Chimeric probes were synthetized targeting fusion RNA sequences for 2230 selected breakpoints and exon junction regions of aberrant RNAs, including EGFRvIII, MET exon 14 skipping, ARv7 and ARv9. We also targeted the full coding region of 27 genes whose change in expression may suggest translocations and mutations, or have diagnostic value. Fusions were called by a custom pipeline using 3 fusion callers and a machine-learning algorithm. The PCR-based Archer FusionPlex assays and RT-PCR followed by Sanger were used as orthogonal validation methods.

## Results

- This single RNA fusion assay can detect at least 1194 unique known fusions pairs involving 1104 genes with 250 core fusion genes compared to 63 or 47 fusion genes from other commercially available NGS assays.
- CLIA assay validation was conducted on 146 unique clinical FFPE samples from various tumor types.
- 206 unique genes were detected in 1 or more gene fusions. The assay detected 116/121 high confidence fusions reported in our CLIA Archer Sarcoma and Comprehensive Thyroid & Lung (CTL) assays. 41 additional fusions not detected by Archer were confirmed by Sanger sequencing.
- The assay has 95.8% sensitivity (141/147) and 100% specificity, as all fusions were confirmed by either orthogonal assay.
- We also detected MET exon 14 skipping, EGFRvIII with 100% sensitivity and 100% specificity. Importantly, in two samples we detected MET exon 14 skipping not predicted from DNA mutation analysis showing the sensitivity of the approach.
- 55 novel fusions were detected by capturing only one of the partner genes.

## Conclusions and Future Directions

We developed a novel and efficient breakpoint targeted fusion detection RNA-seq assay from extracted TNA from FFPE samples that can comprehensively profile thousands of clinically actionable RNA fusions and aberrant RNAs in solid tumors. Future directions include validating the built-in gene expression module to complement even further the characterization of solid tumors by integrating the full spectrum of RNA alterations from this assay with DNA sequencing assays reporting mutations/CNVs thus enhancing Precision Oncology alternatives for cancer patients.

## Data

### Gene Fusions Detection: RNA vs. DNA Detection

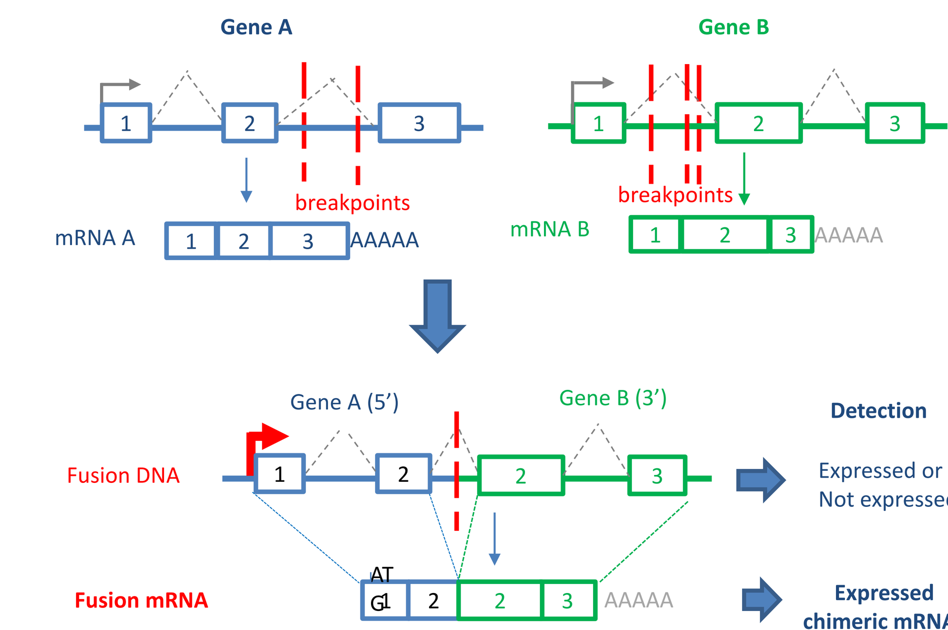


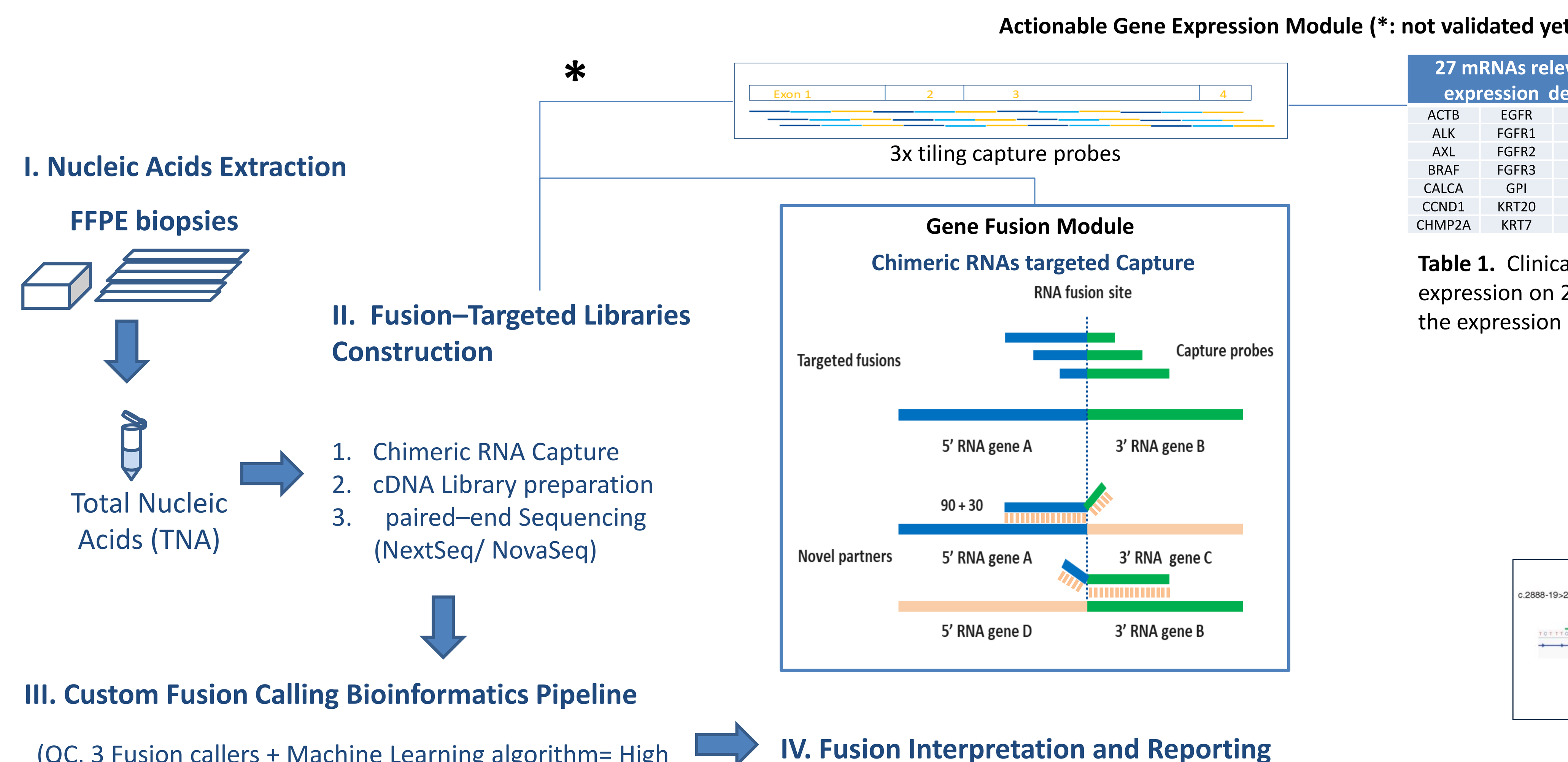

**Figure 1:** Schematic representation of gene fusions breakpoints and the advantage for fusion detection on mRNA.

### Solid Tumor Relevant Gene Fusions and Aberrant Transcripts

| 250 clinically relevant fusion genes | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| ABL1 | C11orf95 | CREB1 | ESRP1 | HERPUD1 | MAST2 | NOTCH2 | PML | SET | TFE3 |
| ACSL3 | CAMTA1 | CREB3L1 | ETV1 | HEY1 | MEAF6 | NPM1 | POU5F1 | SLC34A2 | TFG |
| ACTB | CANT1 | CREB3L2 | ETV4 | HIP1 | MET | NR4A3 | PPARG | SLC45A3 | THADA |
| ACTL6A | CAPZA2 | CREBBP | ETV5 | HMGA2 | MET e14 * | NRG1 | PRCC | SND1 | THRAP3 |
| AFDN | CARS1 | CRTC1 | ETV6 | HMGN2P46 | MKRN1 | NTRK1 | PRKACA | SNURF | TMPRSS2 |
| AFF1 | CBFA2T3 | CRTC3 | EWSR1 | HNRNPA2B1 | MLLT1 | NTRK2 | PRKAR1A | SRF | TP63 |
| AFF3 | CCDC170 | CSF1 | EZR | IL2RB | MLLT10 | NTRK3 | PRKD1 | SRGAP3 | TPM3 |
| AFF4 | CCDC6 | CTLA4 | FAM131B | IRF4 | MLLT11 | NUP214 | PRKD2 | SS18 | TPM4 |
| AHRR | CCNB3 | CTNNB1 | FEV | ITK | MLLT3 | NUP98 | PRKD3 | SSX1 | TPR |
| AKAP9 | CCND1 | CTNNBL1 | FGFR1 | JAK2 | MN1 | NUTM1 | PTPRK | SSX2 | TRIM24 |
| AKT3 | CCND2 | DDIT3 | FGFR2 | JAZF1 | MPRIP | NUTM2A | RAD51B | SSX4B | UBTF |
| ALK | CCND3 | DDX3X | FGFR3 | KAT6A | MRTFB | NUTM2B | RAF1 | STAT6 | USP6 |
| AR | CD274 | DDX5 | FGFR4 | KDM5A | MSH2 | OMD | RANBP2 | STIL | VTI1A |
| ARv7* | CD74 | DHH | FLI1 | KIAA1549 | MYB | PAN3 | RARA | STRN | WDFY2 |
| ARv9* | CDH11 | DNAJB1 | FLT3 | KIF5B | MYBL1 | PATZ1 | RASGEF1A | SUZ12 | WIF1 |
| ARID1A | CDK4 | DUX4 | FOXO1 | KIT | MYC | PAX3 | RET | SYK | WT1 |
| ASPSCR1 | CDK6 | EGFR | FOXP1 | KLK2 | MYH9 | PAX7 | RHEBL1 | TACC3 | WWTR1 |
| ATF1 | CDKN2D | EGFRvIII* | FRK | KMT2A | MYLK | PAX8 | ROS1 | TAF15 | YAP1 |
| ATIC | CHCHD7 | ELK4 | FUS | KNL1 | NAB2 | PBX1 | RPS6KC1 | TAL1 | YWHAE |
| AXL | CIC | ELL | GLI1 | KRAS | NCOA1 | PCM1 | RSPO3 | TBL1XR1 | ZNF444 |
| BCOR | CIITA | EML4 | GLIS1 | LIFR | NCOA2 | PDGFB | RUNX1 | TCF12 | |
| BCR | CLTC | EPC1 | GLIS2 | LMNA | NCOA4 | PDGFRA | RUNX1T1 | TCF3 | |
| BRAF | CNBP | EPS15 | GLIS3 | LPP | NDRG1 | PDGFRB | SDC4 | TCF7L2 | |
| BRCA1 | COA5 | ERBB2 | GNAS | MAML1 | NFATC2 | PHF1 | SEC31A | TEAD1 | |
| BRCA2 | COL1A1 | ERG | GOPC | MAML2 | NFIB | PIK3CA | SEPTIN6 | TEAD2 | |
| BRD4 | COL1A2 | ESR1 | HAS2 | MAST1 | NOTCH1 | PLAG1 | SEPTIN9 | TEAD3 | |

**Table 1.** the actionable genes reported on the universal fusion comprehensive fusions panel.

### RNA Fusions Capture-based Targeted RNA-seq Assay Workflow




| 27 mRNAs relevant due to expression deregulation | | | |
|---|---|---|---|
| ACTB | EGFR | MET | RPL10 |
| ALK | FGFR1 | NTRK1 | RPL19 |
| AXL | FGFR2 | NTRK2 | SLC5A5 |
| BRAF | FGFR3 | NTRK3 | THADA |
| CALCA | GPI | PTH | TTF1 |
| CCND1 | KRT20 | RAB7A | VCP |
| CHMP2A | KRT7 | ROS1 | |

**Table 1.** Clinically relevant expression on 27 genes on the expression module.

**Figure 2:** A new Solid Tumor Gene Fusion RNA-Seq assay workflow. Starting with FFPE total nucleic acid extraction, the assay involves an RNA fusion sequence- targeted, hybridization capture-based, stranded pair-ended RNA-sequencing. Key step resides on custom based design of capture baits mapping to the chimeric RNA sequence` as deduced from chromosomal breakpoints in annotated private and public databases.

### CLIA Validation Samples Cohort

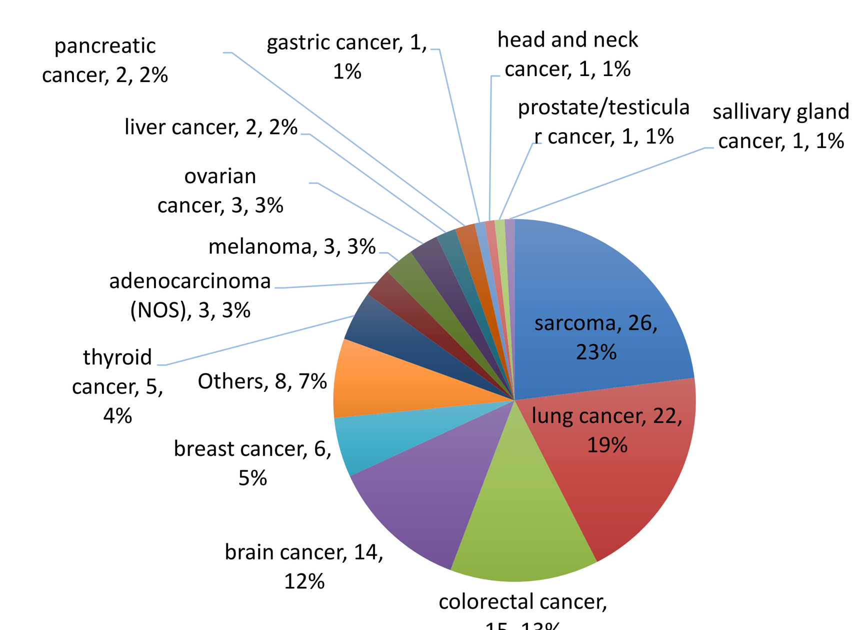



**Figure 3:** Disease representation of samples analyzed in the CLIA/CAP assay validation.

### CLIA Validation Fusion Genes

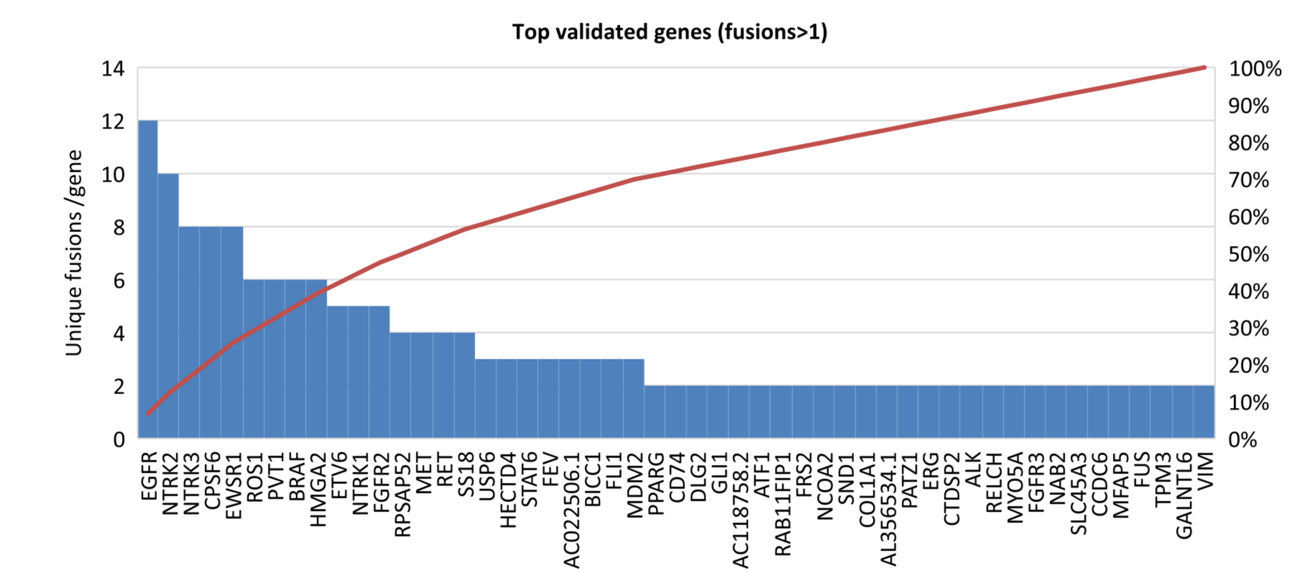



**Figure 4.** Fusion genes frequency. From 206 different genes, partners on the fusions detected in this validation, 51 genes were found as partners of >1 unique gene fusions.

### New Fusions Confirmation: RT-PCR +Sanger Sequencing

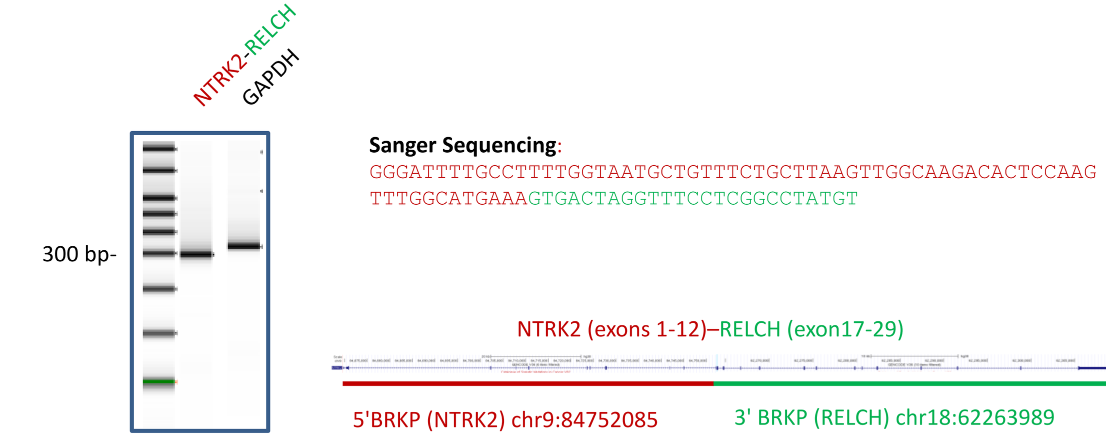


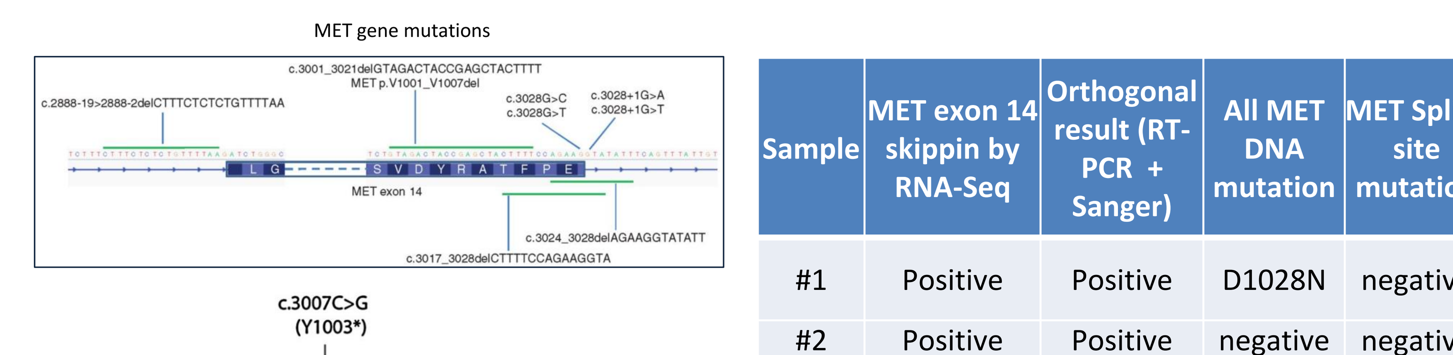

**Figure 5.** Novel fusions confirmation. All novel fusions detected based on the capture by a probe hybridizing on a known fusion gene were validated as the fusion shown here.

### Assay Performance

| Variant Type | Tested tumor cellularity | Sensitivity | Specificity |
|---|---|---|---|
| All RNA Variants | 20% | 95.8% | 100.0% |
| RNA Fusions (gene A -gene B) | 20% | 95.5% | 100.0% |
| Splicing/mutant transcripts (**MET exon 14 skipping**) | 20% | 100.0% | 100.0% |
| Mutant derived transcripts (**EGFRvIII**) | 20% | 100.0% | 100.0% |

**Table 3**. Performance results from accuracy experiments.

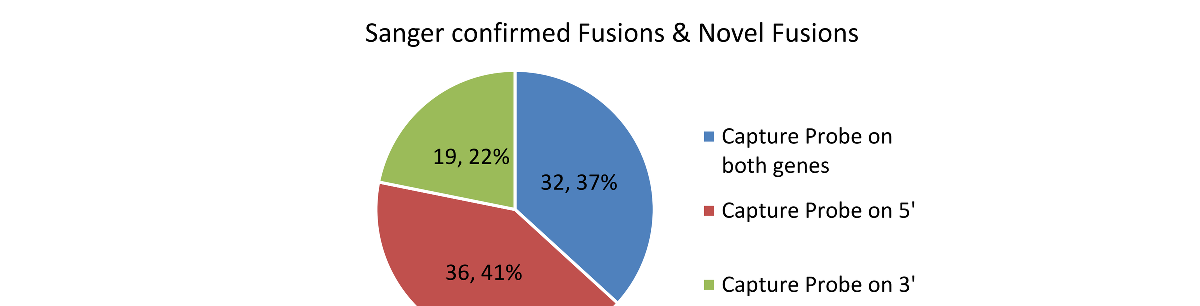
### RNA-seq vs. DNA-seq Mutations Testing for Splicing Alterations




| Sample | MET exon 14 skippin by RNA-Seq | Orthogonal result (RT-PCR + Sanger) | All MET DNA mutation | MET Splice site mutation |
|---|---|---|---|---|
| #1 | Positive | Positive | D1028N | negative |
| #2 | Positive | Positive | negative | negative |

**Figure 6.** Diagram of splicing inducing MET DNA mutations and clinical results from RNA-seq- vs. DNA sequencing results for 2 MET exon 14 Positive samples also analyzed by a CLIA validated NGS DNA test. 83 total samples were tested for MET exon 14 skipping.

### New Fusions and New Partner Fusion Genes Detection




- **Known Fusions**: 2230 custom capture probes for known gene fusions
  - ✓ **1194** unique gene pairs
- **Novel fusions:** one sided capture of novel fusion partners
  - ✓ Probes for breakpoints on **1104** unique partner genes enabling the capture of fusions to anywhere on the transcriptome.

**Figure 7.** Coverage by capture probes on new fusions confirmed by RT-PCR +Sanger sequencing. Percentage of fusions targeted by the probes at both sides or one side of the fusion is indicated.