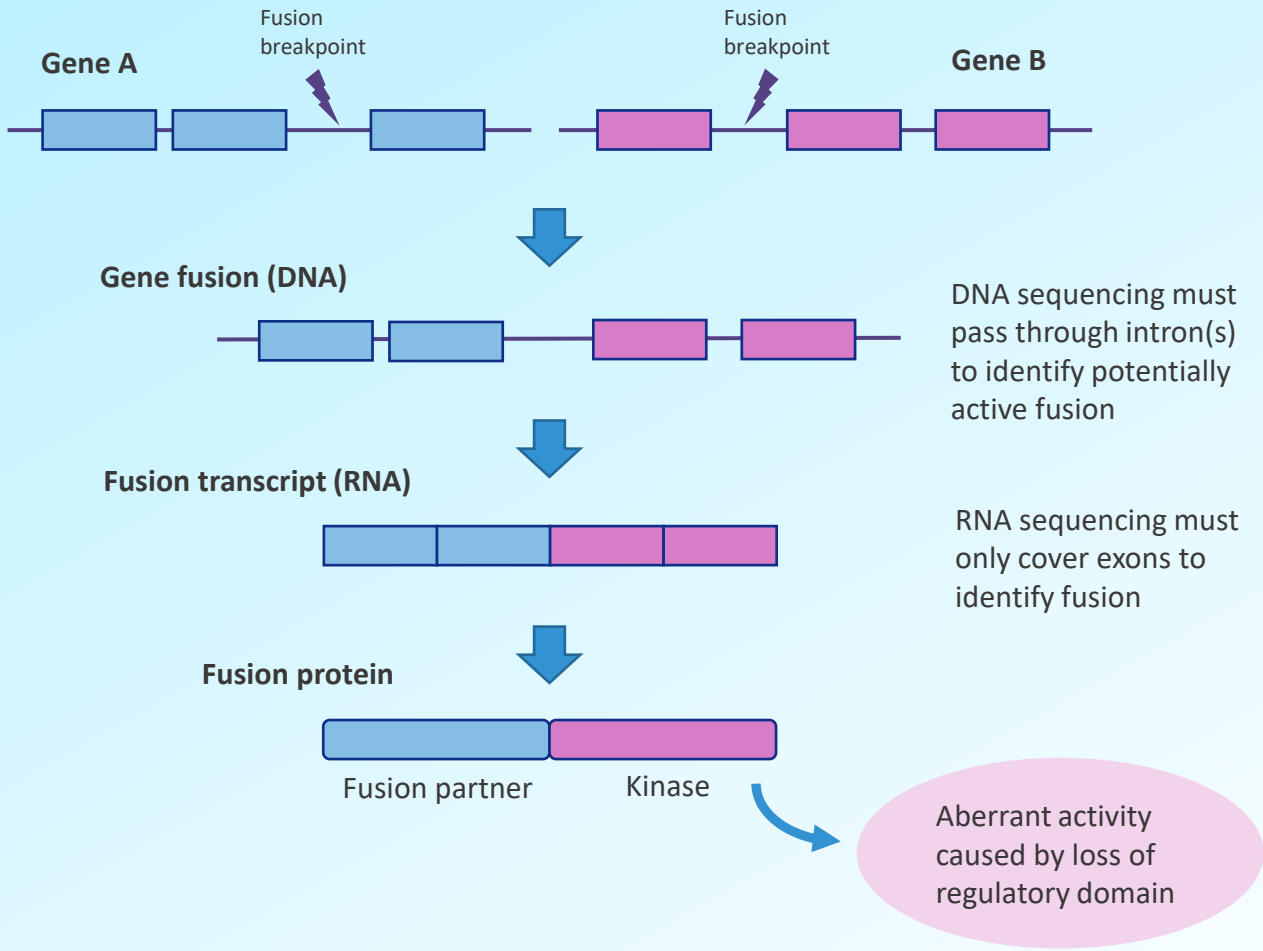
DNA vs RNA Based NGS Detection of Novel Gene Fusions

- Tyrosine kinase fusions that act as driver mutations lack regulatory domains, leading to aberrant signaling activity
- Fusion partners are variable and can be unique, creating challenges for detection
- DNA-based NGS can only detect fusions that occur within relatively short introns that are well-covered by the sequencing panel
- Long introns may also contain repetitive sequences found elsewhere in genome, complicating DNA sequencing
- RNA-based NGS is more straightforward and also provides confirmation that a full-length transcript is present
- FISH can also identify novel fusions but has a high rate of false positives and false negatives



NGS, next-generation sequencing; FISH, fluorescence in situ hybridization.

Benayed R et al. *Clin Cancer Res*. 2019;25:4712-4722.

NCCN Recommendations for Biomarker Testing Modalities in NSCLC

Gene(s)	Real-time PCR	Sanger sequencing	NGS	IHC	FISH
EGFR	✓	With tumor enrichment	Preferred	----	----
KRAS	✓	With tumor enrichment	✓	----	----
ALK	Unlikely to detect novel fusions	----	✓	✓	✓
ROS1	Unlikely to detect novel fusions	----	DNA-based may under-detect some fusions	Low specificity, requires confirmation	May under-detect *FIG-ROS1* fusion
***BRAF* V600E**	✓	With tumor enrichment	✓	Available but not recommended	----
NTRK1/2/3	✓	----	DNA-based may under-detect *NTRK1* or *NTRK3* fusions	Complicated by baseline expression in some tissues	Requires at least 3 probe sets to be comprehensive
***MET* ex14**	----	----	RNA-based may improve detection	----	----
RET	Unlikely to detect novel fusions	----	RNA-based preferred	----	May under-detect some fusions
ERBB2 (HER2)	✓	✓	Preferred	----	----

EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; NGS, next-generation sequencing; PCR, polymerase chain reaction. National Comprehensive Cancer Network (NCCN). NCCN Clinical Practice Guidelines in Oncology: Non-Small Cell Lung Cancer. Version 3.2023. Updated April 13, 2023. https://www.nccn.org/guidelines/guidelines-detail?category=1&id=1450.