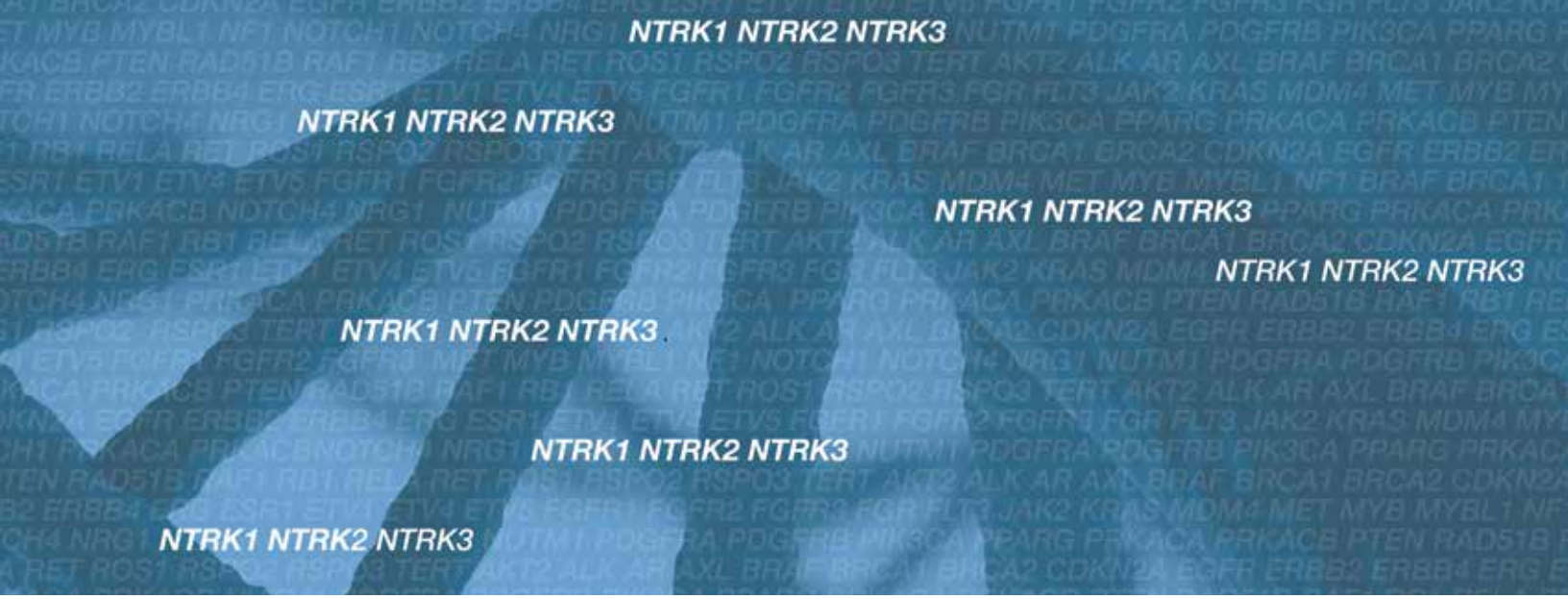

Everything you need to know about *NTRK* testing in precision oncology research

Why *NTRK* testing?

- The *NTRK* gene family encodes the TRK family of proteins, which are oncogenic drivers across multiple tumors in adults and children.
- In TRK fusion cancer, the *NTRK* gene fuses with an unrelated gene, causing overexpression and permanent activation of the TRK fusion proteins. [2,3,6]
- TRK fusion cancer occurs in a broad range of tumor types with varying prevalence across both pediatric and adult patients. [2]

What is the pathogenic mechanism of the TRK proteins?

- The three *NTRK* genes *(NTRK1, NTRK2,* and *NTRK3)* each encode a separate TRK protein as TRKA, TRKB, and TRKC, respectively. [1,2]
- As transmembrane proteins, these kinases function by ligand-dependent transmission of extracellular signals to the nucleus, activating cell growth, proliferation, and survival pathways, such as the MAPK/ERK and PI3K/AKT pathways. [1,2,3]
- Unless silenced, *NTRK* gene fusion leads to the expression of a chimeric protein, which retains the TRK kinase domain but not the ligand-binding domain. [1] *NTRK* genes tend to fuse with housekeeping genes. Once fused, they are constitutively active due to the genetic alteration. Therefore, *NTRK* gene fusions can lead to the development of solid tumors in a variety of tissue types. [2,3]

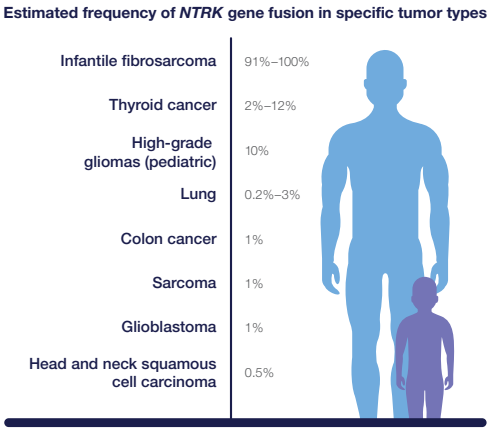

Figure 1. Estimated frequency of *NTRK* gene fusion in specific tumor types. Source: Sciavolina P (2015 Nov) Tropomyosin-Related Kinases (TRK) Making Headway in Head and Neck Cancers. Target Oncol.; Cocco E, Scaltriti M, Drilon A (2018 Oct) *NTRK* fusion-positive cancers and TRK inhibitor therapy. *Nat. Rev. Clin. Oncol.*

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How do you test for *NTRK* fusions?

While *NTRK* gene fusions were one of the first oncogenes identified, they are not routinely tested for and/or included on all test platforms. [1,2] There are several technologies that can test for *NTRK* gene fusions; however, only sensitive, specific, and multiplexing assays can reliably detect them. [1,2]

Immunohistochemistry (IHC) staining with pan-TRK antibodies detect TRK proteins A, B, and C, which may be expressed in both wild-type and fusion proteins. Protein expression may not be the result of a gene fusion event. [6]

DNA fluorescence *in situ* hybridization (FISH) testing may have limited utility in uncovering *NTRK* gene fusions because it is not designed for multiplexing. In order to detect fusions at multiple locations, such as the three *NTRK* genes, multiple FISH tests would need to be run. [7]

Reverse transcription polymerase chain reaction (RT-PCR) is designed to identify only known translocation partners and breakpoints and cannot identify novel breakpoints or novel fusion partners. [9]

Next-generation sequencing (NGS) provides the most comprehensive view of a large number of genes and may identify *NTRK* gene fusions as well as other relevant alterations, with minimal sample tissue needed. [3,4] However, it is important to know whether the NGS assay used for *NTRK* testing has the capacity to detect *NTRK* gene fusions. [3,5]

Method	Sensitivity	Specificity	Detection of all fusion genes	Detection of partner	Detection of expression	Screening
IHC	High	Moderate/high	Yes	No	Yes	Yes
FISH	High	High	One per probe	No	No	No
DNA-Seq NGS	Moderate	High	Yes	Yes	No	Yes
RNA-Seq NGS	High	High	Yes	Yes	Yes	Yes

Figure 2. Comparison of testing methods. ESMO recommendations on the standard methods to detect *NTRK* fusions in daily practice and clinical research. *Annals of Oncology* 30: 1417–1427, 2019 doi:10.1093/annonc/mdz204, published online 3 July 2019.

How to choose the most optimal NGS method for *NTRK* testing

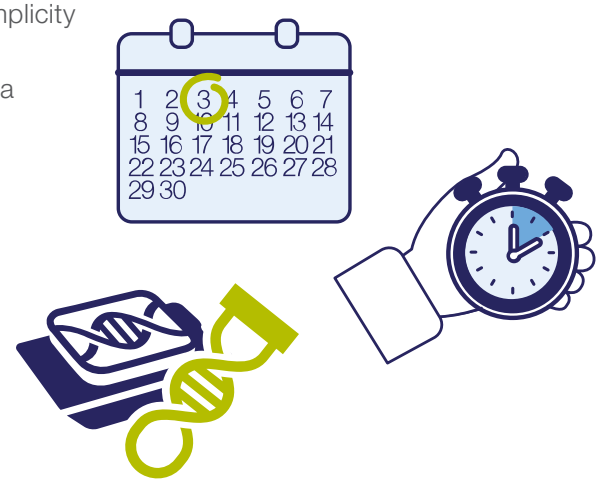
Although NGS is the ideal testing platform for multiple biomarkers while preserving precious sample tissue, not all NGS is the same.

Four key factors to consider when choosing NGS technology for *NTRK* fusion research include:

- **Panel design**—an RNA-based methodology is optimal as it directly detects translocation events between the *NTRK* gene and partner gene. It should also be able to detect not only all the known, but also novel driver and partner combinations.
- **Panel coverage**—*NTRK* gene fusions are yet another biomarker to be tested for some tumor types, such as lung and colon cancer. Due to limited tissue content in many samples, it is favorable to use a testing panel that can detect not only cancer driver fusions including *ALK, ROS1, RET,* and *MET*, but also DNA-based biomarkers such as *EGFR, BRAF, ERBB2, and KRAS* mutations.
- **Sample requirements**—tissue is still the issue, and often the amount available is very limited. Different NGS methods vary significantly in the amount they require, ranging from 10–500 ng of nucleic acid (RNA or DNA), which can have a direct impact on your ability to successfully test all clinical research samples.
- **Completeness of the workflow and implementation support**—NGS workflows are complex. An easy-to-use, integrated workflow with validated protocols from sample to report helps simplify lab operation and test implementation. High-touch consultation service and support from the vendor helps accelerate a lab's validation process to implement the test in a time-efficient manner and save costs.

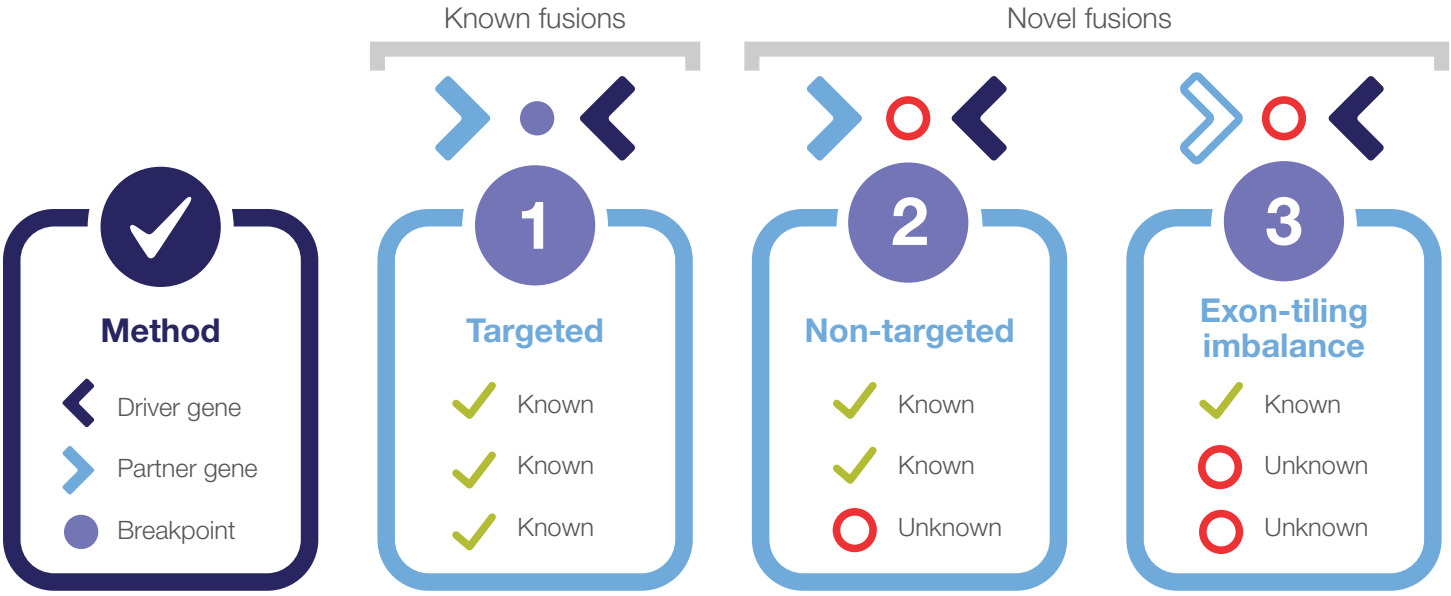
Featured solution

Empower your lab to deliver NGS genomic profiles with the speed and simplicity of immunohistochemistry. The Ion Torrent™ Oncomine™ Precision Assay, combined with the Ion Torrent™ Genexus™ System, will let you experience a whole new world of easy NGS.



- **One-day hands off workflow** with only two user touchpoints and 10 minutes of hands-on time*
- Compatible with **formalin-fixed, paraffin-embedded (FFPE) tissue as well as liquid biopsy samples** and small batch sizes
- **FusionSync** detection technology, which represents a synchronous solution optimized for clinical biomarker research; the process enables comprehensive and sensitive gene-fusion detection based on RNA analysis

Three pillars of FusionSync detection technology–Detect novel but rare, without missing the known and common



Oncomine assays	Number of genes	Validated sample type
Oncomine Comprehensive Assay V3	161	FFPE tissue
Oncomine Focus Assay	52	FFPE tissue
Oncomine Childhood Cancer Research Assay	203	FFPE tissue, blood, and bone marrow
Oncomine Precision Assay	50	Tissue and blood

Figure 3. Other Oncomine assays for *NTRK* fusion detection, all based on RNA sequencing.

* Specimen-to-report workflow will be available after the Ion Torrent™ Genexus™ Purification System and integrated reporting capabilities are added in 2020. Fully integrated specimen-to-report workflow will be available after the Ion Torrent™ Genexus™ Software 6.4 update.

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Oncomine Reporter provides easy-to-read results

With Oncomine™ Solutions, you get a streamlined bioinformatics analysis pipeline optimized for each Oncomine assay—all packaged in a user-friendly experience.

The Ion Torrent™ Oncomine™ Reporter delivers clear and concise reports that present all relevant variants with information about targeted therapies, guidelines, and open clinical trials to facilitate your clinical research.

The report format is fully customizable, allowing you to easily develop a solution fit for your team's needs.

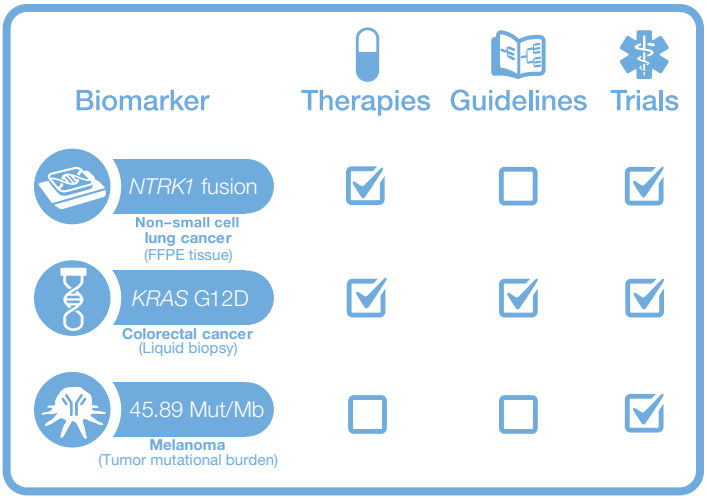

Figure 4. Example content of the report generated by Oncomine Reporter.

Accelerate successful implementation of Oncomine assays in your lab with Analytical Validation Consulting services

Analytical Validation (AV) Consulting service provides technical project management of your lab's AV to help verify that the assay is tested for required parameters. We work with you to optimize and develop your validation workflow while providing data analysis support and template documentation as part of your end-to-end instrument and reagent investment. On average, we can help you complete the validation process 62–75% faster than on your own; and by supplying control samples, data analysis, and reporting, we can help you reduce costs up to 50% for your completed AV.

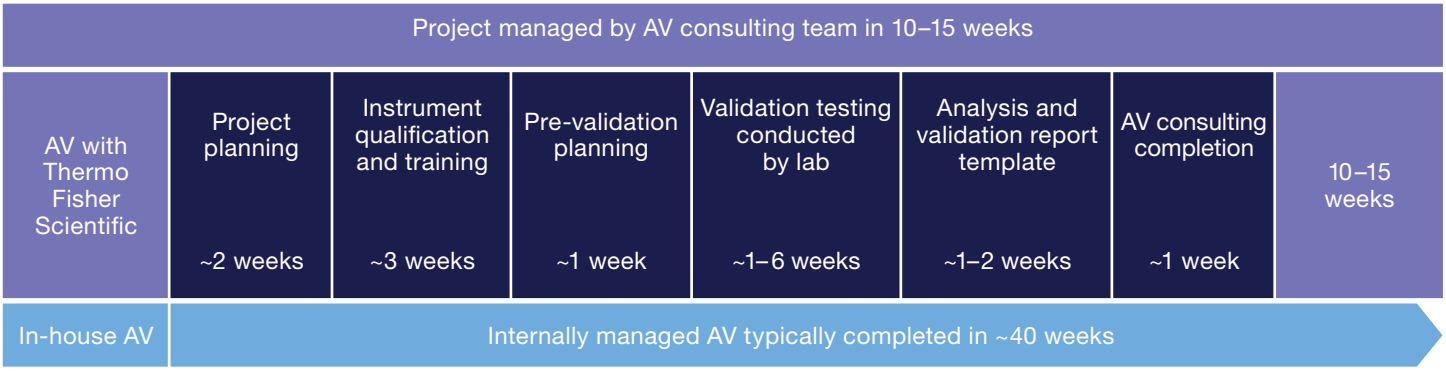

Figure 5. Analytical validation workflow completed 62–75% faster with AV consulting service.

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*Specimen-to-report workflow will be available after the Genexus™ Purification System and integrated reporting capabilities are added later in 2020.

For more information about Oncomine NGS solutions, go to **oncomine.com**

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