干实验过程的方法建立及性能确认

张诗乐 Ph.D, Illumina Inc. Nov 2018

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AGENDA

- General Overview – Oncology Strategy
- Preanalytical Validation
- Analytical Validation
- Genomic biomarkers for Immunotherapy

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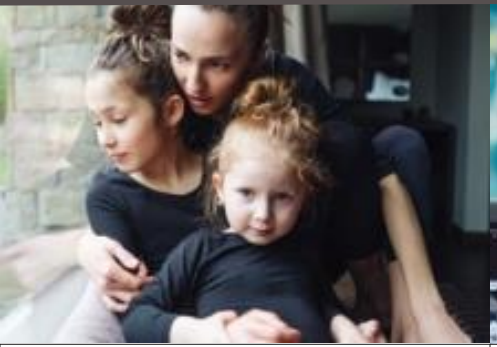
The Cancer Patient Journey

Our strategic focus on the Therapy Selection and Monitoring Segments



Hereditary Factors

Germline

Screening

cfDNA

Therapy Selection

Tissue, cfDNA

Monitoring

cfDNA

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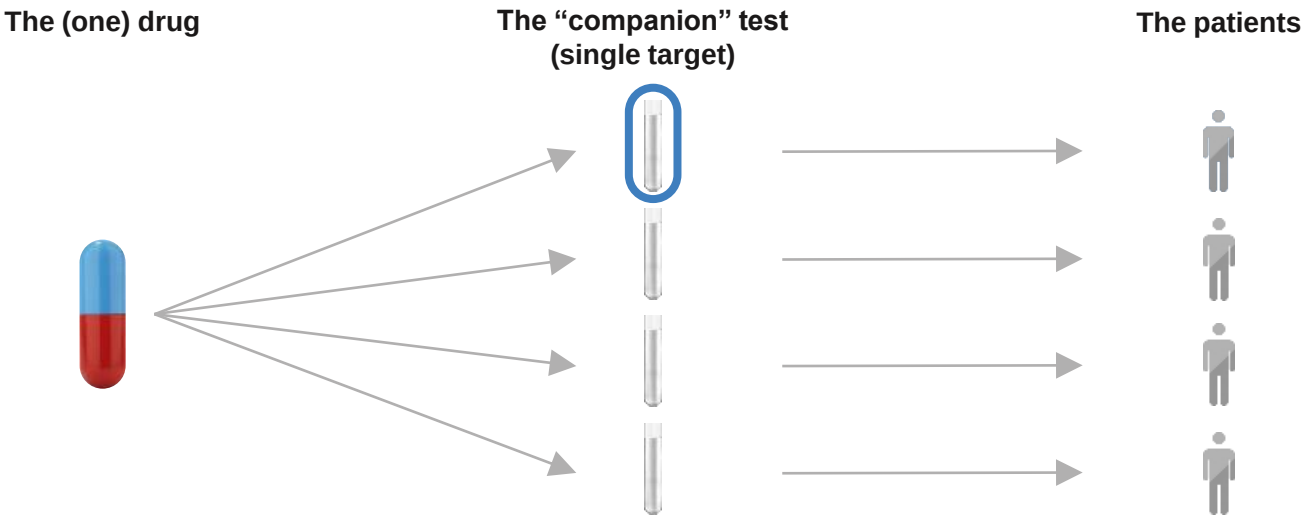
Drug-Centered Oncology Rx

Traditional approach



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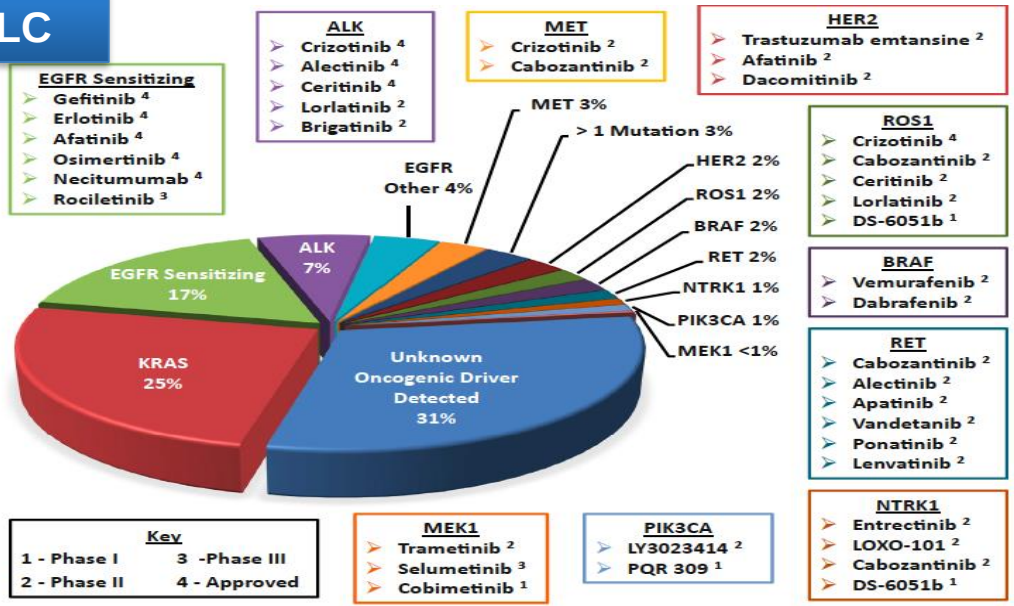
Too many questions for a small amount of tissue

Comprehensive testing can save time, sample and impact patients lives earlier

NSCLC



Frequency of molecular aberrations in various driver oncogenes in lung adenocarcinomas and current available drugs against these oncogenic proteins.

+ New Markers: TMB, MSI

What if, instead of multiple tests, you could perform a **single test** that looked at **all relevant biomarkers** at the same time?

Tsao AS, et al. J Thorac Oncol. 2016;11:613-638.

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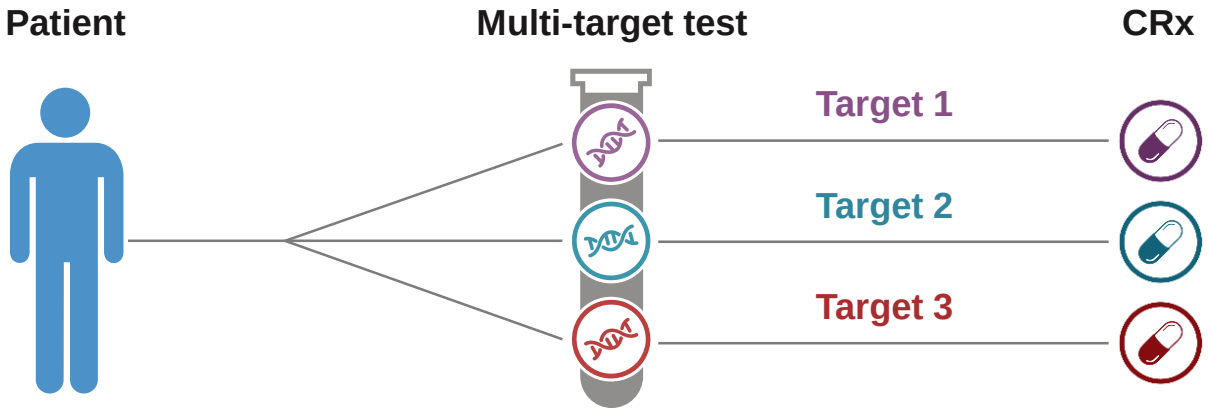
In Progress: Precision Oncology Treatment

From companion diagnostics to companion therapeutics



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Companion Diagnostic Development

Partnering to Power Oncology Precision Medicine

Companion Diagnostic product (under development) based on TruSight™ Tumor 170 content

Loxo to expand oncology menu for NextSeqDx

Companion Diagnostic product (under development) based on TruSight™ Oncology 500 content

Bristol-Myers Squibb to expand oncology menu for NextSeqDx

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TruSight™ Tumor 170 | TruSight™ Oncology 500

Multi Biomarker Panels for Pan-Cancer Analysis

CONTENT	TST170	TSO500
Genes	170	523
Size	0.5Mb	~2Mb
SNVs	✓	✓
InDels	✓	✓
CNVs	✓	✓ *
Fusions	✓	✓ ∞
Splice Variants	✓	✓ ∞
MSI	✓ +	✓
TMB	x	✓

* CNVs calling available after launch
\+ Software solution for MSI calls coming soon
∞ fusions and splice calls enabled by combining TSO500 DNA product with the RNA workflow from TST170

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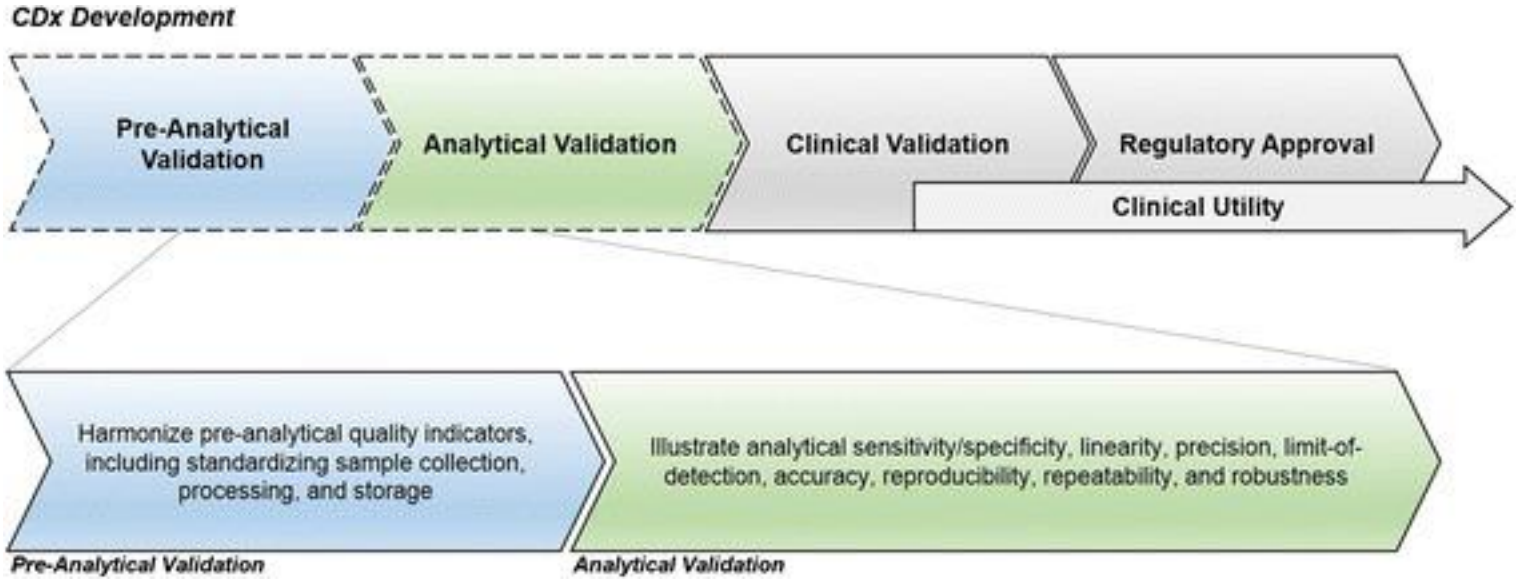
The biomarker development process



Masucci et al. (2016)

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FFPE DNA

- **Pathological review to identify tumor-rich areas for microdissection**
  - Tumor purity information is important for interpretation of the results
  - Limit of detection at 5% variant allele frequency would require tumor purity >10%
- **DNA quantification**
  - Spectrophotometry (such as NanoDrop): Presence of nucleotides; could be single-stranded, highly degraded DNA
  - Fluorometric-based methods (such as Qubit or PicoGreen): Double-stranded DNA, however could be variable fragment size
  - Quantitative RT-PCR is preferable to quantify the amount of amplified templates
    - qPCR reaction determines the fragmentation status and amplification potential of FFPE samples. The status and potential are compared to ACD1 control DNA to calculate a ΔCq value for each sample
    - The higher the ΔCq of the samples, the lower the quality and higher the amount of input DNA required for sequencing

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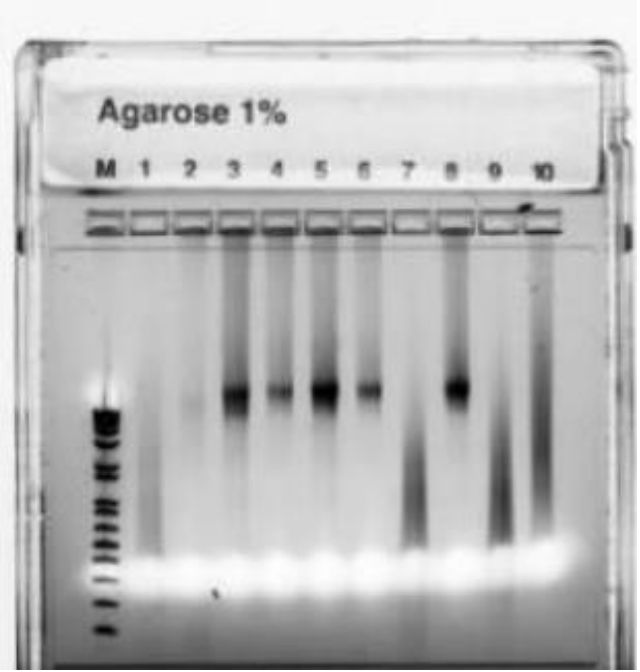
FFPE Nucleic Acids

- **Highly variable within a set**
- **DNA damage**
  - Higher duplicate reads in FFPE libraries
  - Chimeric fragments are more common in FFPE samples
  - Smaller insert size in FFPE samples
  - GC bias is more severe in FFPE samples
  - Noisier variant calling results
  - Noisier CNV results



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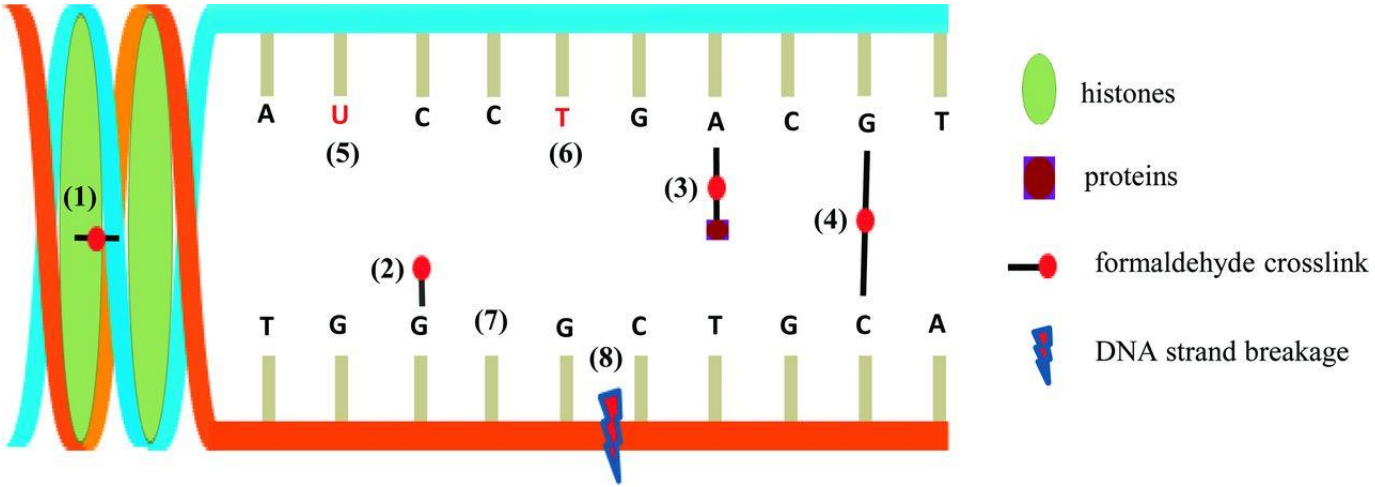
Types of DNA damage in FFPE tissues



Do *et. al. Clinical Chemistry* (2014)

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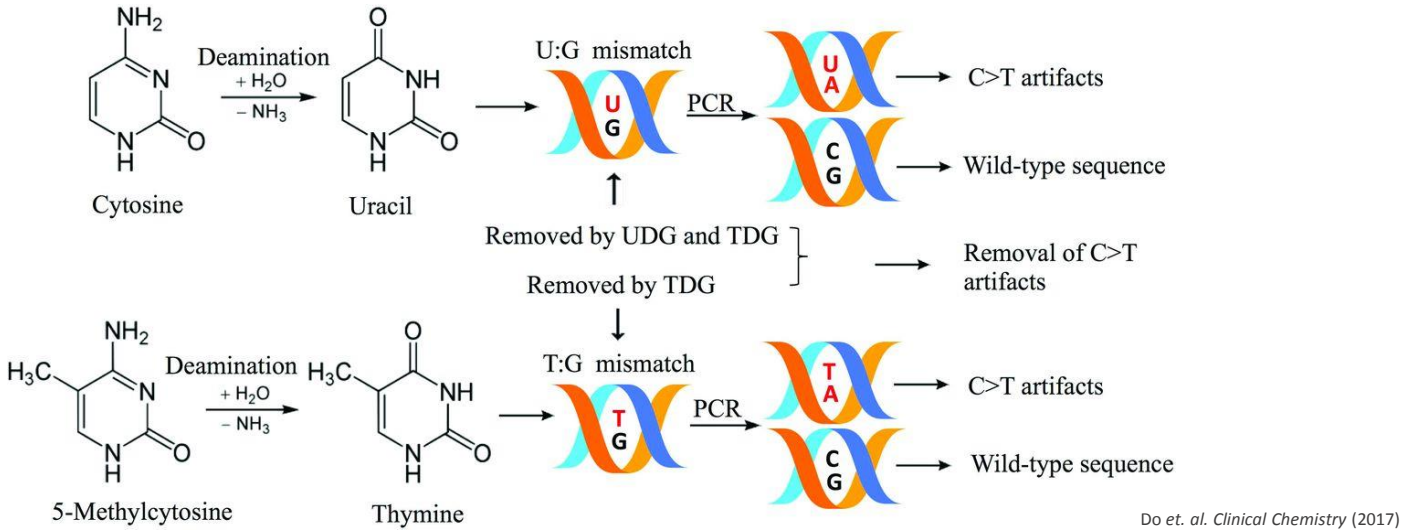
Deamination of Cytosine bases

- **Hydrolytic deamination of cytosine bases: C -> U**
- **Result in artificial C:G >T:A SNVs**
- **Prevalent in CpG dinucleotide**



Do *et. al. Clinical Chemistry* (2017)

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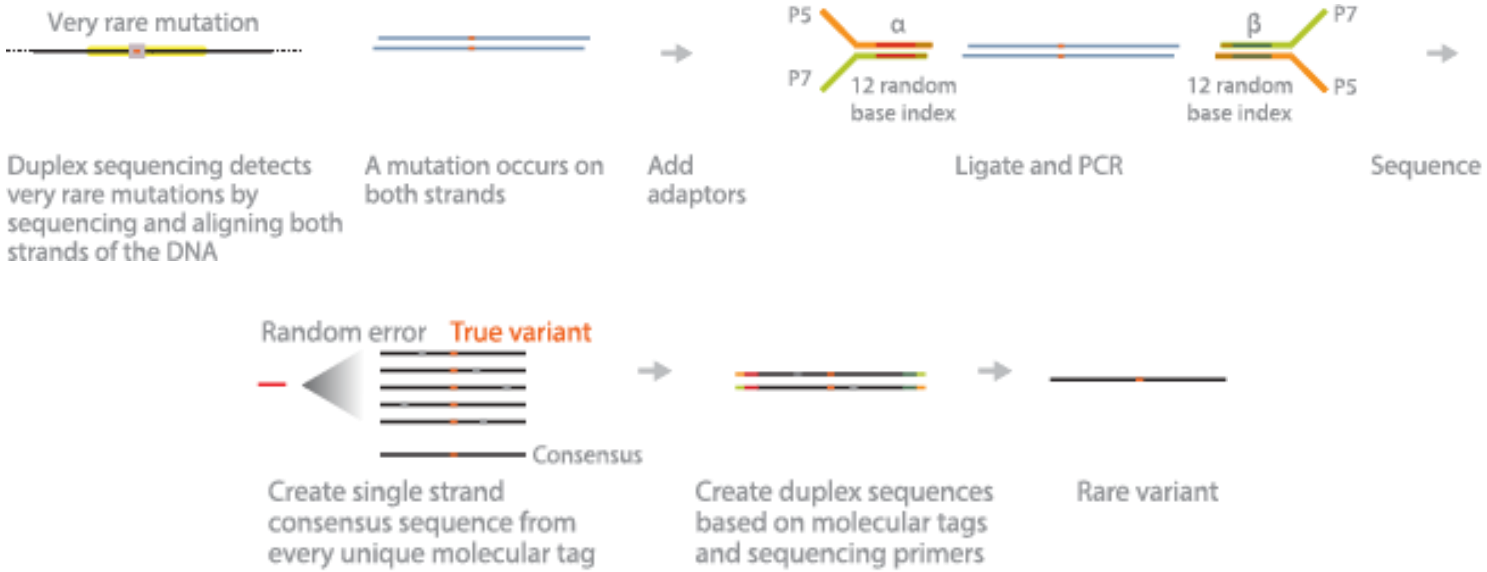
Duplex sequencing



Bioinformatics:
Collapsing reads based on duplex sequences
Build error model based on duplex information

https://www.illumina.com/science/sequencing-method-explorer/kits-and-arrays/duplex-sequencing.html

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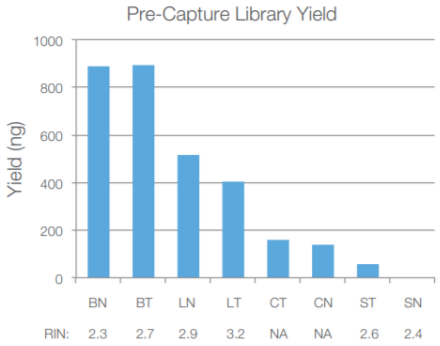
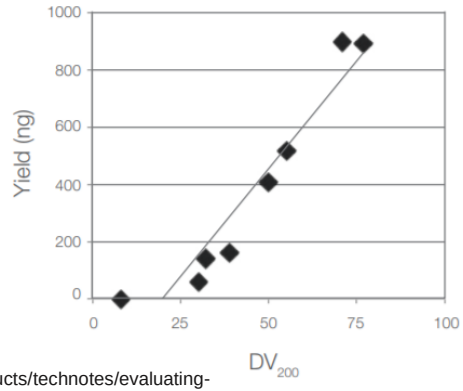
FFPE RNA

- **Traditional quality assessment: RNA Integrity Number (RIN) from RNA electropherogram traces (e.g. Bioanalyzer traces)**
- **DV200 metric**
  - Measure percentage of RNA >200 nucleotides
  - DV200 >30% ensures degraded RNA fragment meet the requirement for efficient target capture
- **DV200 can be calculated from a Bioanalyzer trace by performing a Smear Analysis**

Sample	RIN	DV_{200}*
Breast Normal	2.3	77
Breast Tumor	2.7	71
Lung Normal	2.9	55
Lung Tumor	3.2	50
Colon Normal	N/A	32
Colon Tumor	N/A	39
Stomach Tumor	2.4	30
Stomach Normal	2.6	8





https://www.illumina.com/content/dam/illumina-marketing/documents/products/technotes/evaluating-rna-quality-from-ffpe-samples-technical-note-470-2014-001.pdf

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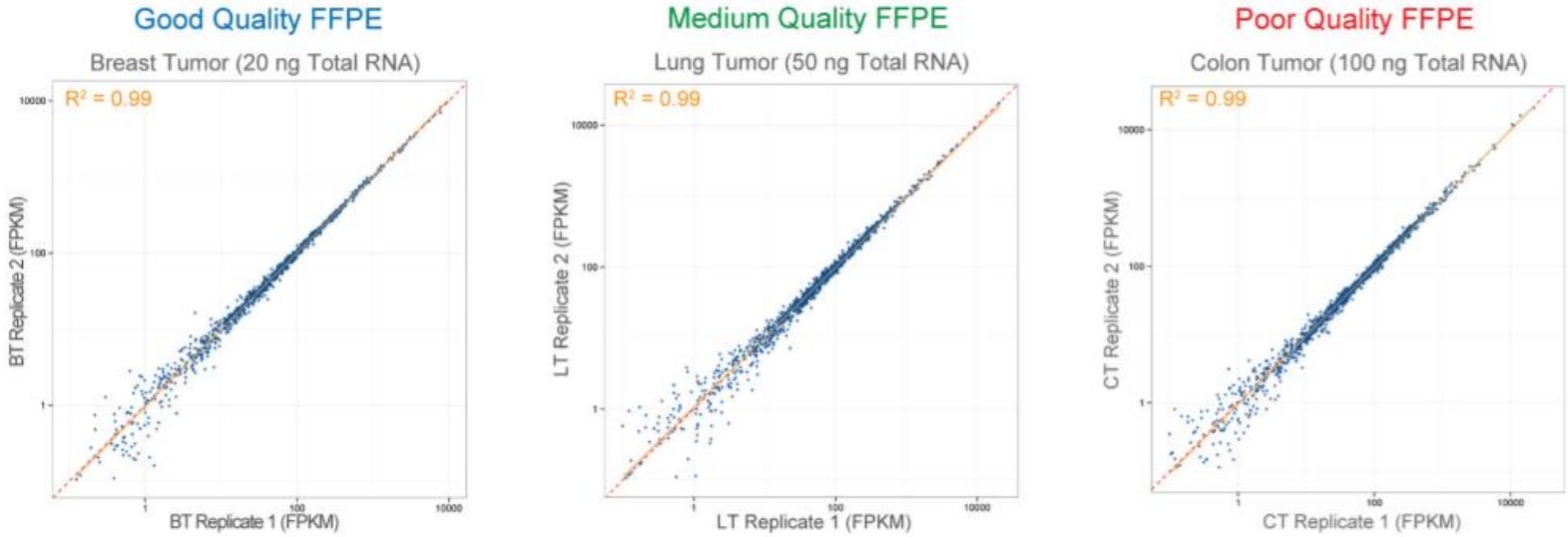
High concordance of gene expression between replicates across FFPE samples of varied quality



https://www.illumina.com/content/dam/illumina-marketing/documents/products/technotes/evaluating-rna-quality-from-ffpe-samples-technical-note-470-2014-001.pdf

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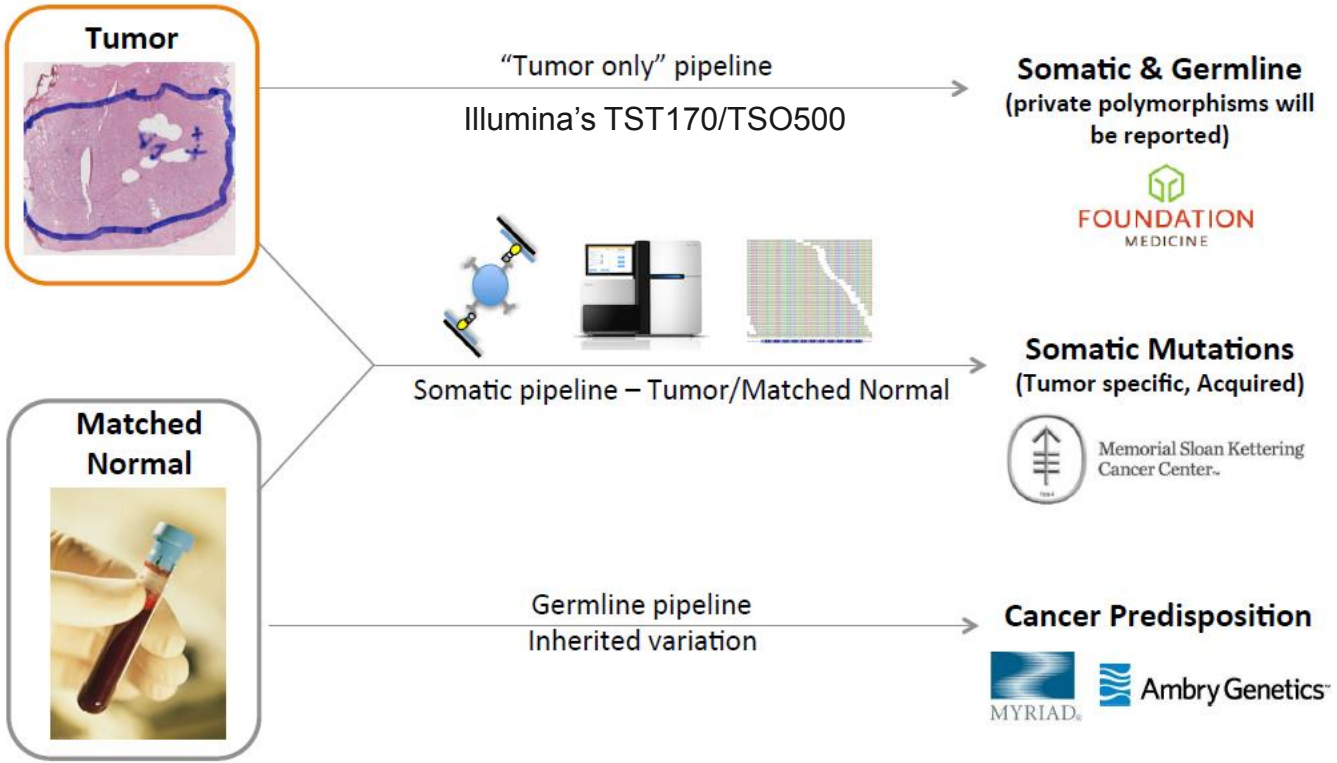
Current NGS Oncology Market



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Tumor/normal vs. tumor only

- **Tumor/normal**
  - To distinguish somatic mutations from germline variants
  - To capture false positive calls caused by poorly behaved genomic regions
  - To confirm germline variants that are associated with tumor development (BRCA genes, MMR genes etc)

- **Tumor-only**
  - Matched normal specimens

    are not routinely collected in clinical practice
  - Tumor/normal analysis has significant cost increase even if normal is sequenced at a lower depth

Practical consideration in patient management

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Build robust pipeline for tumor only workflow

- **Tumor/normal**
  - To distinguish somatic mutations from germline variants
  - To capture false positive calls caused by poorly behaved genomic regions
  - To confirm germline variants that are associated with tumor development (BRCA genes, MMR genes etc)

- **Tumor-only**
  - Removal of common germline variants documented in public databases, e.g. dbSNP, 1000G, ExAC and gnomAD

Tumor-only analyses			
	Candidate mutations per tumor	Candidate mutations per tumor after removal of common germline variants (dbSNP)	Alterations in actionable genes after dbSNP filter
Total alterations	1401	382	2.41
True positives (somatic)	135 (10%)	133 (35%)	1.61 (67%)
False positives (germline)	1266 (90%)	249 (65%)	0.80 (33%)

WES analysis in Jones et al. ***Science TM***, 2015

  - More population sequencing datasets will help or one-time germline WGS at birth or healthy

Cohort Normal

Baseline Normal

Build robust pipeline for tumor only workflow

- **Tumor/normal**
  - To distinguish somatic mutations from germline variants
  - To capture false positive calls caused by poorly behaved genomic regions
    - If a matched normal is from blood or saliva, it could not capture the systematic noise from sample preservation and nucleic acid extraction
    - Adjacent non-tumor tissues might be hard to collect in clinical practice
  - To confirm germline variants that are associated with tumor development (BRCA genes, MMR genes etc)

- **Tumor-only**
  - Reference non-tumor tissues undergone the same process would capture majority if not all systematic bias
  - Use the systematic biases in reference samples as background filter

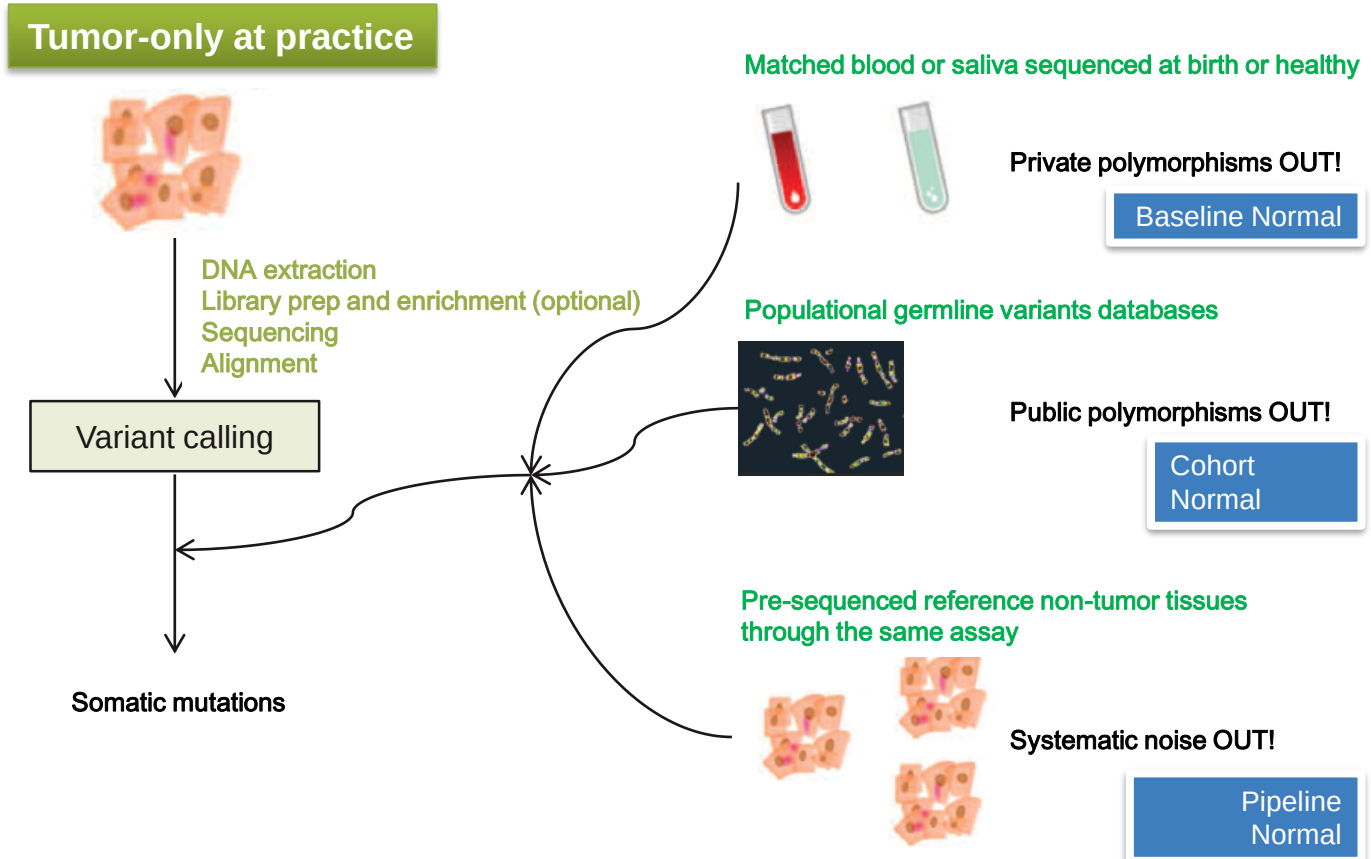
Pipeline
Normal

Build robust pipeline for tumor only workflow

- **Tumor/normal**
  - To distinguish somatic mutations from germline variants
  - To capture false positive calls caused by poorly behaved genomic regions
  - To confirm germline variants that are associated with tumor development (BRCA genes, MMR genes etc)

- **Tumor-only**
  - Serves as a screening test for pathogenic germline variants
  - Once suspect germline variants are identified in the tumor sample, then confirm with germline sequencing

Recommendation for tumor-only workflow



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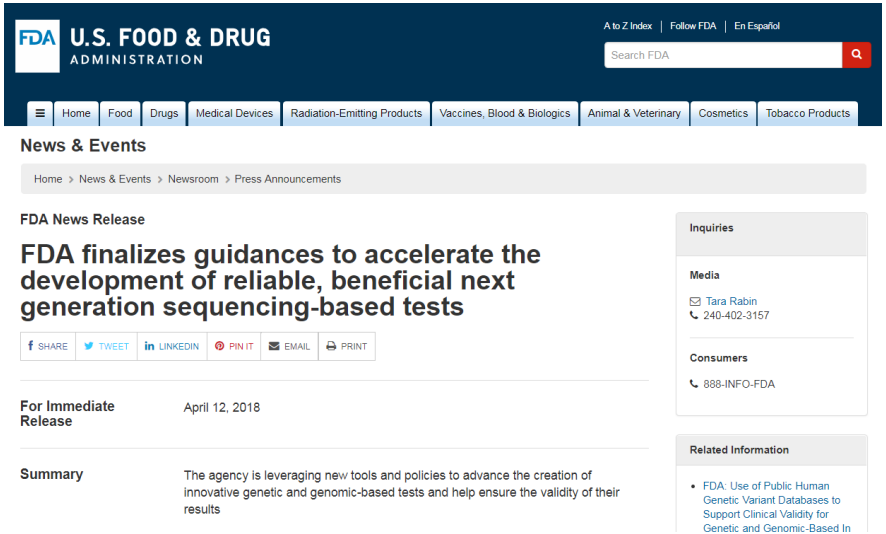
FDA's approach to regulatory oversight for NGS in vitro diagnostic (IVD) tests



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FDA News Release

FDA finalizes guidances to accelerate the development of reliable, beneficial next generation sequencing-based tests

SHARE TWEET LINKEDIN PIN IT EMAIL PRINT

For Immediate Release: April 12, 2018

Summary: The agency is leveraging new tools and policies to advance the creation of innovative genetic and genomic-based tests and help ensure the validity of their results

Inquiries

Media

Tara Rabin
240-402-3157

Consumers

888-INFO-FDA

Related Information

- FDA: Use of Public Human Genetic Variant Databases to Support Clinical Validity for Genetic and Genomic-Based In

"…assure the safety and effectiveness (which, for IVDs, generally means a reasonable assurance of analytical and clinical validity) of these test…"

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Accuracy

- **Positive percent agreement (PPA) : TP/(TP+FN)**
  - Ability to correctly identify variants
- **Negative percent agreement (NPA) : TN/(TN+FP)**
  - Ability to correctly identify wt bases
- **Technical positive predictive value (TPPV) : TP/(TP+FP)**
  - Particular import if variant confirmation will not be performed
- **Minimum acceptable overall and target thresholds for PPA, NPA, and TPPV point estimate and lower bound of 95% confidence interval (CI) of an NGS-based test should be predefined and reported for each variant type claimed by the test**

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No calls in the NGS assay

- **No calls or invalid calls describe the result where a base call is not made, which can be a result of a number of factors**
  - the base level performance not meeting predefined thresholds for quality, resulting in insufficient data to make a variant call.
- **No calls and invalid calls should not be used in PPA, NPA, or TPPV calculations, but should be individually documented as part of the accuracy study results.**
- **Minimum acceptable values for the number of no calls or invalid calls will depend on indications for use and test design.**

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Accuracy calculation

		Comparator Method		Total
		Positive	Negative	
Test	Positive	A	B	A+B
	Negative	C	D	C+D
	No calls or invalid calls	E	F	E+F
	Total	A+C+E	B+D+F	N

- **Percent of no calls or invalid calls : (E+F)/N along with a 95% two-sided confidence interval**
- **PPA : A/(A+C)**
- **NPA: D/(D+B)**
- **TPPV: A/(A+B)**

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Precision (Reproducibility and Repeatability)

- **Reproducibility: NGS tests involves measuring test variability under a variety of specified condition**
  - Samples
  - Runs
  - Reagent lots
  - Days
  - Operators
- **Repeatability: measuring test result variability when using the same operators, the same measuring system, the same operating conditions and the same location, and replicating measurements on the same or similar objects over a short period of time**
- **The percentage of no calls or invalid replicates should be also reported**

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Limit of Detection (LoD)

- Establish and document the input DNA range, that will enable the test to provide expected results in 95% of test runs
- Establish and document that the level of invalid calls or no call results is acceptable
- Establish and document the lower LoD for each variant type, and in different sequence context included in the test's indication for use
- The lower LoD is calculated as the lowest concentration of analyte at which at least 95% of positive calls
- An upper LoD should be established and documented

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Analytical Specificity

Analytical specificity relates to the ability of a test to measure solely the intended analyte.

- **Interference**
  - Identify and document any interfering substances that might reduce the ability to amplify or sequence
- **Cross-reactivity**
  - Access and document the potential for cross-reactivity of known cross-reactive alleles and homologous regions (e.g., pseudogenes), based on the targets that will be interrogated by the test
- **Cross-contamination**
  - Develop, validate and document methods to detect carryover or cross-contamination between patient specimens or samples

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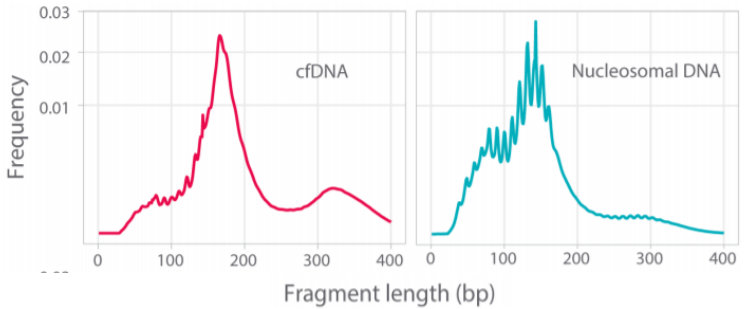
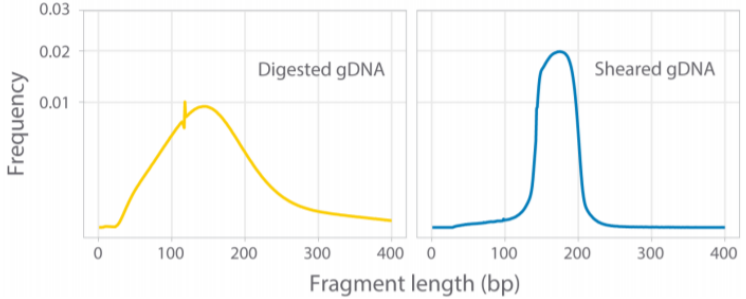
Reference materials

- **Synthetic mimic libraries, cell lines, synthetic vectors, company or WHO and NIST reference**
  - NA12878 from NIST: Genome in a Bottle (GIAB) Consortium
  - FFPE tissue-based controls are available from companies
    - Horizon Dx, Acrometrix (Thermo Fisher, Inc), Seracare Life Science

- **Other concerns for reference materials**





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- **Genomic biomarkers for immunotherapy**

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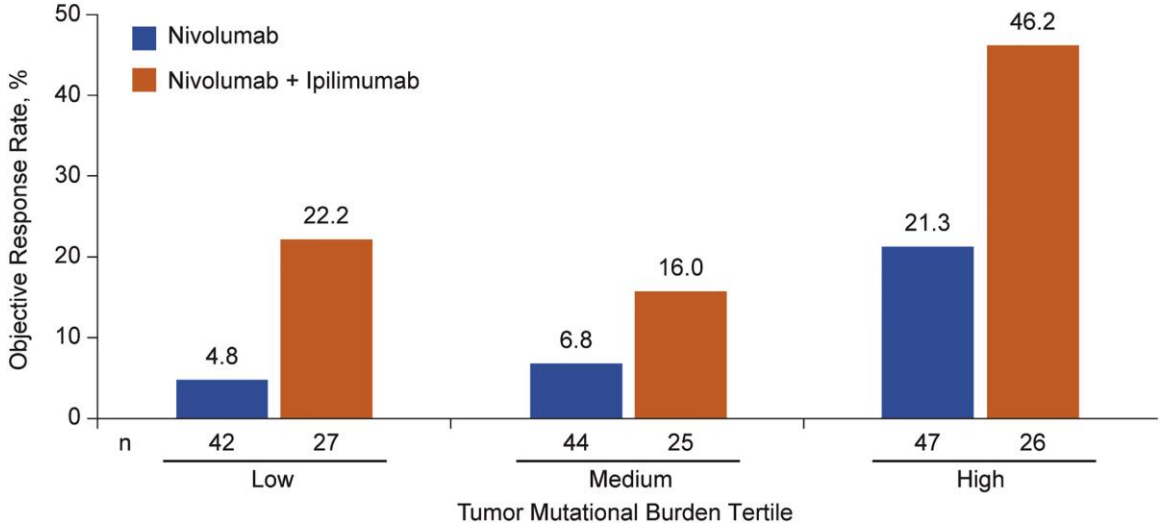
Cancer Immunotherapy Biomarker Advancement

Tumor Mutation Burden correlation to therapy outcomes

Not every tumor responds well to IO therapy. High Tumor Mutation Burden has been linked to better responses

Results from Clinical Trials demonstrate the link between High Tumor Mutation Burden and better response to immunotherapies.



TUMOR MUTATION BURDEN (TMB): The measurement of mutations carried by tumour cells. A new predictive marker being used in response to IO therapies

Hellmann et al., 2018, Cancer Cell 33, 853–861

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TruSight™ Tumor 170 | TruSight™ Oncology 500

Multi Biomarker Panels for Pan-Cancer Analysis

CONTENT	TST170	TSO500
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TMB	x	✓

* CNVs calling available after launch
\+ Software solution for MSI calls coming soon
∞ fusions and splice calls enabled by combining TSO500 DNA product with the RNA workflow from TST170

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TruSight™ Oncology 500 Content

Pan-cancer DNA + RNA analysis: Consolidate multiple tests into one*

Single Assay – 523 Genes

Detection of currently relevant DNA & RNA variants for multiple types of cancer. Some key biomarkers included:

Lung	Melanoma	Colon	Ovary	Breast	Gastric	Bladder	Myeloid	Pan-Cancer
AKT1	BRAF	AKT1	BRAF	AKT1	BRAF	MSH6	ALBL1	MSI
ALK	CTNNB1	BRAF	BRCA1	AR	KIT	PMS2	ASXL1	NTRK
BRAF	GNA11	HRAS	BRCA2	BRCA1	KRAS	TSC1	CALR	
DDR2	GNAQ	KRAS	KRAS	BRCA2	MET		CEBPA	
EGFR	KIT	MET	PDGFRA	ERBB2	MLH1		ETV6	
ERBB2	MAP2K1	MLH1	FOXL2	FGFR1	PDGFRA		EZH2	
FGFR1	NF1	MSH2	TP53	FGFR2	TP53		FLT3	
FGFR3	NRAS	MSH6		PIK3CA			GATA2	
KRAS	PDGFRA	NRAS		PTEN			IDH1	
MAP2K1	PIK3CA	PIK3CA					IDH2	
MET	PTEN	PMS2					JAK2	
NRAS	TP53	PTEN					KIT	
PIK3CA		SMAD4					MPL	
PTEN		TP53					NPM1	
RET							RUNX1	
TP53							SF3B1	
TMB							SRSF2	
							TP53	

* The recommended product to evaluate DNA + RNA variants is the TSO500 DNA+RNA bundle (PN: 20028216) – commercially available in January -2019

For Research Use Only. Not for use in diagnostic procedures.

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TruSight™ Oncology 500

Premium tumor profiling solutions; Include all genes covered by FoundationOne and MSK-IMPACT

Premium Content

- Our assays are **comprehensive**, with **high coverage of relevant biomarkers**, such as the ones present in **NCCN** Guidelines and **Clinical Trials**.
- Enrichment enables **better detection of novel** fusions and splice variants.
- **TSO500 can detect both TMB+MSI**. Detecting both markers is key in immuno-oncology

	TSO500 DNA+RNA
Total # of Genes	523
SNVs, InDels	523
Amplifications	59
Fusions	55
Splice Variants	3
TMB	YES
MSI	YES
Presence in Guidelines	42
Presence in Clinical Trials	>1,600

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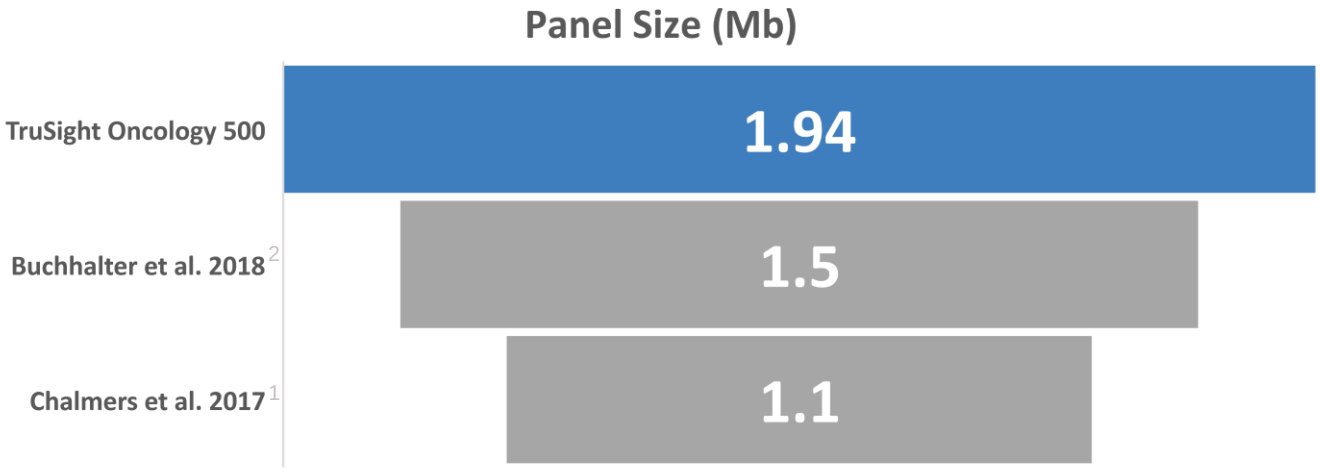
TruSight™ Oncology 500 | Highly Accurate TMB Scoring

Panel size of 1.94 Mb surpasses minimum requirements

Recent Evidence shows that TMB can accurately calculated by targeting ≥1.5 Mb of coding genome.[2] Larger Panels correlate with greater sensitivity and have increased correlation with WES.[1,2]



1. Chalmers et al, Genome Medicine 2017 9:34;
2. Buchhalter et al., Int J Cancer. 2018 Sep 21. doi: 10.1002/ijc.31878.

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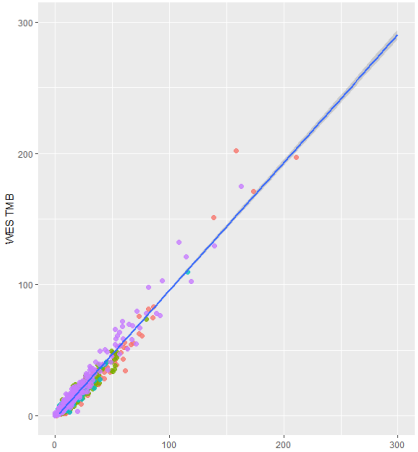
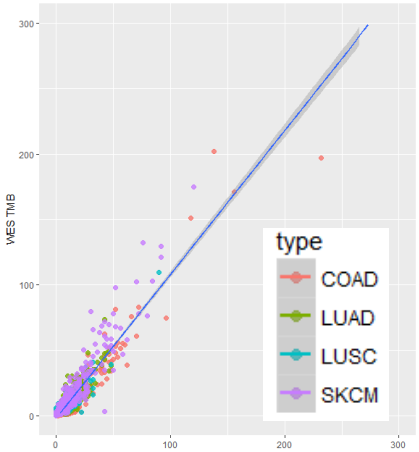
TruSight™ Tumor 170 | TruSight™ Oncology 500

When it comes to TMB detection, size matters

WES vs. TSO500 R^2 = 0.97

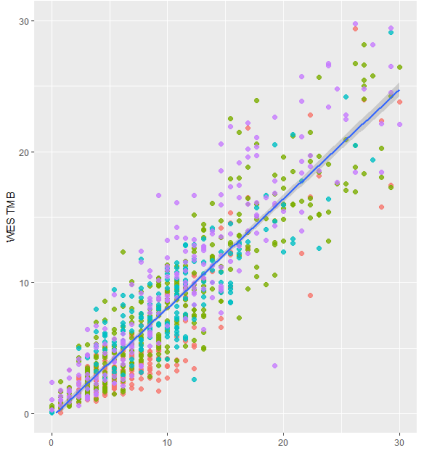


WES vs. TST170 R^2 = 0.89

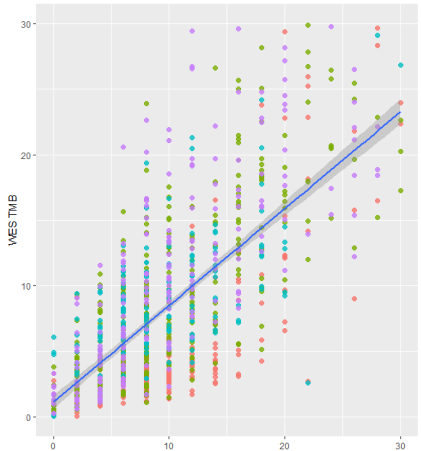


All samples

WES vs. TSO500 R^2 = 0.84



WES vs. TSO170 R^2 = 0.51



Samples in TMB range between 0-30

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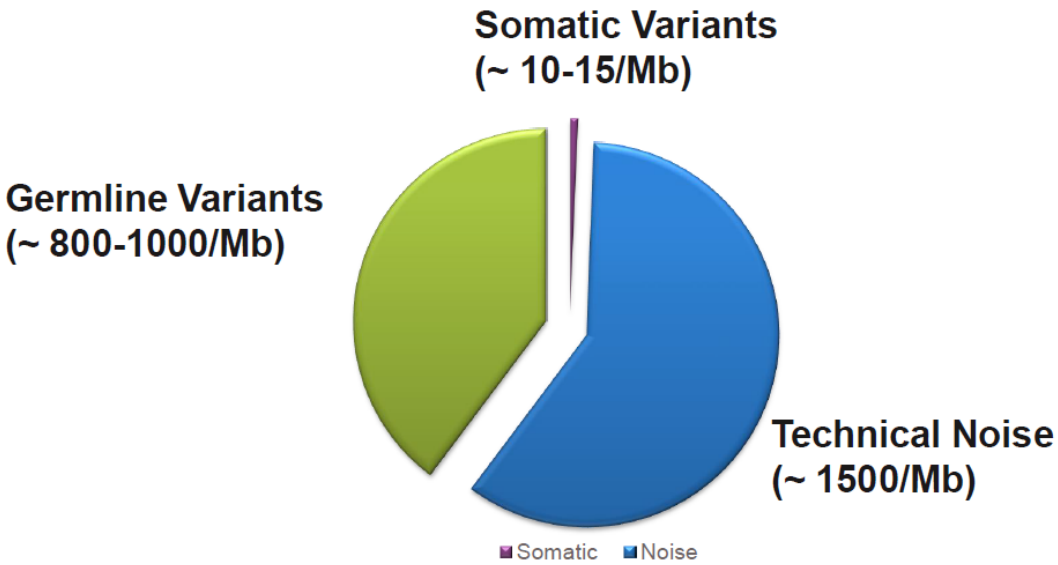
TMB in tumor only variant callilng

- TMB in tumor only setting is a “needle in a haystack” problem
- Main challenge is to effectively remove technical noise (FFPE artifacts) and germline variants



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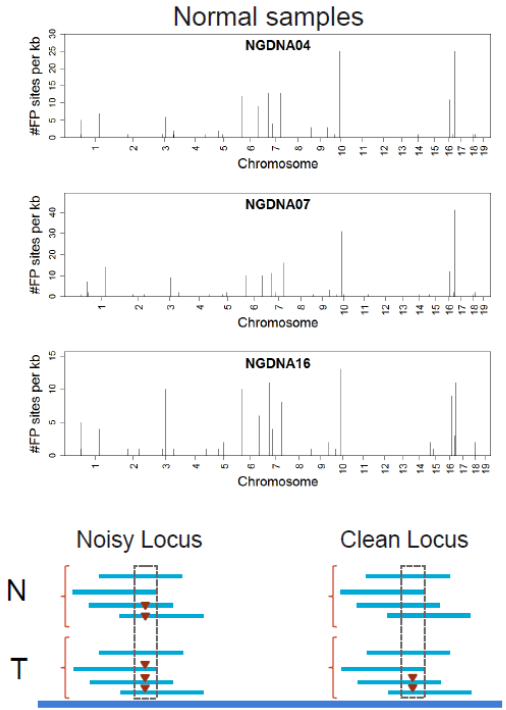
False positive removal with normal baseline



- Systematic noisy regions exist across samples
- These usually represent low complexity regions in the genome
- A cohort of normal FFPE samples can capture such recurrent error, leading to improved specificity
- This approach removes 80-90% of FPs in a given sample
- However, sample specific noise can not be removed by normal baseline

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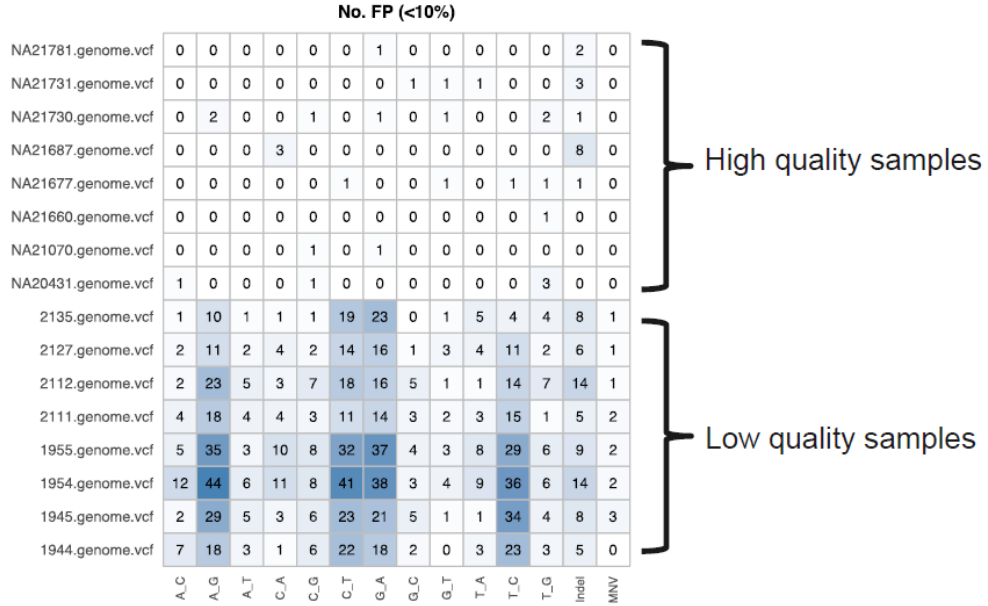
Deamination contributes heavily to FPs



- After removing site specific errors, No. FP is ~5-20/Mb in High quality sample and 200-400/Mb in Low quality sample
- No. FP is a associated with sample quality and strong deamination signature leads to **C:G->T:A** (deamination of C) or **A:T->G:C** (deamination of A) variants

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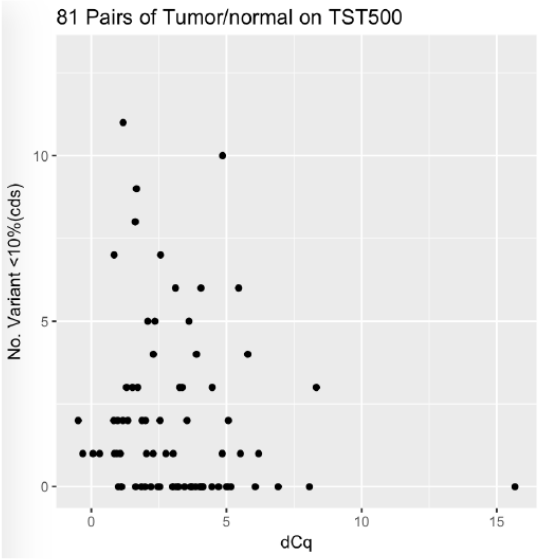
Specificity evaluation on FFPE



- All samples pass 99.999% specificity
- Specificity is not correlated with sample quality (dCq):
  - Very important not to bias TMB measurement

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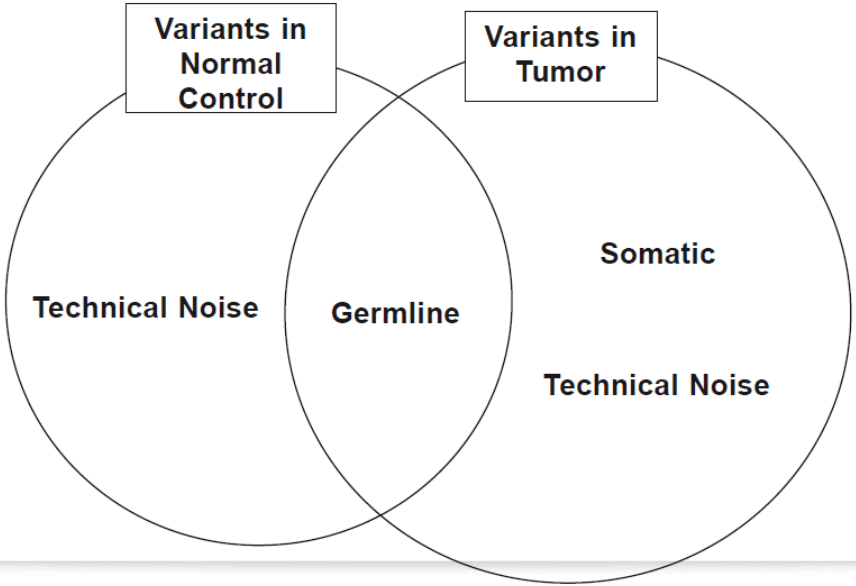
Germline filtering – the challenge with tumor only workflow

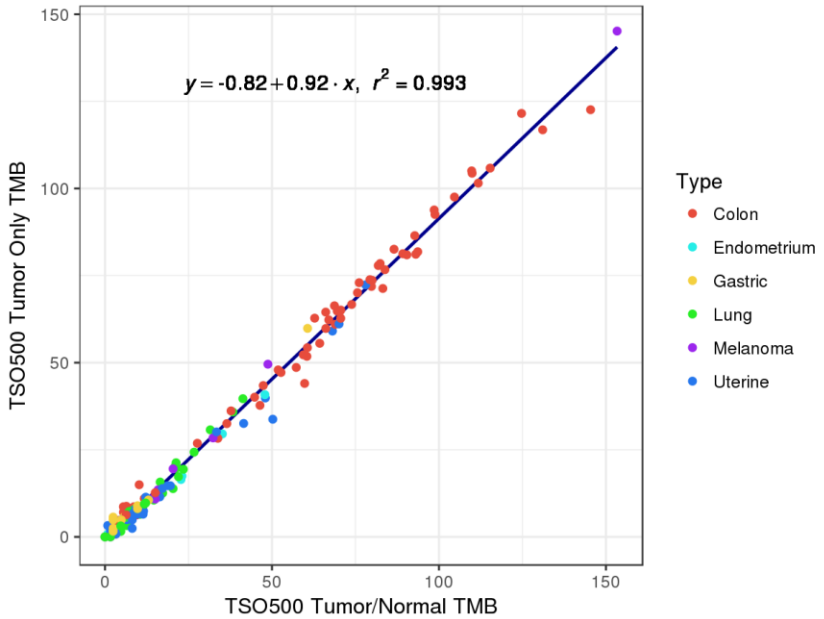
- If both tumor and normal samples are available, we can use the variant calling results from the normal sample to filter out germline variants.
- There are ~800/Mb germline variants in each individual, some are commonly observed in population, some are rare.



TruSight™ Oncology 500 | Highly Accurate TMB Scoring

Combining panel size with proprietary pipeline for high precision

Tumor-Only TMB Scoring



- 170 FFPE Samples tested: colon, endometrium gastric, lung, Melanoma, Uterine
- Tumor/Normal compared to tumor-only
- Illumina's germline filtering algorithm is key to enable tumor-only analysis
- 99.3% correlation (R^2)

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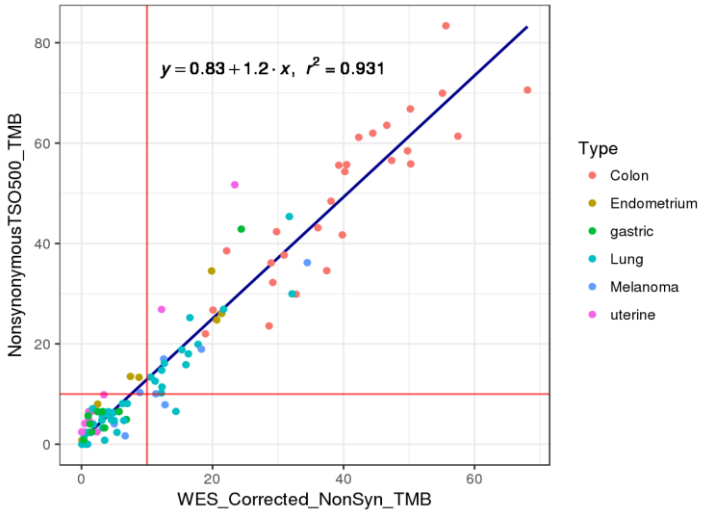
TMB Detection pipeline maximizes sensitivity, specificity and accuracy

Bioinformatic Attribute	TSO500
Includes non-syn + syn SNVs	✓
Includes InDels	✓
5% LOD	✓
Enrichment Chemistry	✓
FFPE and Seq Error Artifact Removal	✓
500 – 1,000x false positive reduction	✓
Removal of variants in low confidence regions	✓
Removal of driver mutations	✓
Germline filtering using variant databases, allele frequency and machine learning	✓

TruSight™ Oncology 500 | Highly Accurate TMB Scoring

Combining panel size with pipeline for high precision



Tumor/Normal whole exome sequencing was compared at Illumina to the tumor only TSO 500 workflow, across 108 FFPE tissue samples. TMB results showed high correlations between the two assays.

Performance at TMB threshold of 10mut/MB

	10 mutation/Mb cutoff
Positive % Agreement	94.7%
Negative % Agreement	96.1%
Overall % Agreement	95.4%

	WES T/N TMB High	WES T/N TMB Low
TSO500 TMB High	**54**	**3**
TSO500 TMB Low	**2**	**49**

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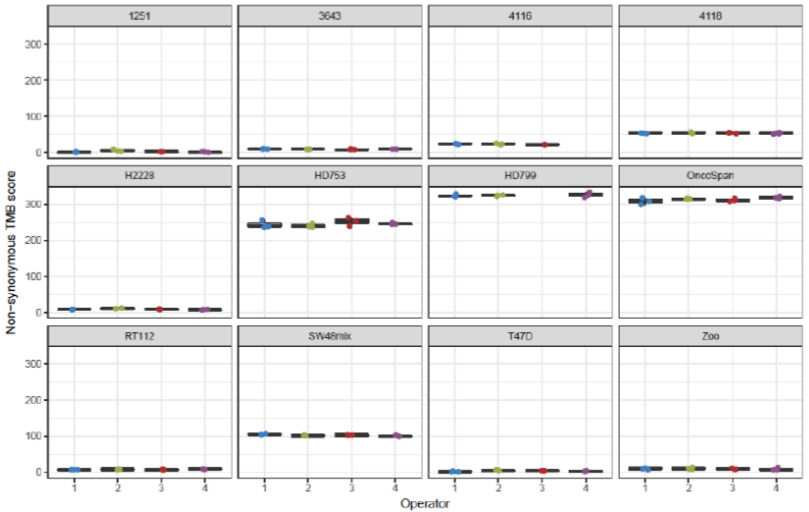
Reproducibility: TMB

Reproducibility
TMB score

- FFPE and cell line DNA
- 12 replicates tested across 4 operators to determine TMB score

Robust TMB scoring across operators



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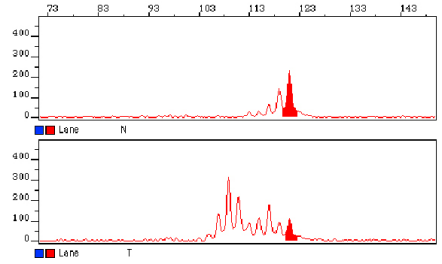
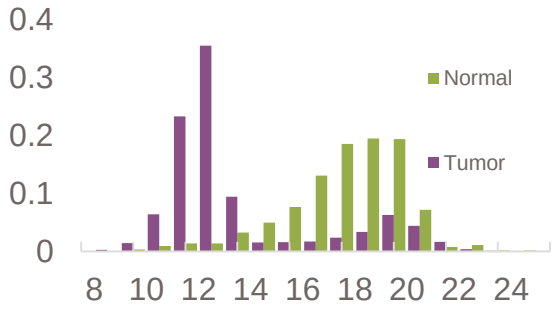
Challenges for MSI calling for tumor only workflow

- **Distribution of multiple alleles instead of a single allele in repetitive regions**
  - Probe synthesis
  - PCR (polymerase slippage)
  - Enrichment bias (AT drop, TC drop)
  - Sequencing error
  - Mapping and variant calling
- **Lack of prior knowledge**
  - Need to rely on noncanonical sites
- **Tumor only workflow**
  - Genetic diversity for normal samples
  - Ethnicity specific sites
  - Tumor samples contain mixed tumor and normal cells
- **A robust metric to capture the distribution shift for each MSI site**





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Enable Tumor Only Testing with baseline samples

- **Use 61 normal samples for baseline distribution of same MSI sites**
- **Jensen-Shannon Distance between baseline samples**
  - Pairwise calculation for any two BL samples
    - discrete distribution of relative alleles : BL1 = Pr [X=x] ; BL2= Pr[X=x]
    - JS <- 0.5 * (sum(BL1 * log(BL1 / m)) + sum(BL2 * log(BL2 / m)); m <- 0.5 * (BL1 + BL2)
    - d1 <- sqrt (JS)
- **Jensen-Shannon Distance between the test sample to Baseline samples**
  - Pairwise calculation for one BL sample and the test sample
    - BL1 = Pr [X=x] ; T= Pr[X=x]
    - JS <- 0.5 * (sum(BL1 * log(BL1 / m)) + sum(T * log(T / m)); m <- 0.5 * (BL1 + T)
    - d2 <- sqrt (JS)
- **Comparison between two Jensen-Shannon Distance distributions**
  - One sided t-test: d1 < d2, p<0.01
  - d2-d1>=0.1
- **Final score:**
  - Number of unstable sites / number of total sites tested
  - Similar to MSI-PCR

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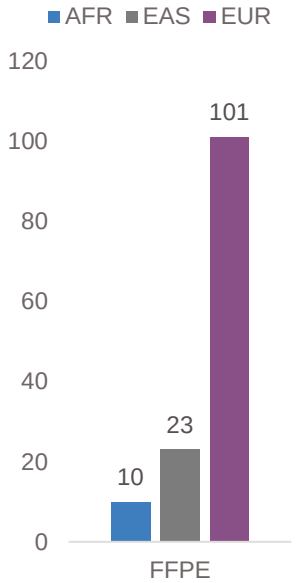
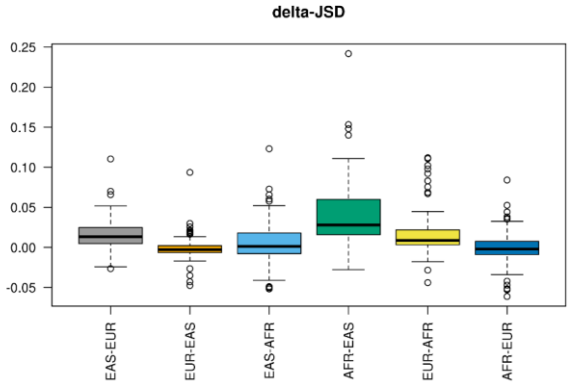
Identify MSI sites with ethnicity bias



$\Delta JSD = avg(JSD_{between}) - avg(JSD_{within})$



- **175 sites with at least 20 supporting reads for minimum 5 samples of each ethnicity group**
- **Identified 45 sites with >=0.1 ΔJSD based on pair-wised comparison between three ethnicity groups**

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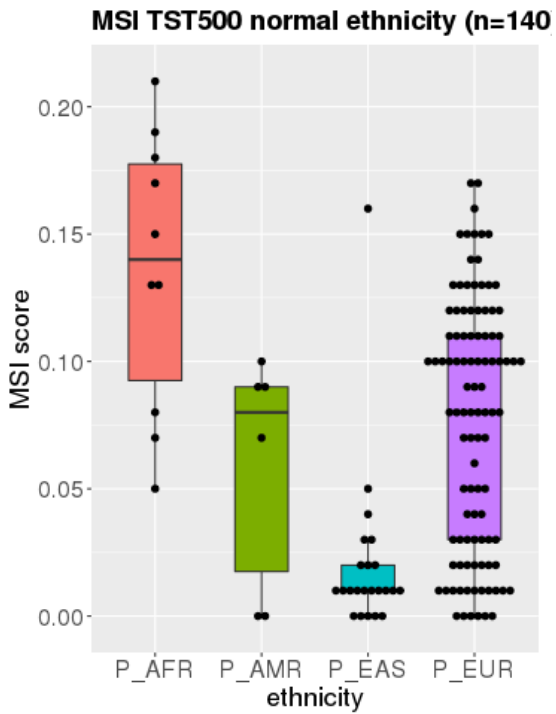
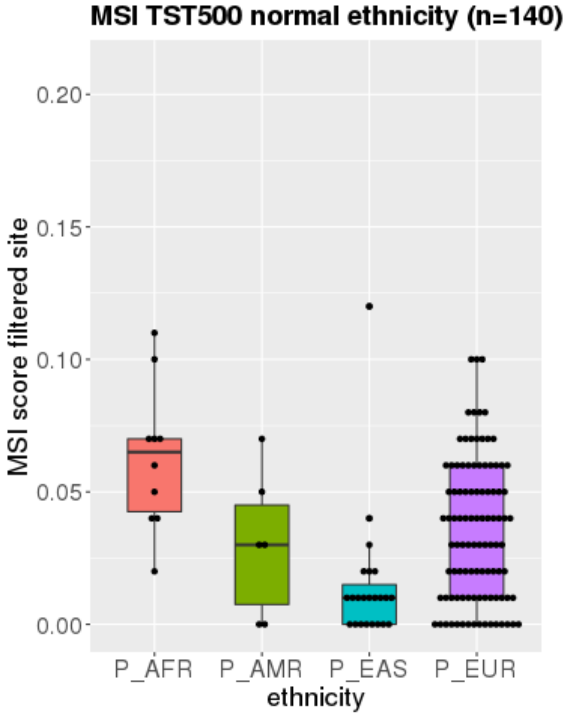
Normal sample MSI-score with





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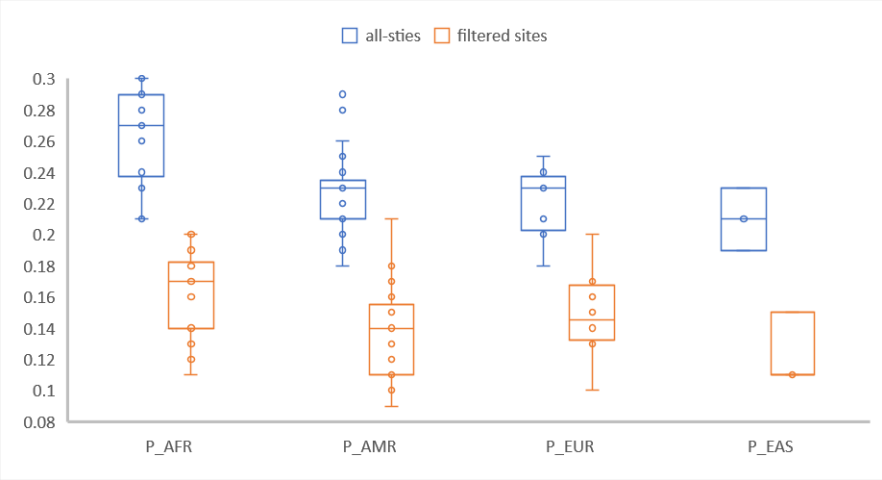
Use cell line sample as a validation cohort



- For filtered sites MSI score, the highest value is sample IHW09101 (MSI score: 0.21)
- This is annotated with Warao SA Indian ethnicity

- 10 IHW and 48 coriell
- P_AFR (n=22)
- P_AMR (n=25)
- P_EUR (n=8)
- P_EAS (n=3)

Warao

A Warao family in their canoe.

Total population
(20,000)
Regions with significant populations
Venezuela, Guyana, Suriname, Trinidad and Tobago
Languages
Warao language
Religion
Traditional beliefs, Christianity

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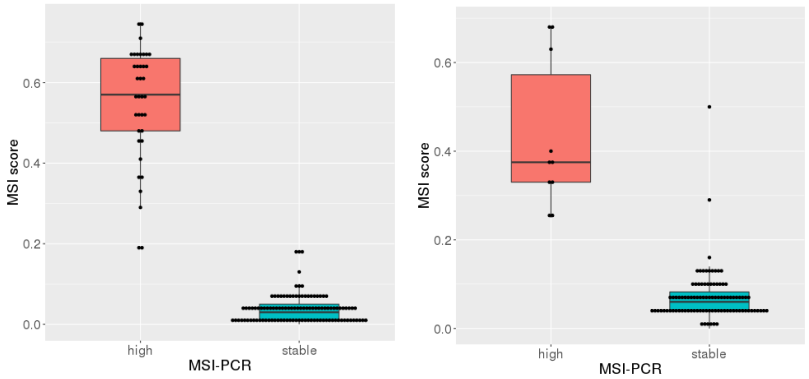
TruSight™ Oncology 500 | Highly Accurate MSI Detection

Tumor-only MSI workflow, Precise MSI detection

Tumor-Only MSI Detection



	Training set	Validation set
PPA	97.3% (85.8%-99.9%)	100% (69.2%-100.0%)
NPA	100% (96.7%-100.0%)	98.0% (92.7%-99.8%)
OPA	99.3% (96.3%-100.0%)	98.1% (93.4%-99.8%)

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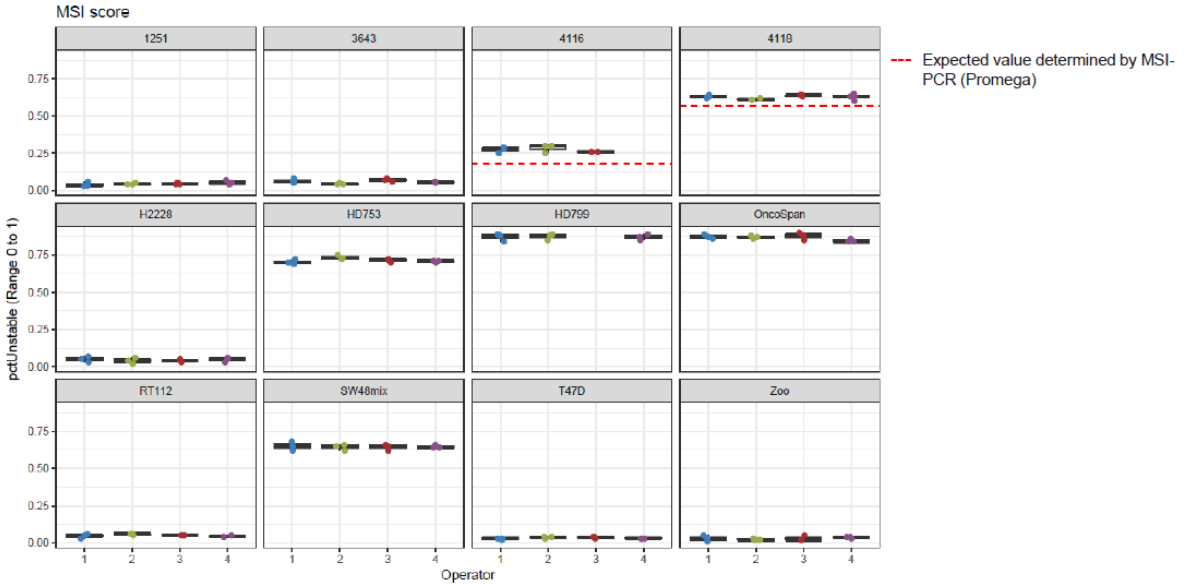
Reproducibility: MSI

Reproducibility
MSI status

- FFPE and cell line DNA
- 12 replicates tested across 4 operators to determine MSI score

Robust MSI scoring for all samples across operators



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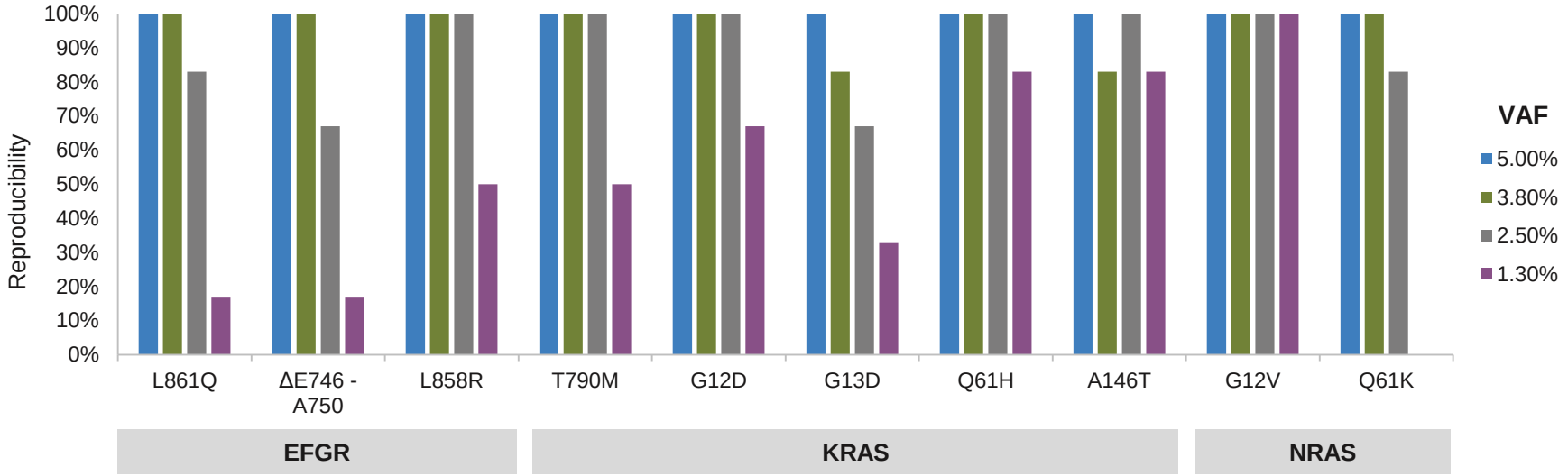
TruSight™ Oncology 500 | Highly Accurate Small Variants Detection

Precise Assay chemistry combined with sophisticated variant calling pipeline



FFPE cell line samples were tested at 40 ng input and VAF ranges of 1.3% - 5.0%, 6 replicates each. VAFs with ≥95% sensitivity are highlighted in green, while VAFs below 95% sensitivity are in yellow.

Illumina data on file, 2018

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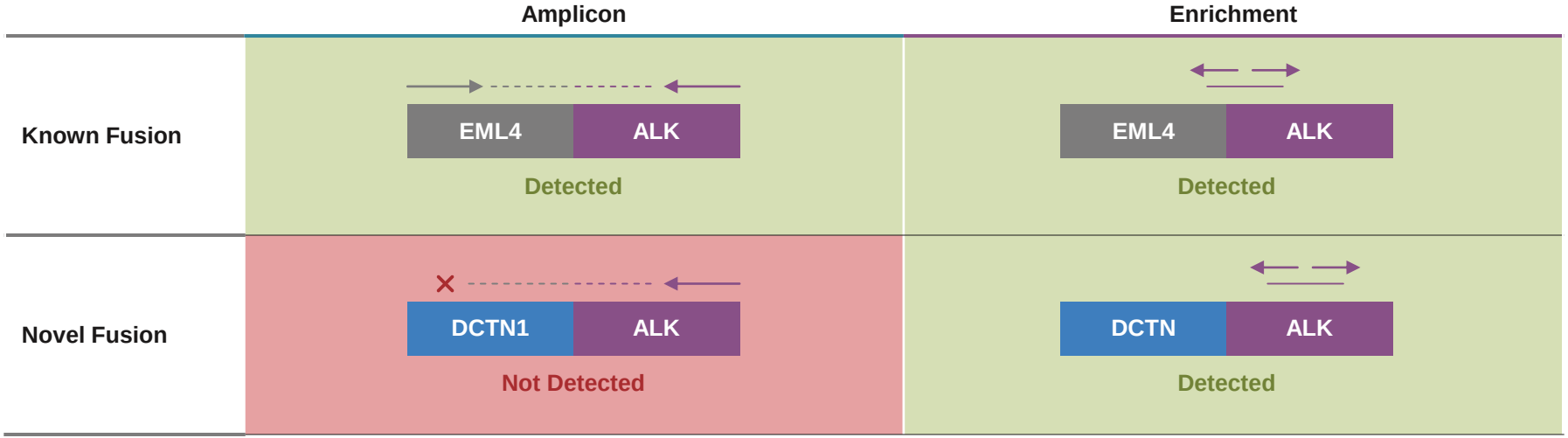
TruSight™ Oncology 500 | Ability to Detect Novel Fusions

Enrichment based assay combined with probe design algorithm



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5624911/

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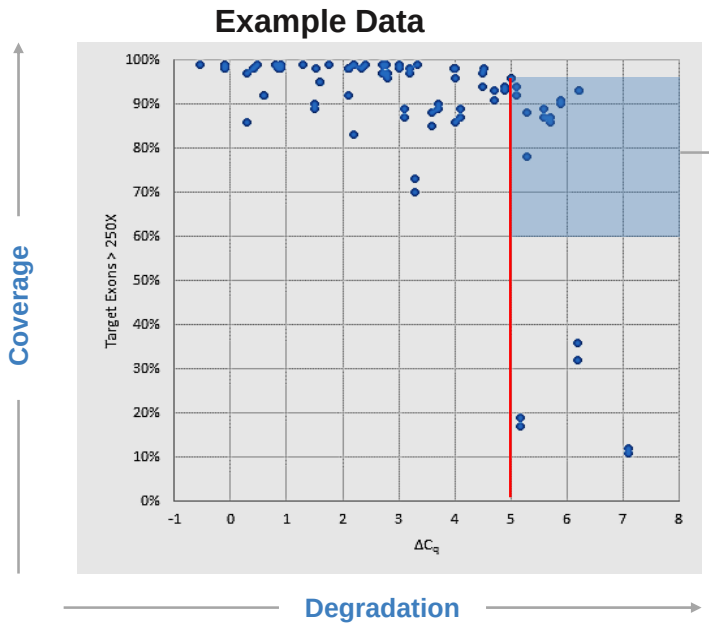
TruSight™ Oncology 500 | Tolerance to Sample Degradation

Enrichment based assay can generate yield from highly degraded samples



Our enrichment based assay and our design algorithm, enable our assays to generate results from even highly degraded samples (fragments <100bp), increasing sensitivity.

Ability to generate data even from highly degraded samples due to enrichment technology. Amplicon technologies tend to have more difficulty and have less sensitivity to such samples

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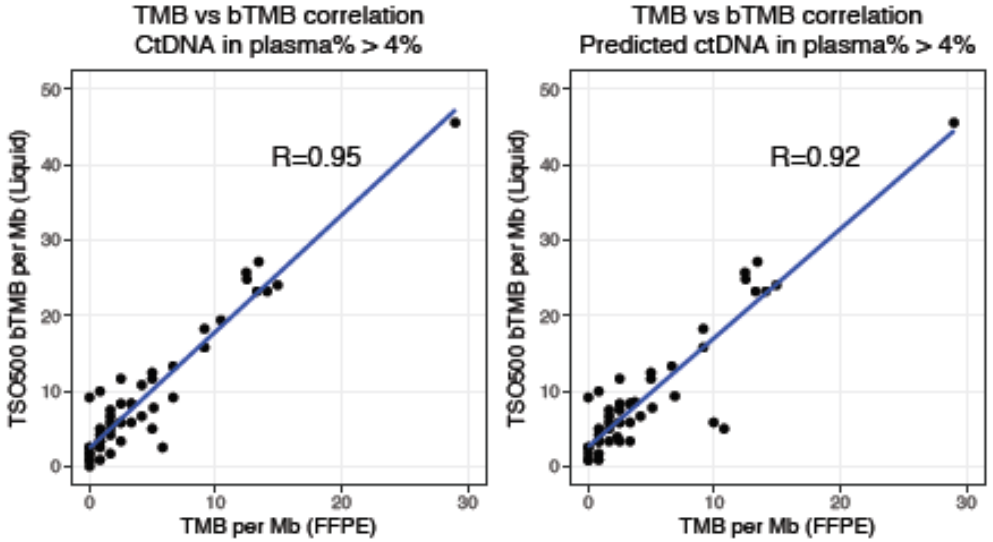
TruSight™ Oncology 500

Future-Proof: UMIs incorporated to enable Liquid Samples analysis (under development)

TMB evaluation from Liquid Samples (under development)



UMIs designed by our patented algorithm provide state-of-the-art error correction

128 samples, across 4 tissue types were included. Matched FFPE tissue andplasma tested.

The fraction of ctDNA in plasma was calculated

bTMB is highly correlated with tissue TMB in samples with high ctDNA fraction (**R=0.95**).
Performance is equivalent with the predicted tumor fraction by plasma only (**R=0.92**)

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TruSight™ Oncology 500 | Partnerships

Partnering with leading institutions to realize the vision of Precision Oncology

15 Early Access Partner Labs

…and expanding

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TruSight™ Tumor 170 | TruSight™ Oncology 500

Similar and automated workflows

Nucleic Acid to Variant Report in 3–4 days

	FFPE Specimens	Sample Prep	Automated Library Prep and Enrichment	Sequencing	Analysis
TST170	• Multiple tissue types supported	• Commercial DNA/RNA extraction kits	• TruSight™ Oncology Kit • DNA Probes – 152 genes • RNA Probes -55 genes • Automation with Hamilton or Beckman	• NextSeq™ • NextSeq™ 550Dx* • NovaSeq™ - under development * For In vitro diagnostic use	3rd Party Examples: • AMR: Pierian Dx • EU: Sophia Dx • S. Korea: NGene Bio
TSO500			• TruSight™ Oncology + UMI kit • DNA Probes - 523 genes • (optional: combine with TST170 RNA workflow) • Automatable workflow		

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APPENDIX – ADDITIONAL PRODUCT INFORMATION

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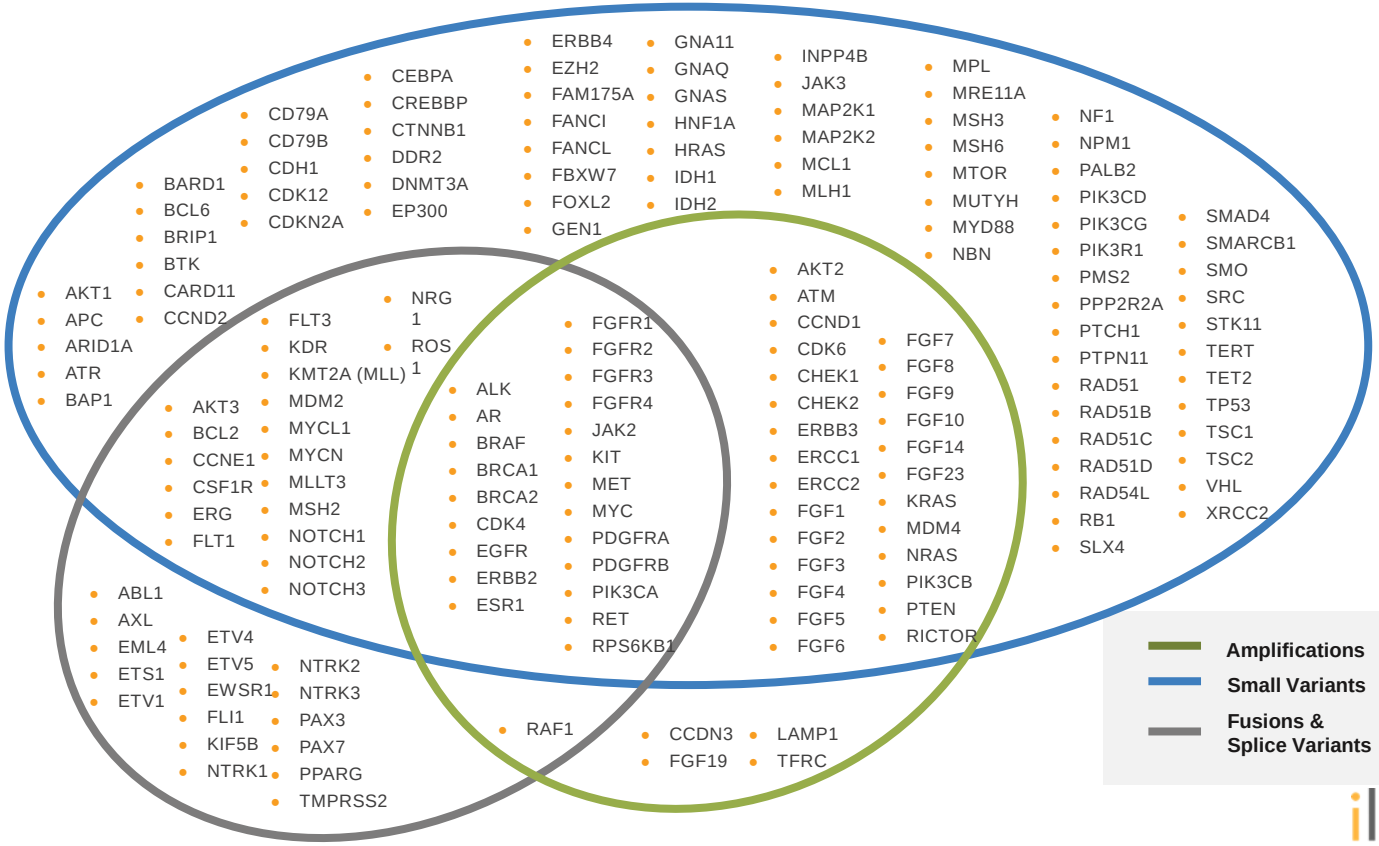
TruSight Tumor 170 Content

Gene List and Variant Classification



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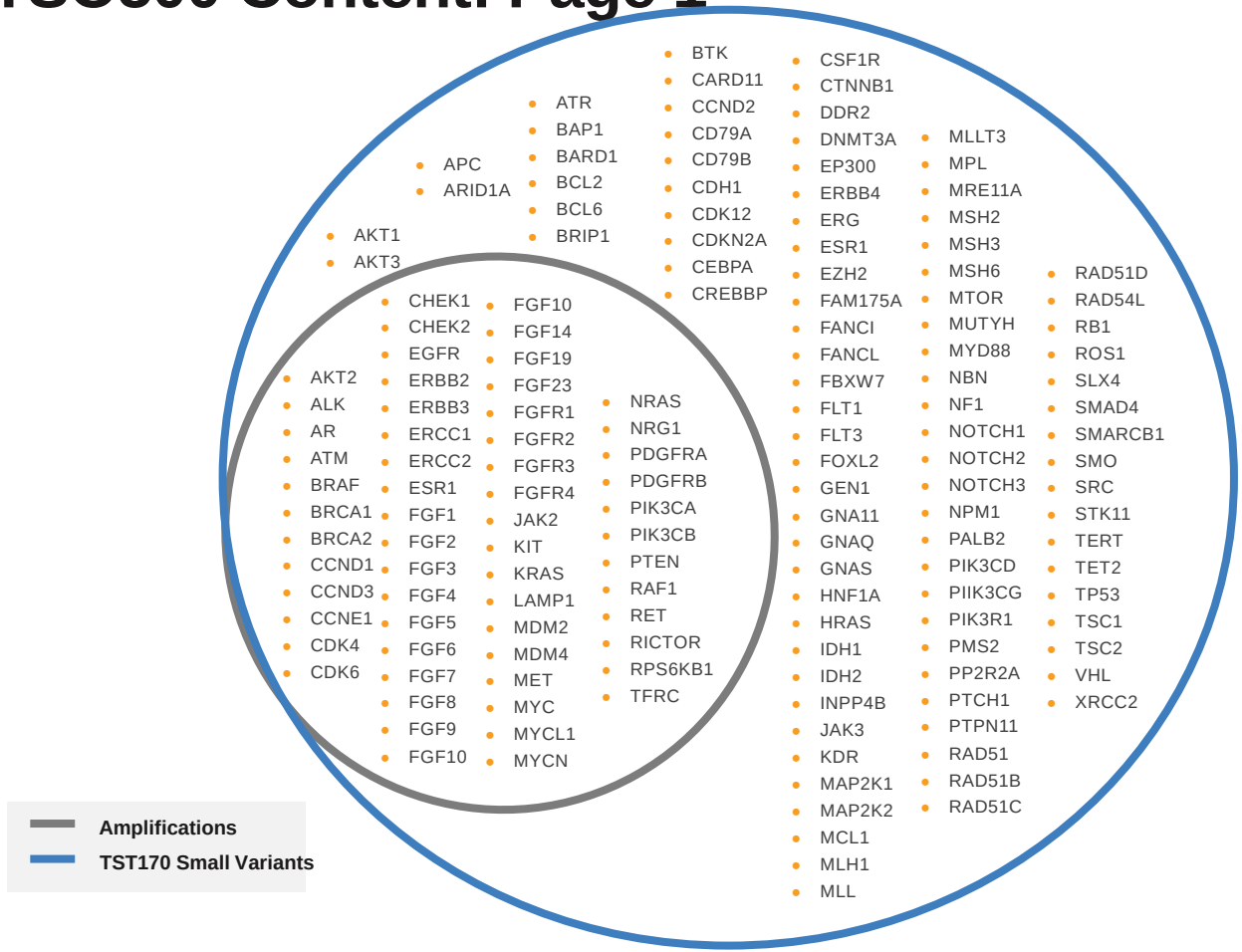
TSO500 Content: Page 1



TSO500 Content: Page 2

Small Variants
Fusions

- ABL1
- ABL2
- ACVR1
- ACVR1B
- ALOX12B
- ANKRD11
- ANKRD26
- ARAF
- ARFRP1
- ARID1B
- ARID2
- ARID5B
- ASXL1
- ASXL2
- ATRX
- AURKA
- AURKB
- AXIN1
- AXIN2
- AXL
- B2M
- BBC3
- BCL10
- BCL2L1
- BCL2L11
- BCL2L2
- BCOR
- BCORL1
- BCR
- BIRC3
- BLM
- BMPR1A
- BRD4
- BTG1
- C11orf30
- CALR
- CASP8
- CBFB
- CBL
- CD274
- CD276
- CD74
- CDC73
- CDK8
- CDKN1A
- CDKN1B
- CDKN2B
- CDKN2C
- CENPA
- CHD2
- CHD4
- CIC
- CRKL
- CRLF2
- CSF3R
- CSNK1A1
- CTCF
- CTLA4
- CTNNA1
- CUL3
- CUX1
- CXCR4
- CYLD
- DAXX
- DCUN1D1
- DDX41
- DHX15
- DICER1
- DIS3
- DNAJB1
- DNMT1
- DNMT3B
- DOT1L
- E2F3
- EED
- EGFL7
- EIF1AX
- EIF4A2
- EIF4E
- EML4
- EPCAM
- EPHA3
- EPHA5
- EPHA7
- EPHB1
- ERCC3
- ERCC4
- ERCC5
- ERRFI1
- ETS1
- ETV1
- ETV4
- ETV5
- ETV6
- EWSR1
- FAM123B
- FAM46C
- FANCA
- FANCC
- FANCD2
- FANCE
- FANCF
- FANCG
- FAS
- FAT1
- FGF19
- FH
- FLCN
- FLI1
- FLT4
- FOXA1
- FOXO1
- FOXP1
- FRS2
- FUBP1
- FYN
- GABRA6
- GATA1
- GATA2
- GATA3
- GATA4
- GATA6
- GID4
- GLI1
- GNA13
- GPR124
- GPS2
- GREM1
- GRIN2A
- GRM3
- GSK3B
- H3F3A
- H3F3B
- H3F3C
- HGF
- HIST1H1C
- HIST1H2BD
- HIST1H3A
- HIST1H3B
- HIST1H3C
- HIST1H3D
- HIST1H3E
- HIST1H3F
- HIST1H3G
- HIST1H3H
- HIST1H3I
- HIST1H3J
- HIST2H3A
- HIST2H3C
- HIST2H3D
- HIST3H3
- HLA-A
- HLA-B
- HLA-C
- HNRNPK
- HOXB13
- HSD3B1
- HSP90AA1
- ICOSLG
- ID3
- IFNGR1
- IGF1
- IGF1R
- IGF2
- IKBKE
- IKZF1
- IL10
- IL7R
- INHA
- INHBA
- INPP4A
- INSR
- IRF2
- IRF4
- IRS1
- IRS2
- JAK1
- JUN
- KAT6A
- KDM5A
- KDM5C
- KDM6A
- KEAP1
- KEL
- KIF5B
- KLF4
- KLHL6
- KMT2B
- KMT2C
- KMT2D
- LAMP1
- LATS1
- LATS2
- LMO1
- LRP1B
- LYN
- LZTR1
- MAGI2
- MALT1
- MAP2K4
- MAP3K1
- MAP3K13
- MAP3K14
- MAP3K4
- MAPK1
- MAPK3
- MAX
- MDC1
- MED12
- MEF2B
- MEN1
- MGA
- MITF
- MST1
- MST1R
- MYB
- MYOD1
- NAB2
- NCOA3
- NCOR1
- NEGR1
- NF2
- NFE2L2
- NFKBIA
- NKX2-1
- NKX3-1
- NOTCH4
- NSD1
- NTRK1
- NTRK2
- NTRK3
- NUP93
- NUTM1
- PAK1
- PAK3
- PAK7
- PARK2
- PARP1
- PAX3
- PAX5
- PAX7
- PAX8
- PBRM1
- PDCD1
- PDCD1LG2
- PDK1
- PDPK1
- PGR
- PHF6
- PHOX2B
- PIK3C2B
- PIK3C2G
- PIK3C3
- PIK3R2
- PIK3R3
- PIM1
- PLCG2
- PLK2
- PMAIP1
- PMS1
- PNRC1
- POLD1
- POLE
- PPARG
- PPM1D
- PPP2R1A
- PPP6C
- PRDM1
- PREX2
- PRKAR1A
- PRKCI
- PRKDC
- PRSS8
- PTPRD
- PTPRS
- PTPRT
- QKI
- RAB35
- RAC1
- RAD21
- RAD50
- RAD52
- RAF1
- RANBP2
- RARA
- RASA1
- RBM10
- RECQL4
- REL
- RFWD2
- RHEB
- RHOA
- RIT1
- RNF43
- RPS6KA4
- RPS6KB2
- RPTOR
- RUNX1
- RUNX1T1
- RYBP
- SDHA
- SDHAF2
- SDHB
- SDHC
- SDHD
- SETBP1
- SETD2
- SF3B1
- SH2B3
- SH2D1A
- SHQ1
- SLIT2
- SMAD2
- SMAD3
- SMARCA4
- SMARCD1
- SMC1A
- SMC3
- SNCAIP
- SOCS1
- SOX10
- SOX17
- SOX2
- SOX9
- SPEN
- SPOP
- SPTA1
- SRSF2
- STAG1
- STAG2
- STAT3
- STAT4
- STAT5A
- STAT5B
- STK40
- SUFU
- SUZ12
- SYK
- TAF1
- TBX3
- TCEB1
- TCF3
- TCF7L2
- TERC
- TET1
- TFE3
- TFRC
- TGFBR1
- TGFBR2
- TMEM127
- TMPRSS2
- TNFAIP3
- TNFRSF14
- TOP1
- TOP2A
- TP63
- TRAF2
- TRAF7
- TSHR
- U2AF1
- VEGFA
- VTCN1
- WISP3
- WT1
- XIAP
- XPO1
- YAP1
- YES1
- ZBTB2
- ZBTB7A
- ZFHX3
- ZNF217
- ZNF703
- ZRSR2

FUSIONS + SPLICE VARIANTS

- ABL1
- AKT3
- ALK
- AR
- AXL
- BCL2
- BRAF
- BRCA1
- BRCA2
- CDK4
- CSF1R
- EGFR
- EML4
- ERBB2
- ERG
- ESR1
- ETS1
- ETV1
- ETV4
- ETV5
- EWSR1
- FGFR1
- FGFR2
- FGFR3
- FGFR4
- FLI1
- FLT1
- FLT3
- JAK2
- KDR
- KIF5B
- KIT
- KMT2A (MLL)
- MET
- MLLT3
- MSH2
- MYC
- NOTCH1
- NOTCH2
- NOTCH3
- NRG1
- NTRK1
- NTRK2
- NTRK3
- PAX3
- PAX7
- PDGFRA
- PDGFRB
- PIK3CA
- PPARG
- RAF1
- RET
- ROS1
- RPS6KB1
- TMPRSS2

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