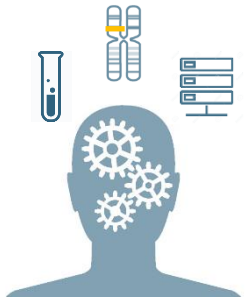
Comprehensive Detection of Genetic Alterations in Circulation of Cancer Patients

Theresa Zhang, PhD
Vice President
Translational Research and Diagnostics

Workshop on Circulating Tumor DNA assays in Clinical Cancer Research, Sept 29-30, 2016

Empowering the fight against cancer

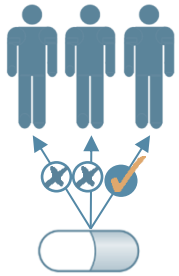


BIOMARKER DISCOVERY

Custom NGS Research & Development

Identify biomarker targets for drug discovery pipeline

Larger, multi-analyte panels in tissue and plasma including whole exome sequencing and neoantigen prediction (RUO)

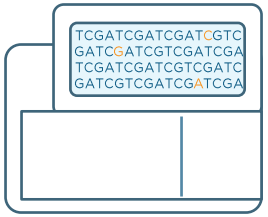


HYPOTHESIS TESTING

Clinical Biomarker & Clinical Trial Services

Prospectively stratify patients for clinical trials

Robust prototype cost and risk profile at early stage (CLIA/IUO)



GLOBAL PATIENT ACCESS

IVD Product Development & Commercialization

Inform patient treatment selection

Decentralized oncology testing system, sequencer agnostic using pre-existing installed based (PROGENEUS)

End to end solution & decentralized NGS diagnostics

...to reach CROs and molecular labs GLOBALLY

End-to-End Components

Co-developed assays

- Platform will run multiple assays
- Tissue or plasma
- Hybrid capture or amplicon PCR based

Reagent kits

All the key reagents and protocols required to prepare genomic libraries for sequencing

On-site server

PGDx supplied server houses bioinformatics pipeline to ensure ownership and security of valuable sample data

Flexible data formats

Sequencing data and analysis integration for co-developed reporting format

Web-based portal

Training and other support tools accessible from a PGDx web-based portal tailored to client laboratory set-up

On-site training

Hands-on training conducted on-site or at PGDx laboratory to build proficiency with methods

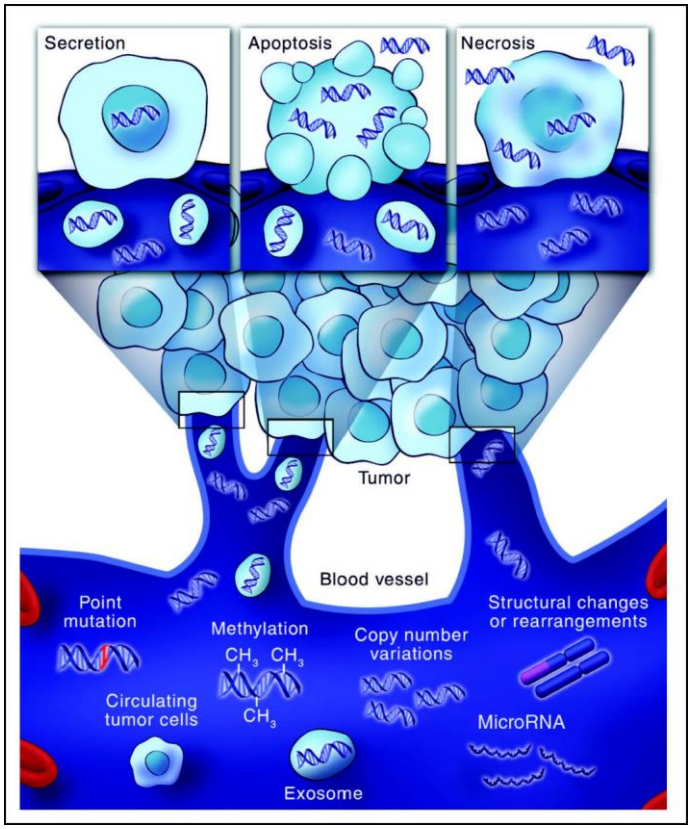
Validation support

Samples provided for well characterized mutations in both FFPE and ctDNA formats

Technical Support

Around the clock expert support for adopting CROs and molecular labs

Circulating cell-free tumor DNA (ctDNA)



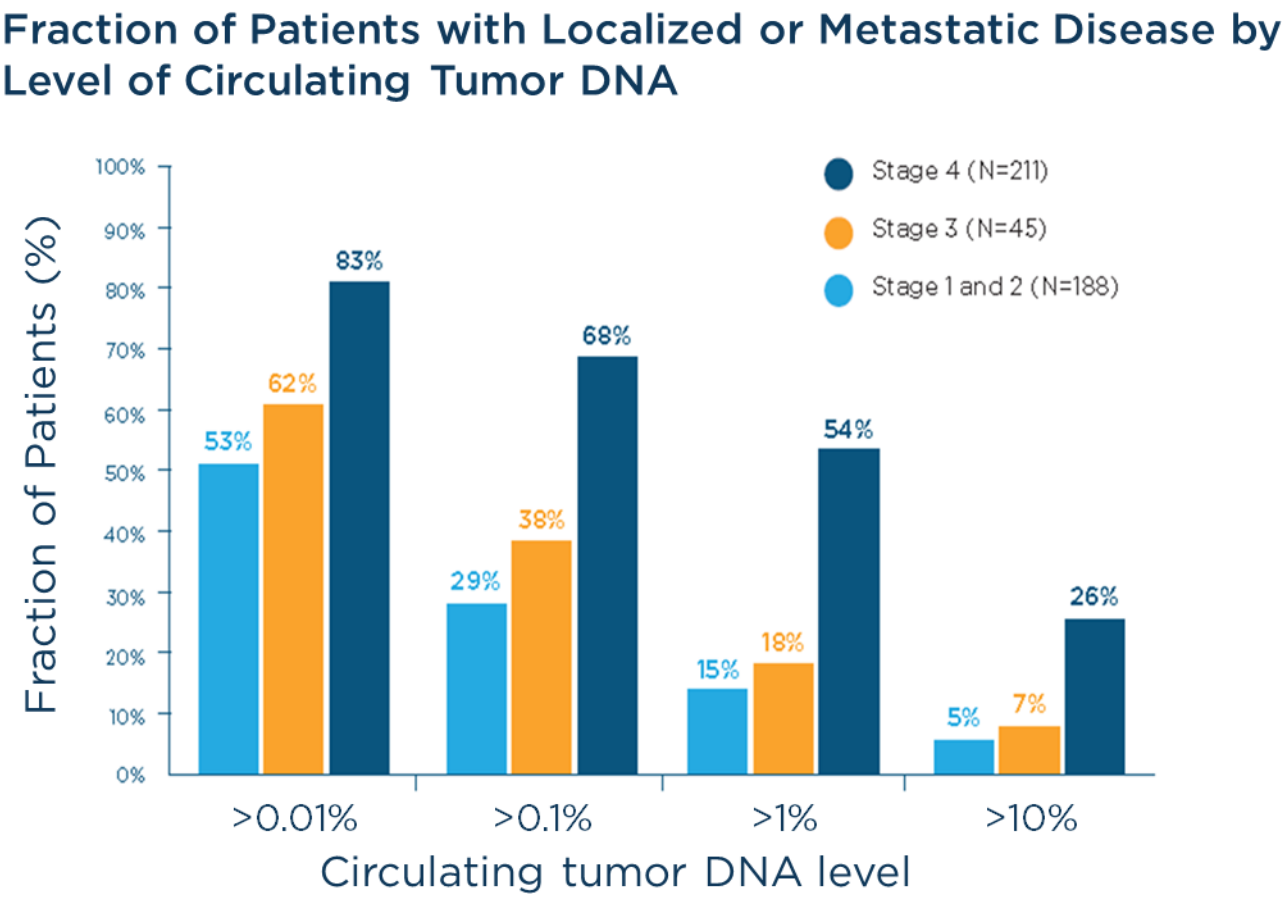
- 180-200bp DNA fragments
- Half Life: 2 hours – Real Time
- Specific to Tumor
- Multi-clones & Multi-lesions

ctDNA Challenge
Very low amounts of ctDNA in the sea of wild- type DNA

Diaz et al., J Clin Oncol. 2014 Feb

Desired lower limit of detection of ctDNA assay

PGD

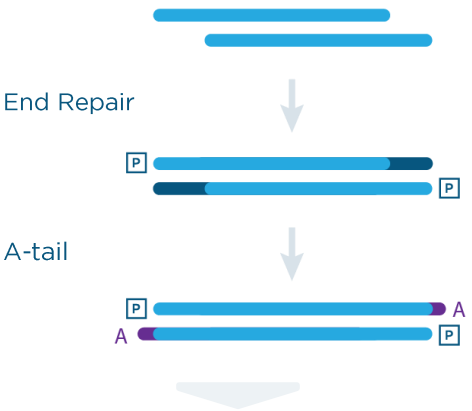


Bettegowda et al. Sci Transl Med. 2014 Feb 19;6(224):224ra24.

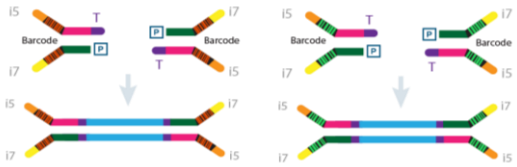
cfDNA Work Flow

Sample Preparation

Cell-Free DNA Derived from Plasma

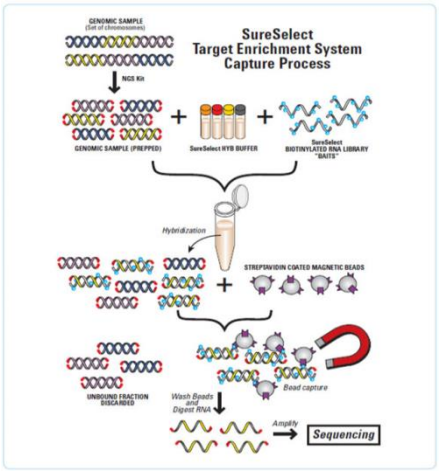


Adaptor Ligation (Molecular Barcoding)



Sequencing

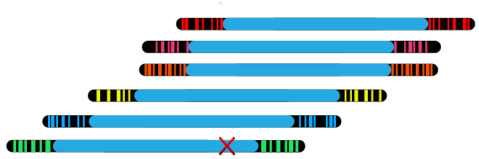
Target Enrichment (Optimized Probe Design)



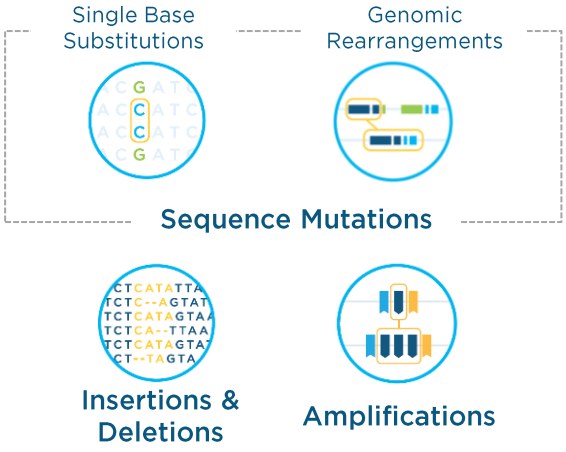
Sequencing

Analysis

Error Suppression



Analysis



Genes evaluated in PlasmaSELECT™ 64

PGD

58
Genes Analyzed
for Sequence Mutations

19
Genes Analyzed
for Amplifications

17
Genes Analyzed
for Rearrangements

*Full coding and specific exon analysis in 58 well characterized cancer genes, as well as †amplification analysis for 19 genes

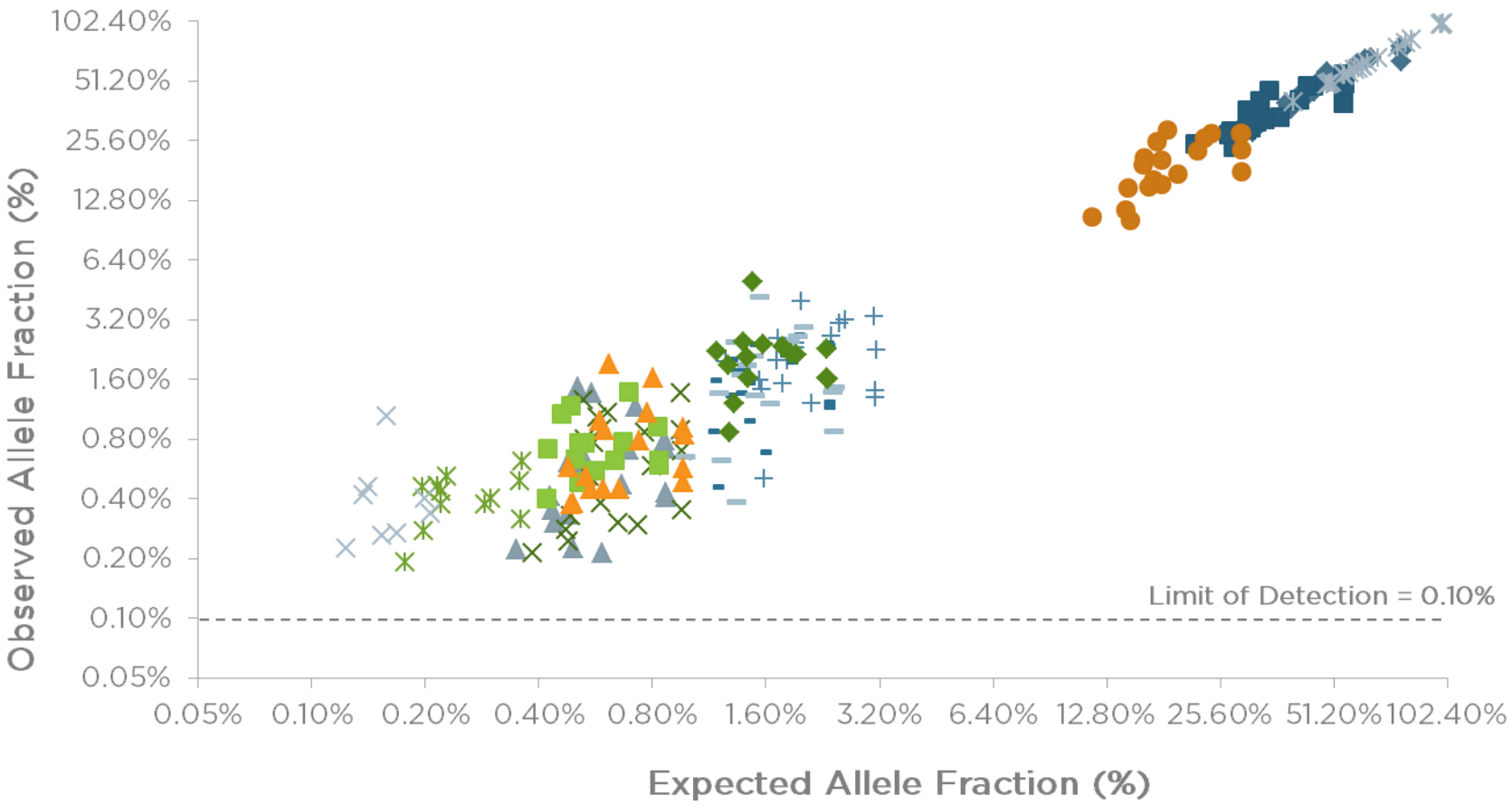
AKT1	CDK4*†	FLT3	MYCN†	PTCH1
ALK*†	CDK6*†	GNAS	NPM1	PTEN
AR*†	CDKN2A	HRAS	NRAS	RB1
ATM	CTNNB1	IDH1	NTRK1	RET*
BRAF*	DNMT3A	IDH2	NTRK2	RNF43
BRCA1	EGFR*†	JAK2	NTRK3	ROS1†
BRCA2	ERBB2*†	KIT*†	PALB2	TERT
BRCA2*	ESR1	KRAS*	PIK3CA†	TP53*
CCND1†	EZH2	MAP2K1	PIK3CB	TSC1*
CCND2†	FGFR1†	MET†	PIK3R1	TSC2
CCND3†	FGFR2†	MTOR	POLD1	VHL
CD274*†	FGFR3†	MYC†	POLE	

Rearrangement analysis for selected regions of 17 well-characterized cancer genes.

ALK	ETV6	MYC	PDGFRB	ROS1
BCR	FGFR1	NTRK1	RAF1	
BRAF	FGFR2	NTRK2	RARA	
EGFR	FGFR3	PDGFRA	RET	

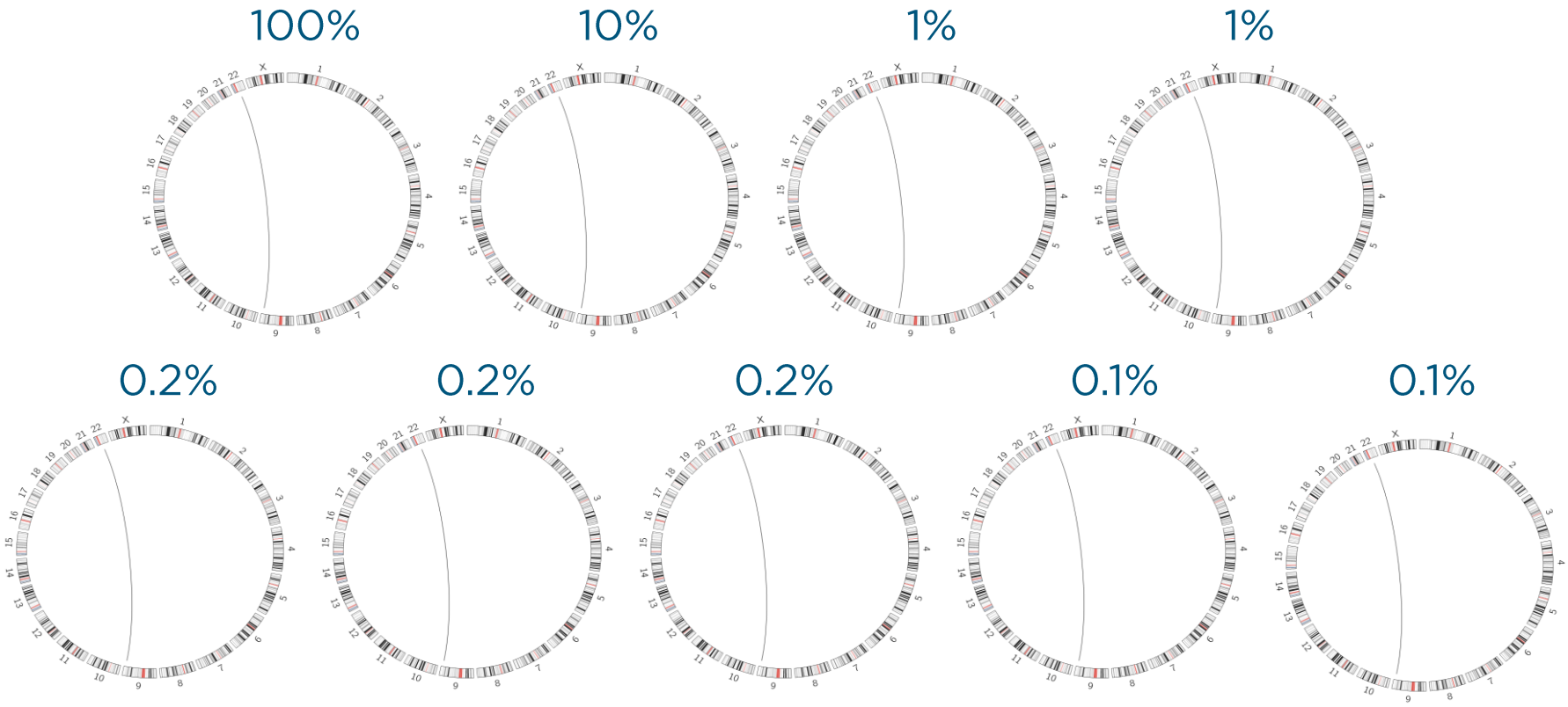
Micro-Satellite Instability (MSI) in plasma coming soon

Detection of Sequence Mutations at Varying Levels of Tumor Contribution Using PlasmaSelect In-Solution Capture Technology



A breast cancer tumor was diluted with matched wild-type DNA to 0.10%, 0.20%, 0.50%, 1.0%, 10%, 25%, 50% and 100% tumor-derived DNA (0.10%, 0.20%, and 0.50% were evaluated in triplicate). For each sequence mutation, the observed allele fraction for the mutations identified in each case are plotted against the expected mutation allele fraction for that case.

Identification of translocations at varying levels of tumor contribution



The chronic myeloid leukemia cell line 562 was titrated with wild-type DNA to 0.10%, 0.20%, 0.50%, 1.0%, 10%, 25%, 50% and 100% tumor-derived DNA (0.10%, 0.20%, and 0.50% were evaluated in triplicate). Similar results were obtained for H2228 containing the *EML4-ALK* translocation and HCC78 containing the *SLC34A2-ROS1* translocation.

Performance Metrics (minimum sample input of 25ng)

PGD

Performance Specification	Mutant Allele Fraction	Sensitivity	Specificity
Sequence Mutations (SBS/Indel)	≥0.50%	99.4%	>99.999%*
Rearrangements	≥0.50%	94.4%	>99%
Amplifications (≥4-fold)	≥20%	97.2%	>99%
Amplifications (≥4-fold)	<20%	*varies depending on level of amplification and tumor content*	

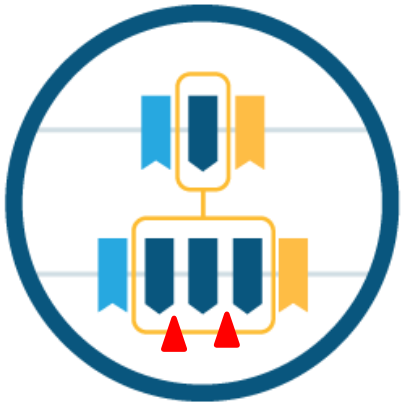
*Per-base specificity provided for sequence mutation analyses

Can we improve the performance of amplification detection?

Ultrasensitive identification of MET amplification in plasma

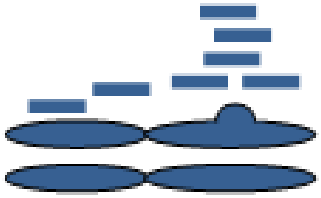
Approach #1:

Detect the novel genomic junctions created by amplifications and deletions



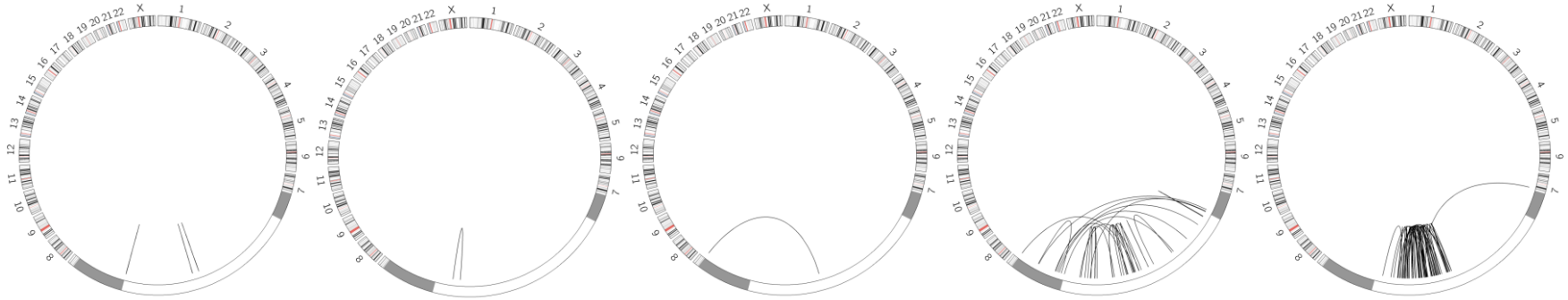
Approach #2:

Compute fold change of read count between MET and reference genomic regions

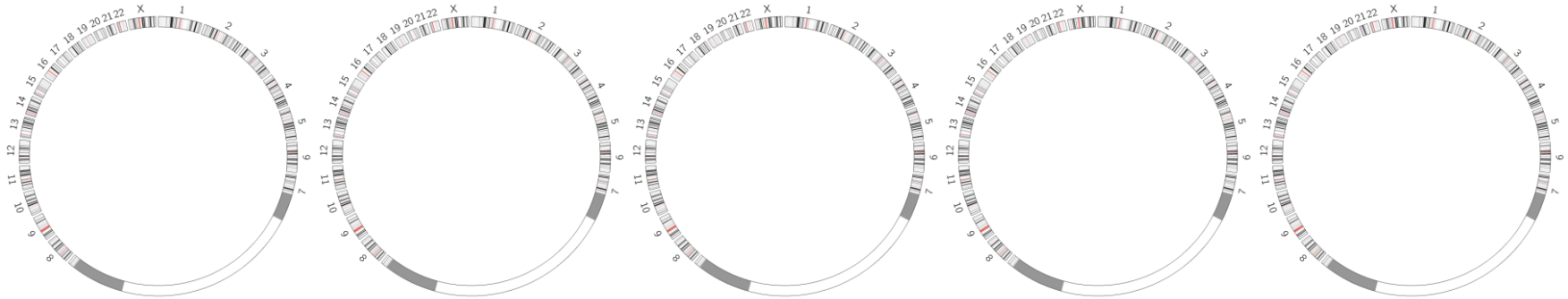


Leary, Sausen, and Kinde et al. Sci Transl Med. 2012 Nov 28;4(162):162ra154.

Analyses of Cell-Free DNA from Patients with *MET* Amplification

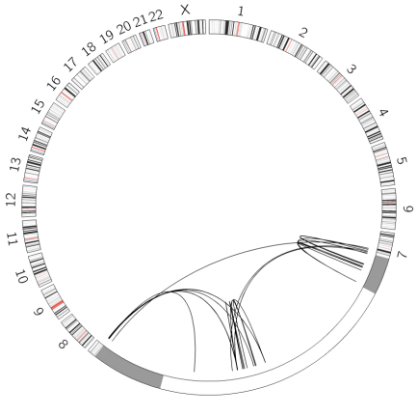


Analyses of Cell-Free DNA from Patients without *MET* Amplification

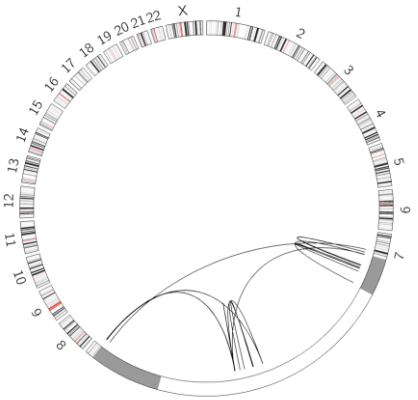


Detection of MET amplification at varying levels of tumor contribution

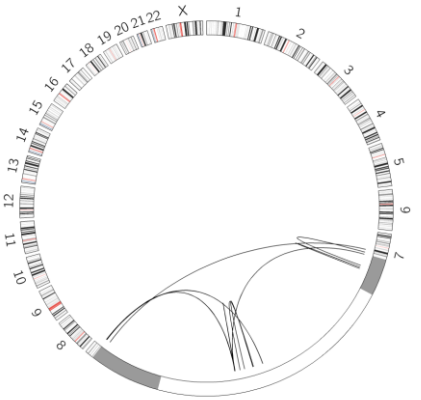
100%
(6,305x)



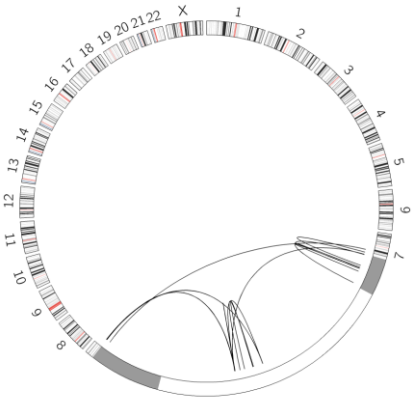
10% Replicate
(3,781x)



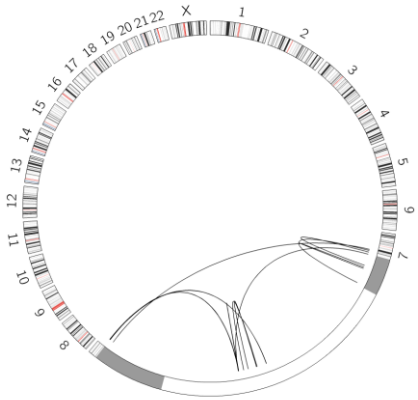
10%
Replicate
(2,376x)



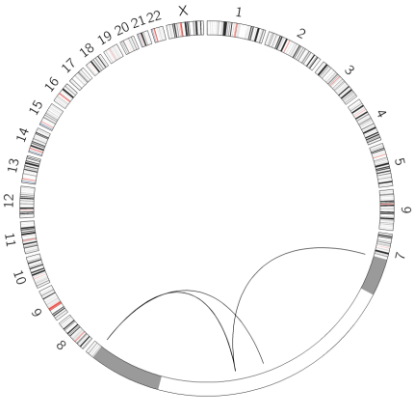
10% Replicate
(2,922x)



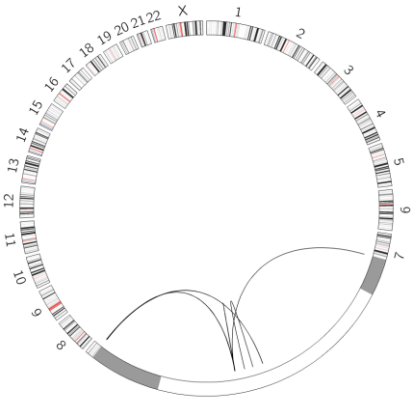
5%
(2,573x)



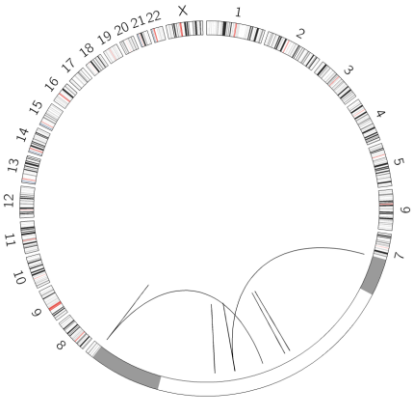
2%
(2,832x)



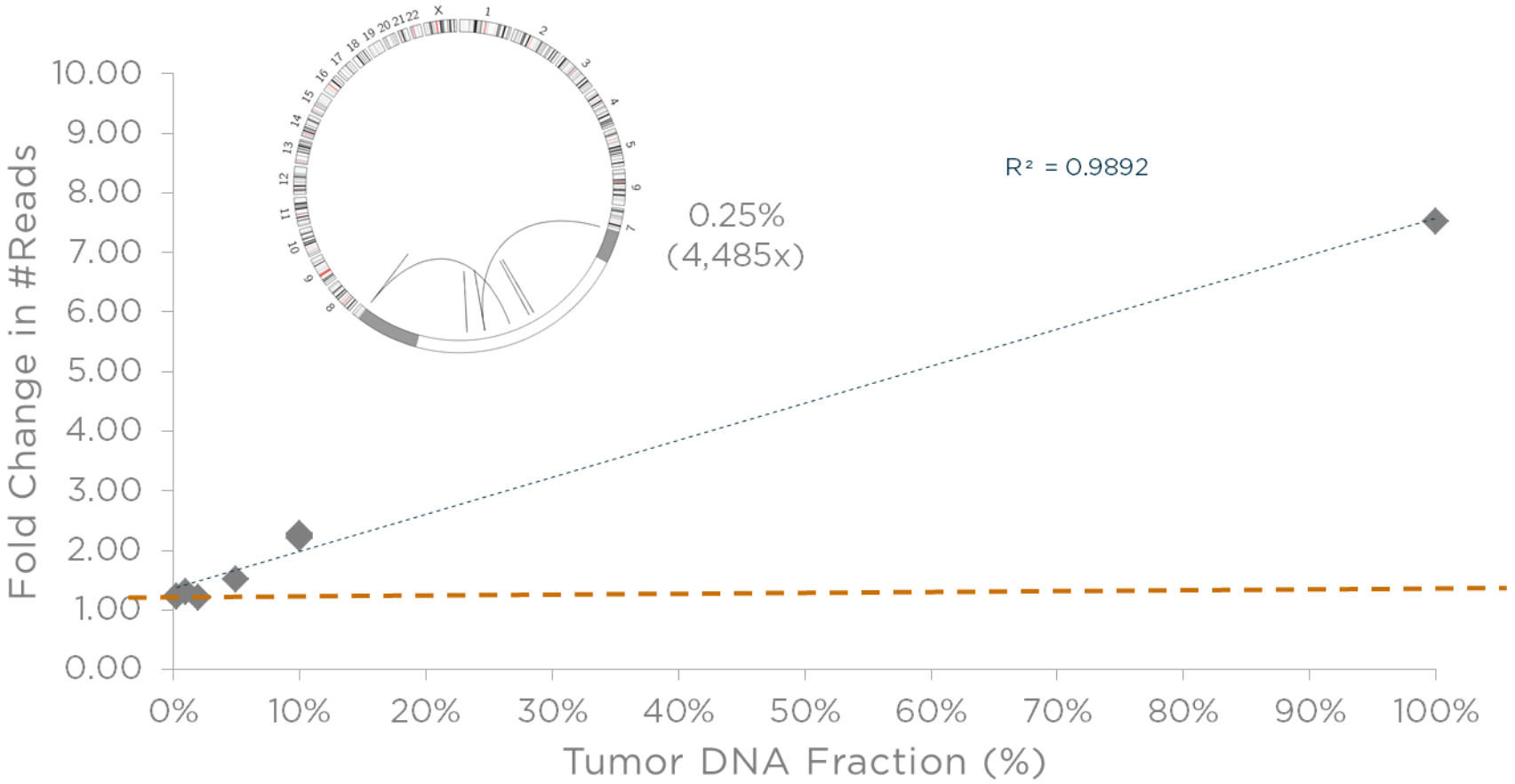
1%
(3,457x)



0.25%
(4,485x)



Evaluation of MET amplification in dilution series (fold change of read count)



Performance of METdetect™

STRUCTURAL ALTERATIONS ASSAY PERFORMANCE				
	Mutant Allele Fraction	Assay Sensitivity	Assay Specificity	Comment
Amplifications	≥0.50%	>95%	>99%	>15 copies
Translocations	≥0.50%	>95%	>99%	

Challenges for validating tests to detect rare structural alterations in plasma

- Lack of positive controls
  - Whole genome sequencing required to identify breakpoints
- Lack of orthogonal test for structural alterations in plasma
- Undefined gold standard tests even for tumor

PGD
Personal Genome Diagnostics

Thank You

Theresa Zhang, PhD
Vice President, Translational Research and Diagnostics
tzhang@personalgenome.com