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Next-generation sequencing of FFPE breast cancers demonstrates high concordance with FISH in calling *HER2* amplifications and commonly identifies additional clinically relevant genomic alterations

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Background

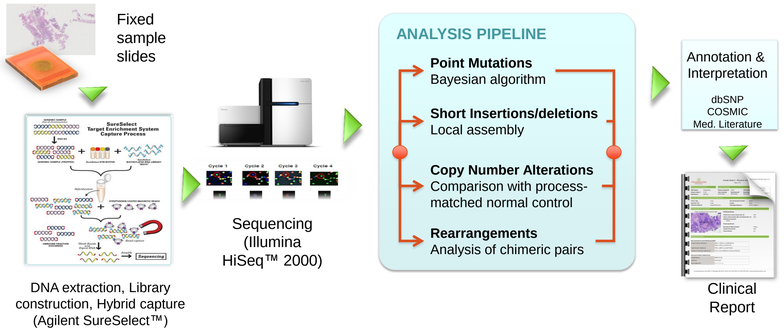
As therapies targeting genomic alterations become increasingly available, next-generation sequencing (NGS) is an attractive option in tumor types where mutational status may drive treatment choice. In addition to its ability to identify base substitutions, insertions and deletions across entire exons of hundreds of cancer-related genes, NGS can detect relevant copy number changes such as amplification of *HER2* in breast tumors. However, for NGS to be clinically applicable, it must reliably analyze FFPE tumor samples and show concordance with the best current diagnostic methods.

Methods

To confirm a clinical role for NGS in detecting copy number alterations, we identified 35 FFPE invasive breast carcinomas previously tested for HER2 status by FISH, including 15 *HER2* positives (≥7 copies) and 20 *HER2* negatives (<4 copies) and sequenced 3,230 exons of 182 cancer genes including *HER2*, in a CLIA certified lab (Foundation Medicine). Average coverage depth of >900X uniquely-mapping reads was obtained. Sequence data were analyzed for *HER2* copy number (blinded to FISH results) based on a statistical model using allele frequencies and coverage depth of *HER2* exons versus a process-matched normal control, classifying cases as *HER2* positive (≥6 average copies), *HER2* negative (≤3 copies), intermediate (4-5 copies) or unknown (<20% tumor purity). The data were also analyzed for additional clinically relevant genomic alterations.

NGS-based Genomic Profiling

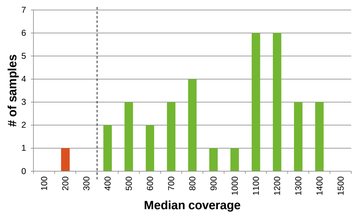
Detection of all genomic alteration classes in 182 cancer-related genes



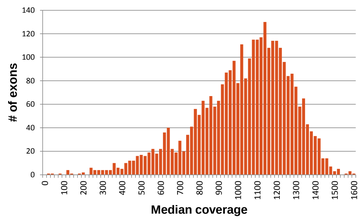
Results

Deep, even coverage is obtained from low input DNA from clinical FFPE samples

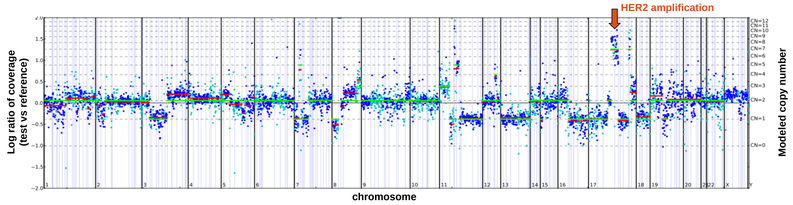
Across all samples in study:



Across all exons in a sample:



HER2 copy number status was determined by modeling genomic aneuploidy



High concordance was observed between *HER2* copy number status determined by FISH and NGS

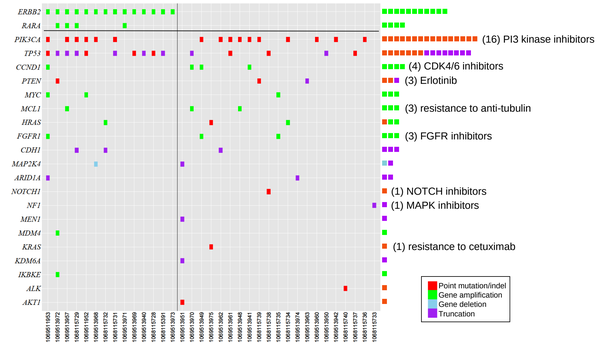
	FISH positive	FISH negative	Total
NGS positive	**13**	0	13
NGS intermediate	1	0	1
NGS negative	**1***	**16**	17
NGS unknown	0	4	4
Total	15	20	35

* NGS detected no evidence of a *HER2* amplification in the single discordant case (diploid)

NGS performance relative to FISH as a gold standard:

- **Accuracy**: 97% (29/30, 95% CI 83-99%)
- **Sensitivity**: 93% (13/14, 95% CI 69-99%)
- **Specificity**: 100% (16/16, 95% CI 81-100%)

Additional genomic alterations that predict sensitivity or resistance to approved or experimental targeted therapies were found in 69% of cases.



Further concordance studies demonstrate >95% accuracy in calling copy-number changes by NGS vs other methods

Tissue (ref)	Gene tested	CNA type	# of samples*	Confirmatory method (CM)	CM+, NGS+	CM- , NGS-	# discordant calls	Accuracy
Prostate (1)	AR	Amp	25	FISH	5,5	20,20	0	**100%**
Prostate (1)	PTEN	Del	22	FISH	6,7	16,15	1	**95%**
Breast (2)	HER2	Amp	42	FISH	6,6	36,36	0	**100%**
H&N (3)	CCND1	Amp	34	IHC†	8,9	26,25	1	**97%**
H&N (3)	PTEN	Del	32^	IHC†	3,3	29,29	0	**100%**

* Number of samples with copy number calls by both NGS and FISH/IHC; † Strong expression for amplification or loss of staining for homozygous deletion; ^ Excludes two cases with PTEN mutation by NGS and loss of staining.

References:

1. H. Beltran, R. Yelensky et al, Eur Urol (2012)
2. Collaboration with L. Pusztai, Yale, *in preparation*
3. Collaboration with C. Boshoff, UCL, *under review*

Conclusions

- *HER2* status determination by NGS from routine FFPE breast tumor demonstrates 97% accuracy relative to *HER2* status determined by FISH
- NGS uncovers actionable genomic alterations that could impact disease management in 69% of invasive breast cancer cases
- NGS demonstrates >95% concordance in calling copy-number changes in additional genes vs FISH/IHC, with discordances including both positive and negative NGS calls.

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