

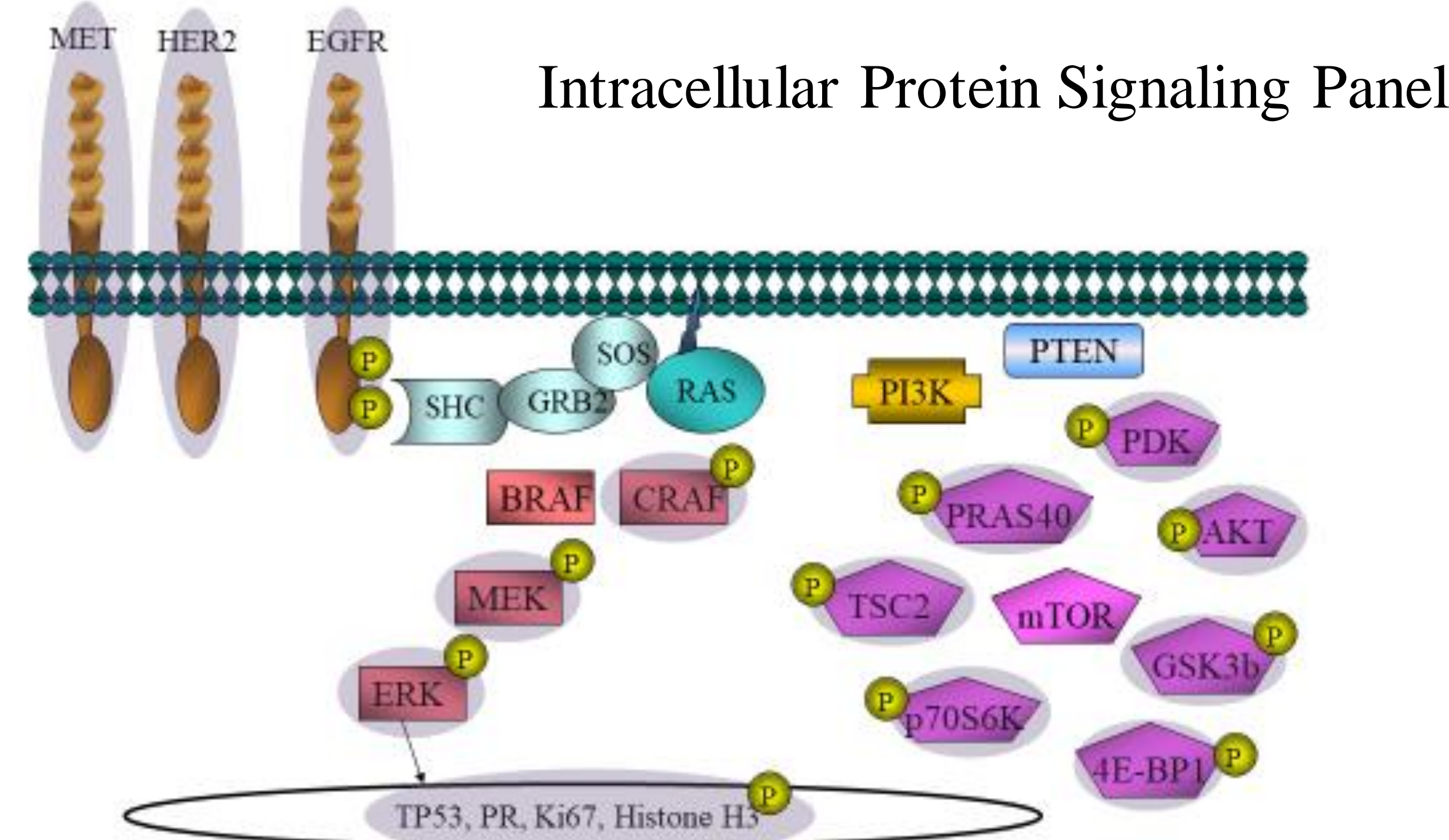
Analytical Validation of a Quantitative Intracellular Protein Signaling Panel for the Analysis of FFPE Breast Cancer Biopsies

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Introduction

Measuring protein expression by immunohistochemistry (IHC) in formalin-fixed, paraffin-embedded (FFPE) tissue is routine in clinical labs. However, current assays are limited to just 1 to 3 biomarkers per slide and histochemical signals are only semi-quantitative. Recently, a multiplexed panel of oligonucleotide-tagged antibodies has been developed (Nanostring 3D Vantage Solid Tumor Panel), comprised of 27 antibodies, including 13 phosphorylated protein targets, specifically designed to interrogate the MAPK and PI3K/mTOR signaling pathways (ref.1). The panel uses the Nanostring nCounter system to quantitate oligonucleotides that are released by UV light after antibody binding. We undertook a validation of this panel with the goal of creating a new clinical assay for core biopsies of solid tumors.



Methods

Four micron sections of formalin-fixed paraffin-embedded (FFPE) cancer cell lines (controls) or 18-21 gauge cores of solid tumors (fixed within 3 minutes of biopsy) were subjected to citrate-based antigen retrieval and incubated overnight with the cocktail of oligo-tagged antibodies. After washing, the oligo-tags were released by UV light and quantitated on the Nanostring nCounter system. A set of 6 FFPE cancer cell lines were selected as positive controls and included on every run to assess antibody performance and support inter-run normalizations. 28 metastatic breast cancer core biopsies were analyzed to serve as a comparative cohort.



Results

- In FFPE cancer cell lines detection of progesterone receptor (high in MCF-7, BT474, T47D) and HER2 (high in HCC1954, SKBr3, BT474) matched reverse-phase protein array data. Pre-incubation of MDA-MB-468 with EGF led to expected increases in p-EGFR, p-MEK1/2, p-ERK1/2, p-AKT and p-S6.
- Among 10 rapidly-fixed FFPE solid tumors, signals for EFGR, p-EGFR, HER2, TP53, TSC2 and p-S6 were all consistent with known genomic alterations.
- There was good correlation with IHC results for PR and HER2 on sections of FFPE breast cancer core biopsies (Fig.1).
- Slide pre-treatment with lambda phosphatase eliminated phospho-protein signals (Fig.2).
- A bioinformatics strategy was developed to allow comparison of samples between runs (Fig 3).
- Analysis of 6 FFPE cancer cell lines selected as positive controls showed excellent inter-run reproducibility ($r \geq 0.92$).
- Data from 28 metastatic breast carcinoma core biopsies were generated to serve as a reference cohort (Fig.4).

Figure 1 - Correlation With IHC

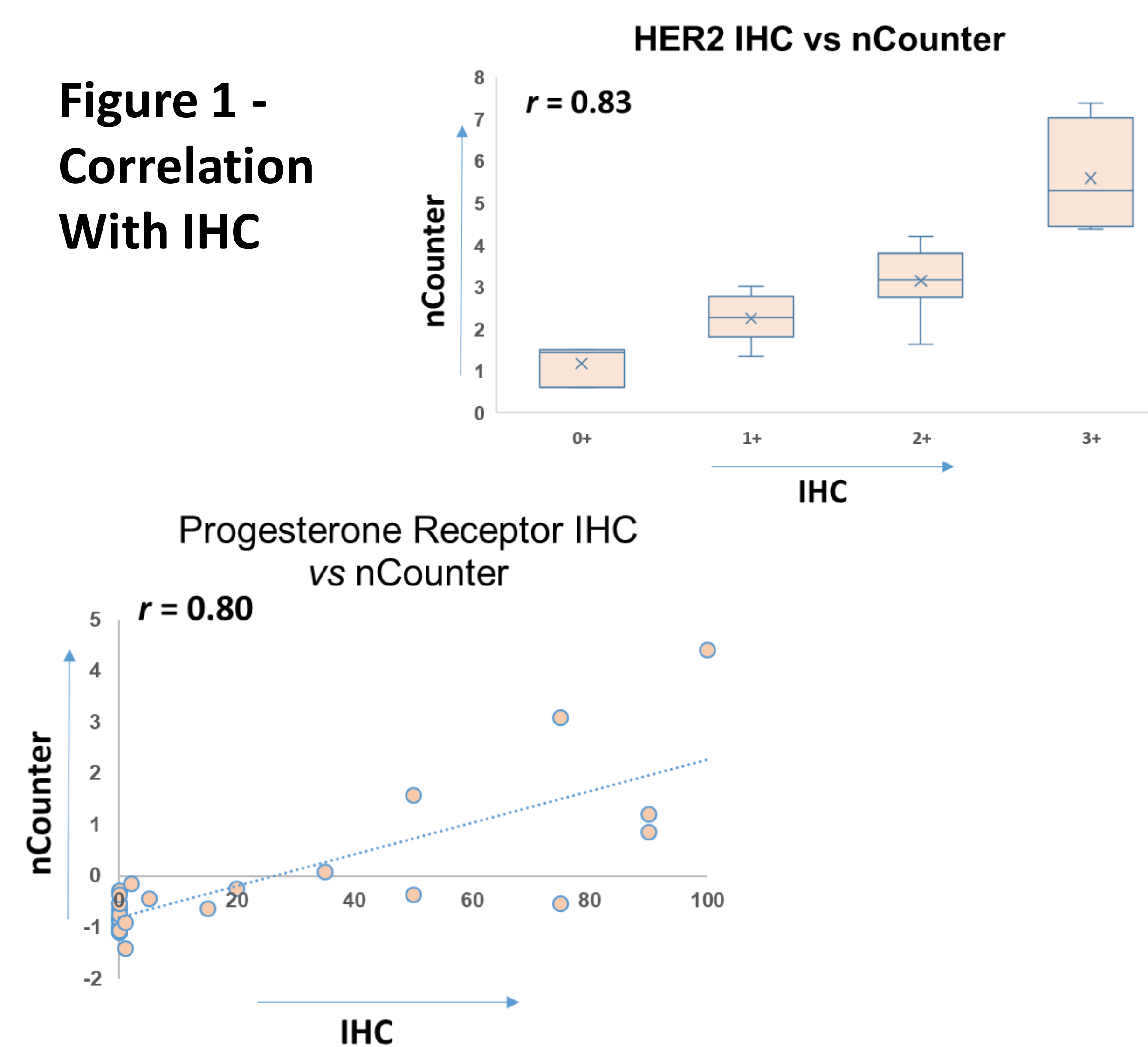


Figure 2 – Specificity of Phosphoprotein Abs

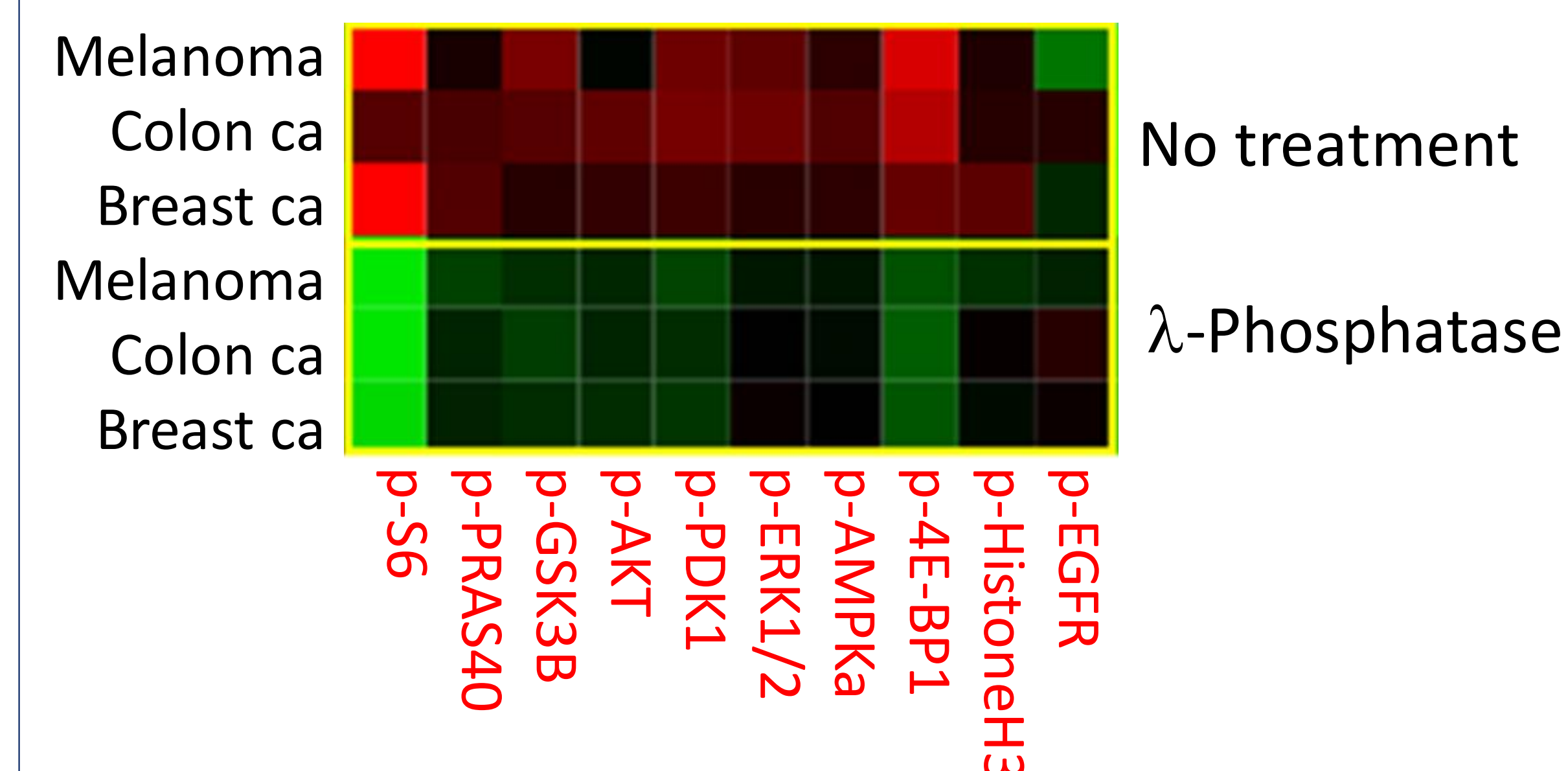


Figure 3 – Bioinformatics Steps

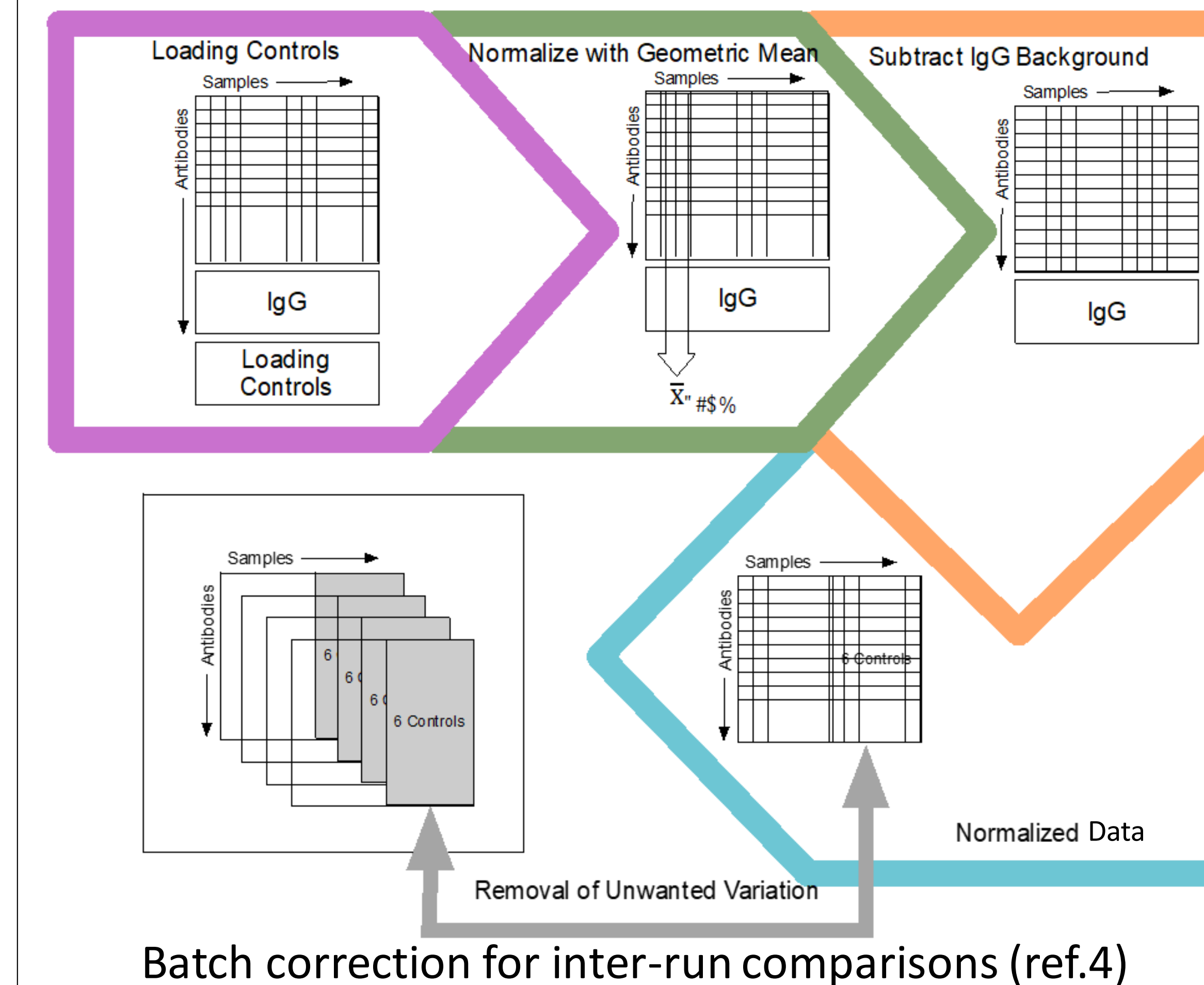
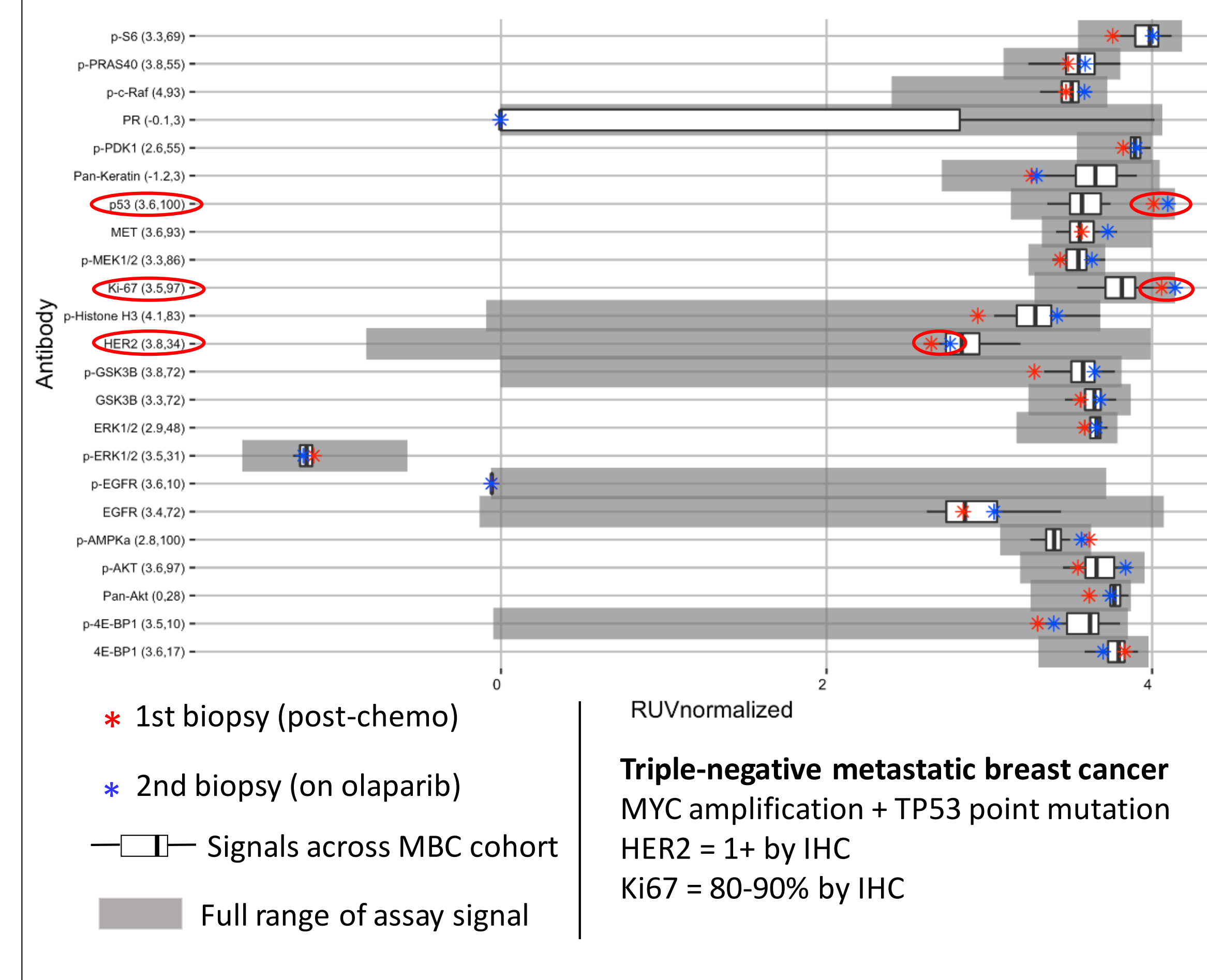


Figure 4 – Comparison of 2 serial biopsies to cohort of 28 metastatic breast cancers



Conclusions

The 3D Vantage Solid Tumor Panel provides a robust, quantitative approach to measuring 27 cell signaling biomarkers from a single section of FFPE tissue in <48 hours. The panel is useful for investigating treatment-induced changes in tumor cell signaling, but there are some limitations: 1) specimens must be fixed within minutes of biopsy to avoid protein de-phosphorylation (ref.2); 2) the assay requires a minimum of 40% tumor fraction; 3) any normal tissue should be excluded. The excellent performance of this slide-based approach suggests that it can be adapted to the new GeoMx Digital Spatial Pathology system (Nanostring), which uses micro-mirrors to focus UV light and release tags only from selected cells of interest. With the appropriate controls and bioinformatics steps, quantitative protein profiling using oligo-tagged antibodies holds great promise for future assays.

References

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