

MammaPrint 70-gene test using targeted RNA sequencing

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BACKGROUND

MammaPrint 70-gene test ^[1]

- In vitro diagnostic microarray-based test to assess a patient's risk for distinct metastasis within 5 years
- Low Risk and High Risk results with no intermediate or indeterminate category
- FDA 510(k) clearances and CE marking for fresh and FFPE tissues on microarray

Blueprint 80-gene test ^[2]

- In vitro diagnostic microarray-based test to assess breast cancer molecular subtypes (Luminal-type, HER2-type, Basal-type)

Next Generation RNA-Seq technology ^[3,4]

- Is becoming a standard method for transcriptome analysis
- Low background signal with a large dynamic range of expression levels
- Multiple ongoing efforts to establish benchmark standards for technical and analytical best practices
- Potential to revolutionize clinical testing

Advantages of MammaPrint 70-gene test on RNA-Seq platform

- Decentralized setting: "in-house solution" to hospitals without compromising the level of clinical utility
- Easier reimboursent process thanks to local processing of the sample
- Involvement of countries with ethical restriction for the exchange of patient material

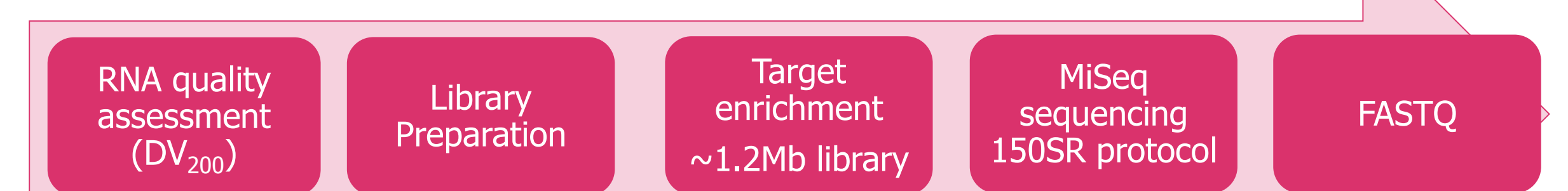
OBJECTIVE

Development of the MammaPrint and Blueprint diagnostic test from the microarray to the RNA-seq platform using NGS targeted RNA sequencing technology (RNA-Seq).

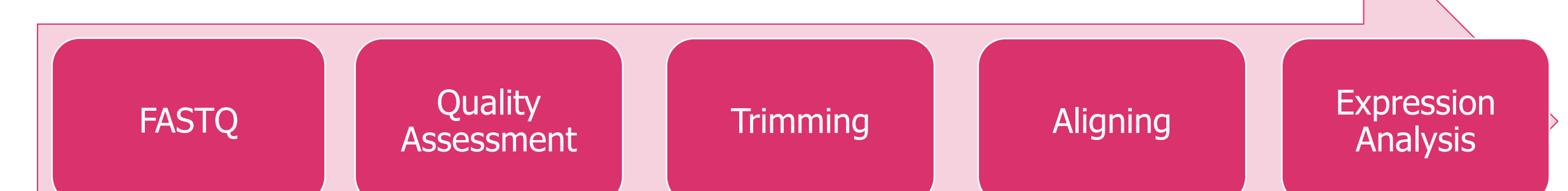
METHODS

- Total RNA Isolated using Qiagen RNeasy FFPE kit
- 85 FFPE samples processed with both on Microarray and RNA-Seq technologies
- 43 FFPE samples underwent two independent RNA isolations and processed with RNA-Seq technology
- 1 FFPE control samples measured over time and sequenced in 14 consecutive runs
- Gene counts (reads) for NGS normalized using Counts per Million (CPM) method

Targeted RNA-Seq laboratory processing (Agilent SureSelect XT)



Targeted RNA-Seq analysis pipeline



RESULTS

- MammaPrint 70-gene and Blueprint 80-gene signatures successfully mapped to the RNA-Seq genes
- On average 1.2 million reads assigned to gene per sample (15 samples on average per run)
- 96.3% reads were mapped to genes (hg19 build 37) with 74.8% reads on-target
- High correlation between the MammaPrint index calculated using the RNA-Seq data and the correspondent Microarray data (Pearson's correlation=0.98) in 85 FFPE samples (**Figure 1**)
- High correlation between the Blueprint indices calculated using the RNA-Seq data and the correspondent Microarray data (Luminal Pearson's correlation=0.98, Basal Pearson's correlation =0.98, HER2 Pearson's correlation =0.94) in 85 FFPE samples
- High correlation between RNA-Seq MammaPrint indices derived from two independent RNA isolations (Pearson's correlation=0.99) for intratumor heterogeneity assessment (**Figure 2**)
- High index reproducibility of 14 consecutive assessments of 1 FFPE control samples (standard deviation \pm 0.03) (**Figure 3**)

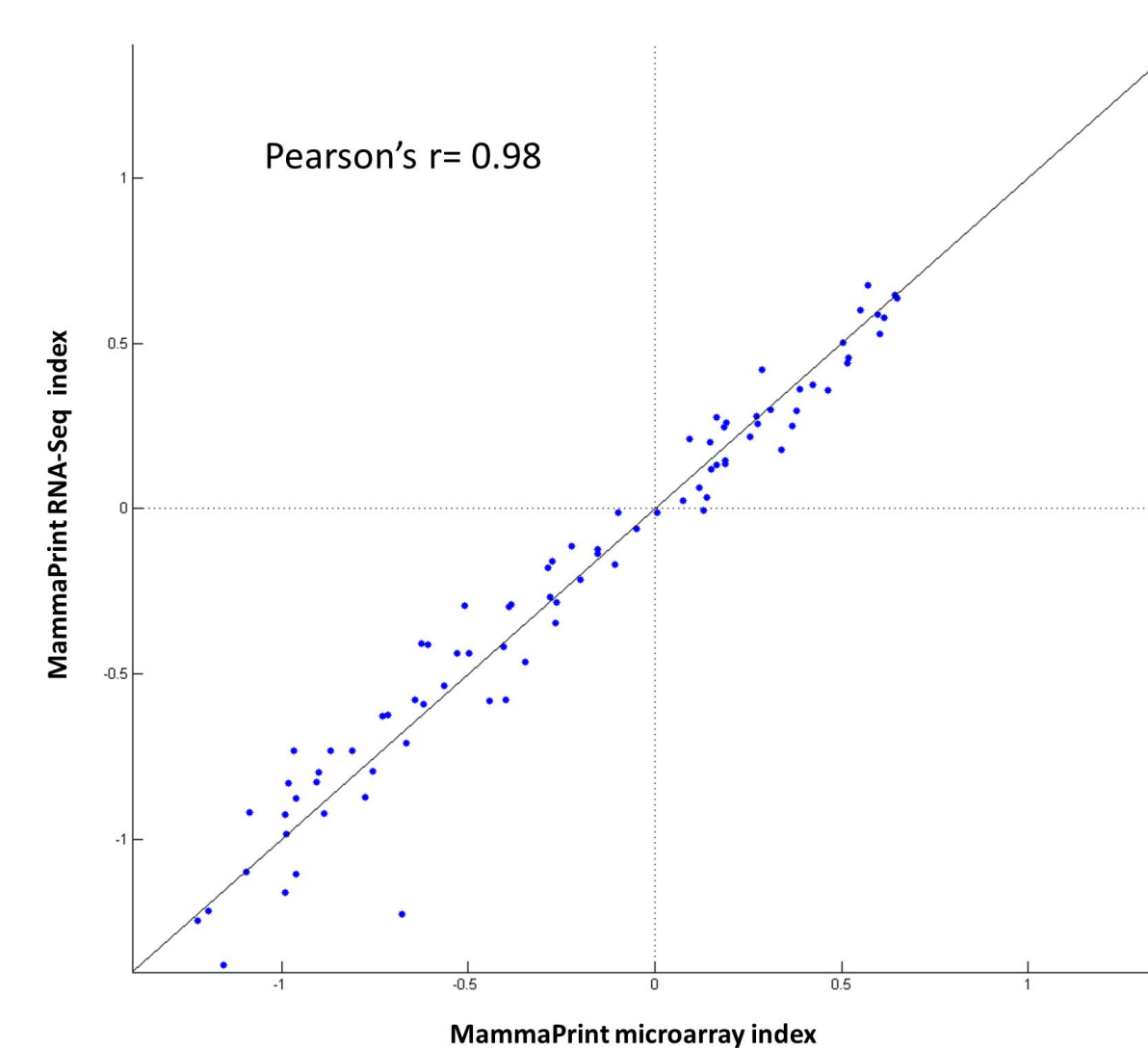


Figure 1: Scatterplot of MammaPrint microarray indices (x-axis) vs MammaPrint RNA-Seq indices (y-axis) in a set of 85 FFPE samples

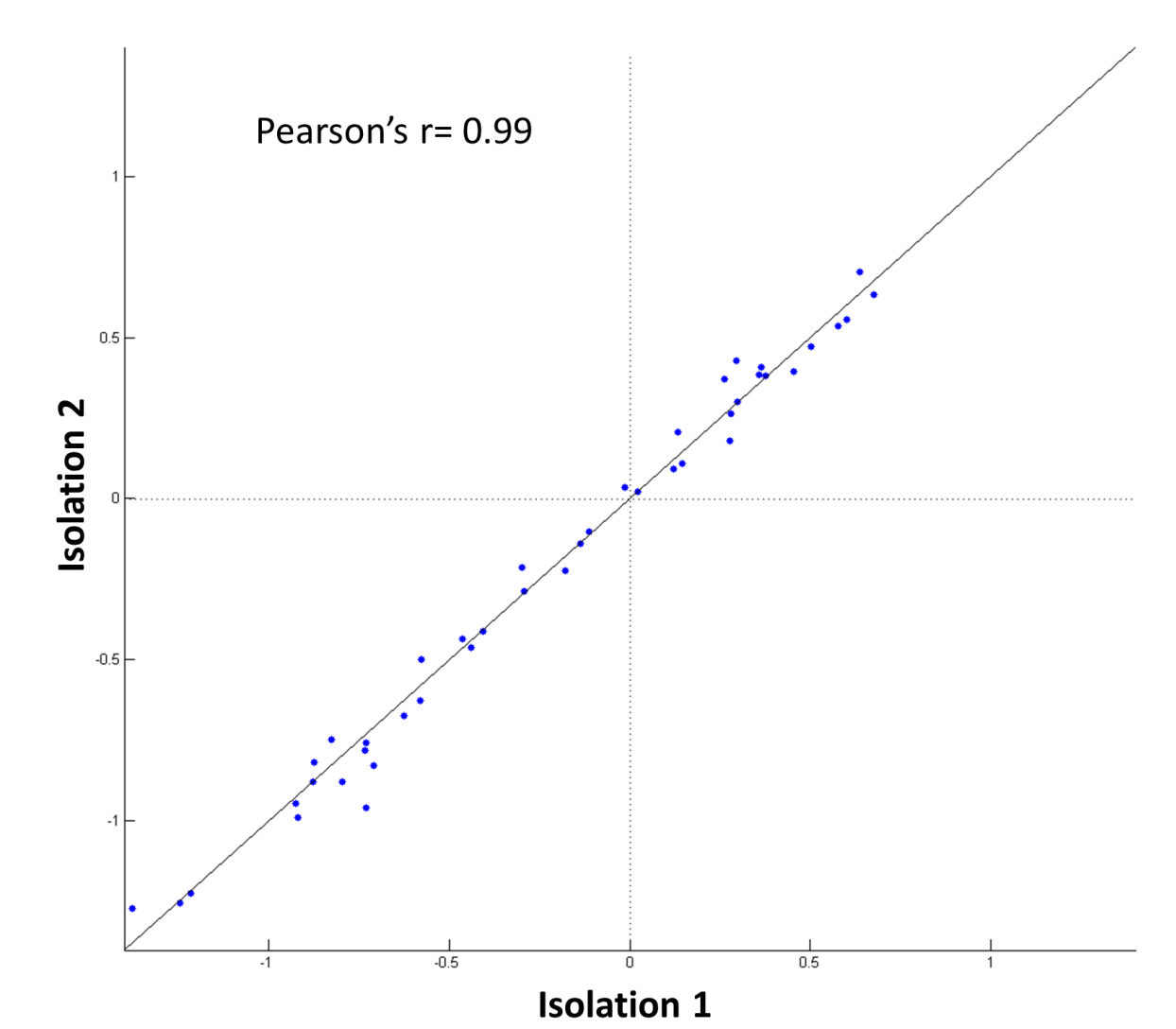


Figure 2: Scatterplot of MammaPrint RNA-Seq indices of isolation 1 (x-axis) vs isolation 2 (y-axis) in a set of 43 FFPE samples

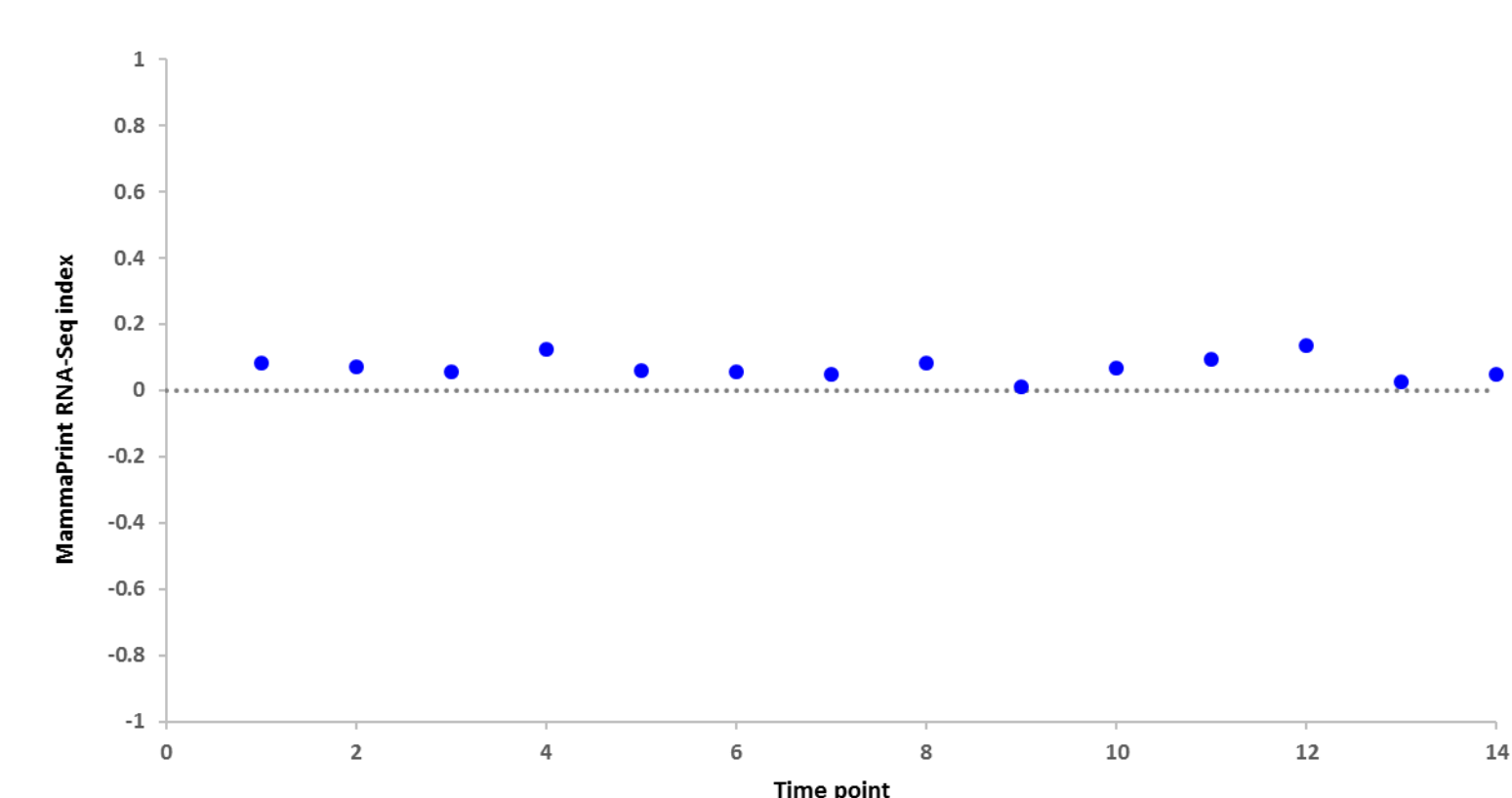


Figure 3: Scatterplot of MammaPrint RNA-Seq indices (y-axis) over time (x-axis) in 1 FFPE control sample

CONCLUSION

Preliminary analyses show that FFPE MammaPrint and Blueprint gene signature readouts generated from Targeted RNA-Seq technology, are highly comparable to the microarray diagnostic test readouts in a series of 85 FFPE early-breast cancer samples.

Further work assessing the stability and reproducibility are ongoing

REFERENCES

- [1] MammaPrint molecular diagnostics on formalin-fixed, paraffin-embedded tissue. Sapino A. J Mol Diagn 2014
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- [3] Translating RNA sequencing into clinical diagnostics: opportunities and challenges. Byron SA. Nat Reviews Genetics, 2016
- [4] A survey of best practices for RNA-seq data analysis. Conesa A. Genome Biol. 2016