

# Evaluating gene expression in FFPE tissues using DASL<sup>®</sup>: Validation by quantitative real-time PCR and immunohistochemistry

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## Summary

- Comparison of expression of 500 cancer-related genes in FFPE blocks of breast cancer specimens using DASL technology, and frozen samples from the same patients using Illumina WG-6 whole genome direct hybridisation, suggests good correlation for *EGR1* and *ERBB2* genes.
- Evaluation of mRNA integrity in FFPE samples by real-time PCR assay for *RPL13A* indicates a wide spread in quality, and is a robust means of filtering inadequate samples prior to DASL analysis
- DASL analysis of *EGR1* and *ERBB2* showed good correlation with TaqMan analysis in filtered samples
- DASL analysis of *EGR1* and *ERBB2* also showed moderate correlations with IHC for respective proteins using conventional scoring algorithms

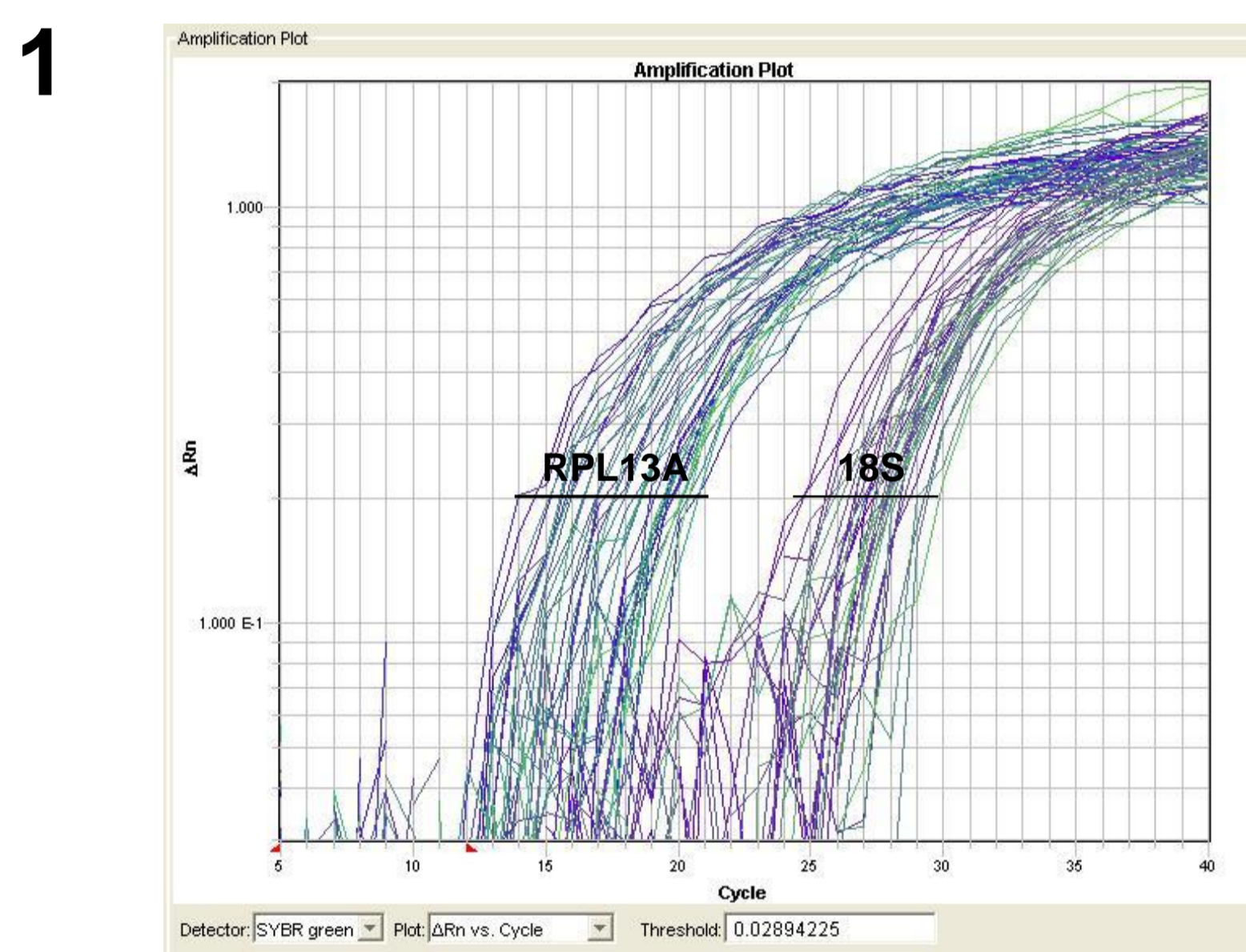
## Introduction

The study of gene expression in conventionally processed tissues is hampered by degradation of mRNA. Such samples are most commonly evaluated using expensive, low multiplex, quantitative PCR methods that can be unreliable due to the limited template sizes. One way to overcome this problem is to use array-based methods. DASL<sup>®</sup> technology relies on random priming for production of cDNA in concert with universal bead-arrays to allow the detection and relative quantitation of expression of specific gene subsets.

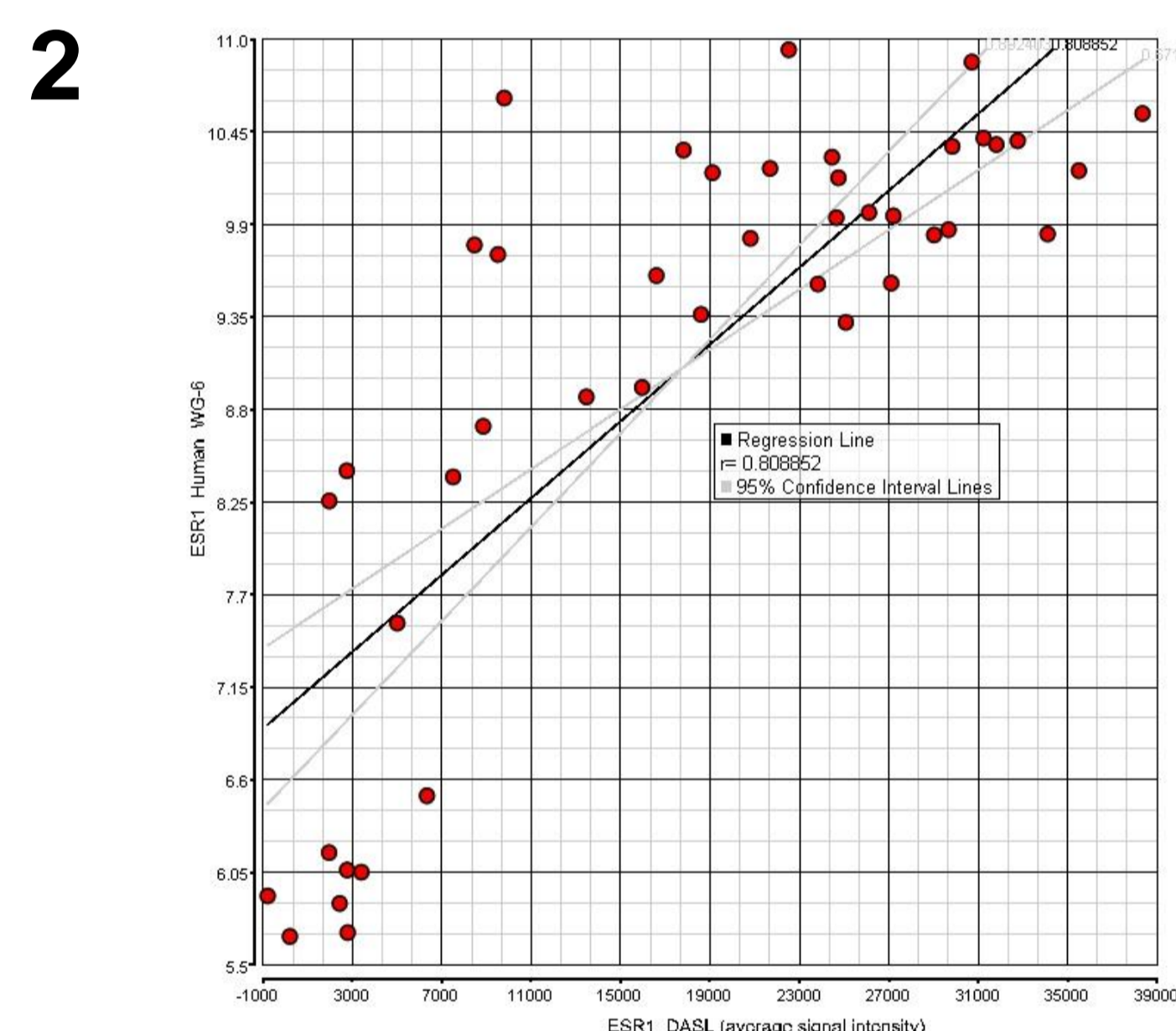
## Materials and methods

- 80 FFPE samples were obtained from early stage breast cancer tumours diagnosed between 1990 and 1992 (Ref 1)
- RNA extracted from two 10 $\mu$ m sections using Qiagen RNeasy FFPE kit
- For real-time PCR analysis, 200ng RNA was reverse-transcribed with High Capacity cDNA Reverse Transcription Kit (Applied Biosystems)
- RNA integrity was assessed using real-time PCR assays for the *RPL13A* and *18S* genes and SYBR Green master mix (Applied Biosystems) (Ref 2)
- DASL analysis was performed on samples using the Illumina Human Cancer Panel (representing 500 cancer-associated genes) and the 96-sample Sentrix Array Matrix (SAM); for each sample 200ng RNA was used in the DASL assay and no repeats were performed
- Data were analysed using BeadStudio software (Illumina Inc.) and Partek Genomics Suite
- *ESR1* (ER; oestrogen receptor) expression was evaluated using a Taqman assay (Hs01046816\_m1, Applied Biosystems) and Taqman Gene Expression Master Mix (Applied Biosystems)
- All real-time PCR assays were performed in quadruplicate

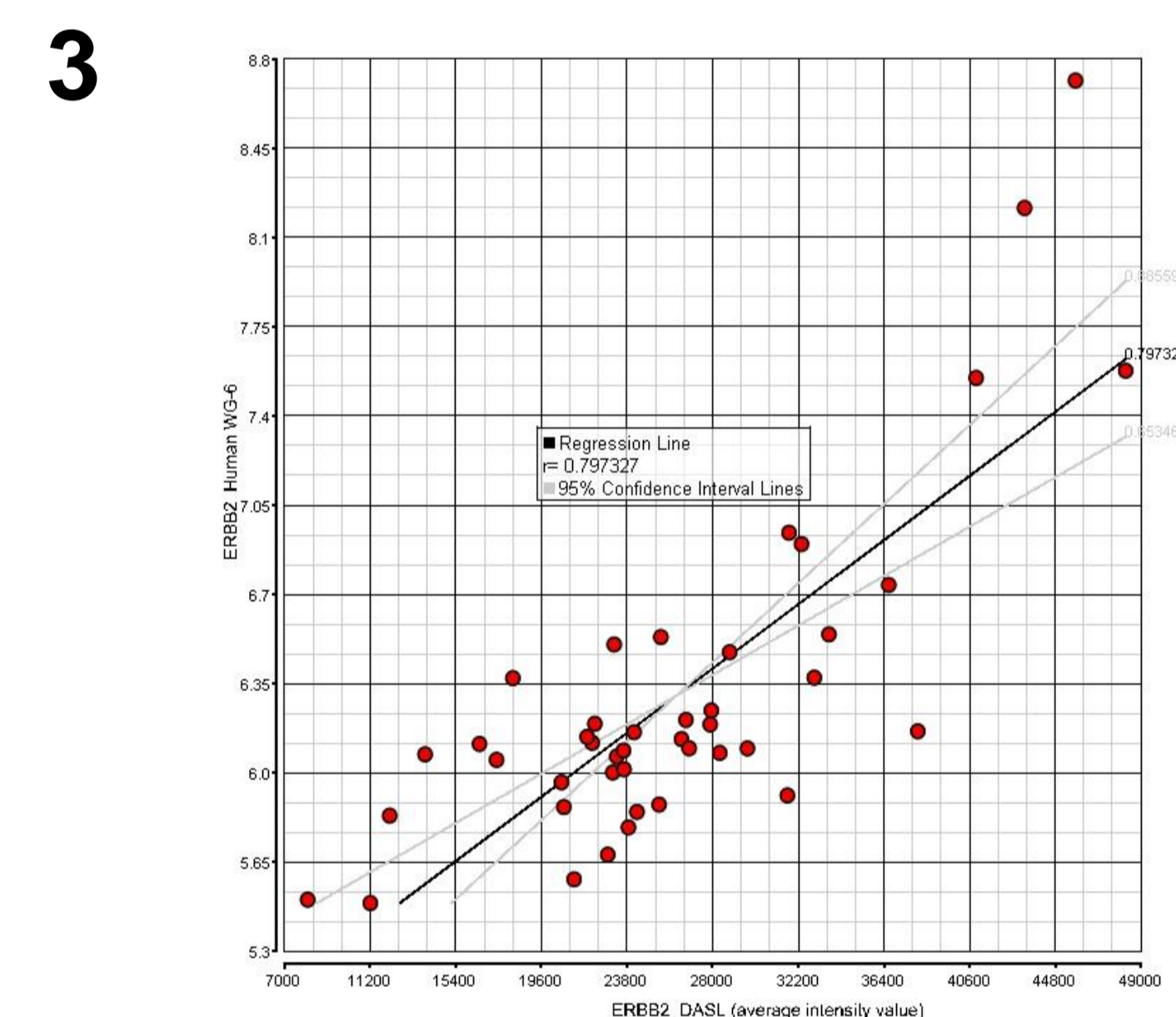
## Results



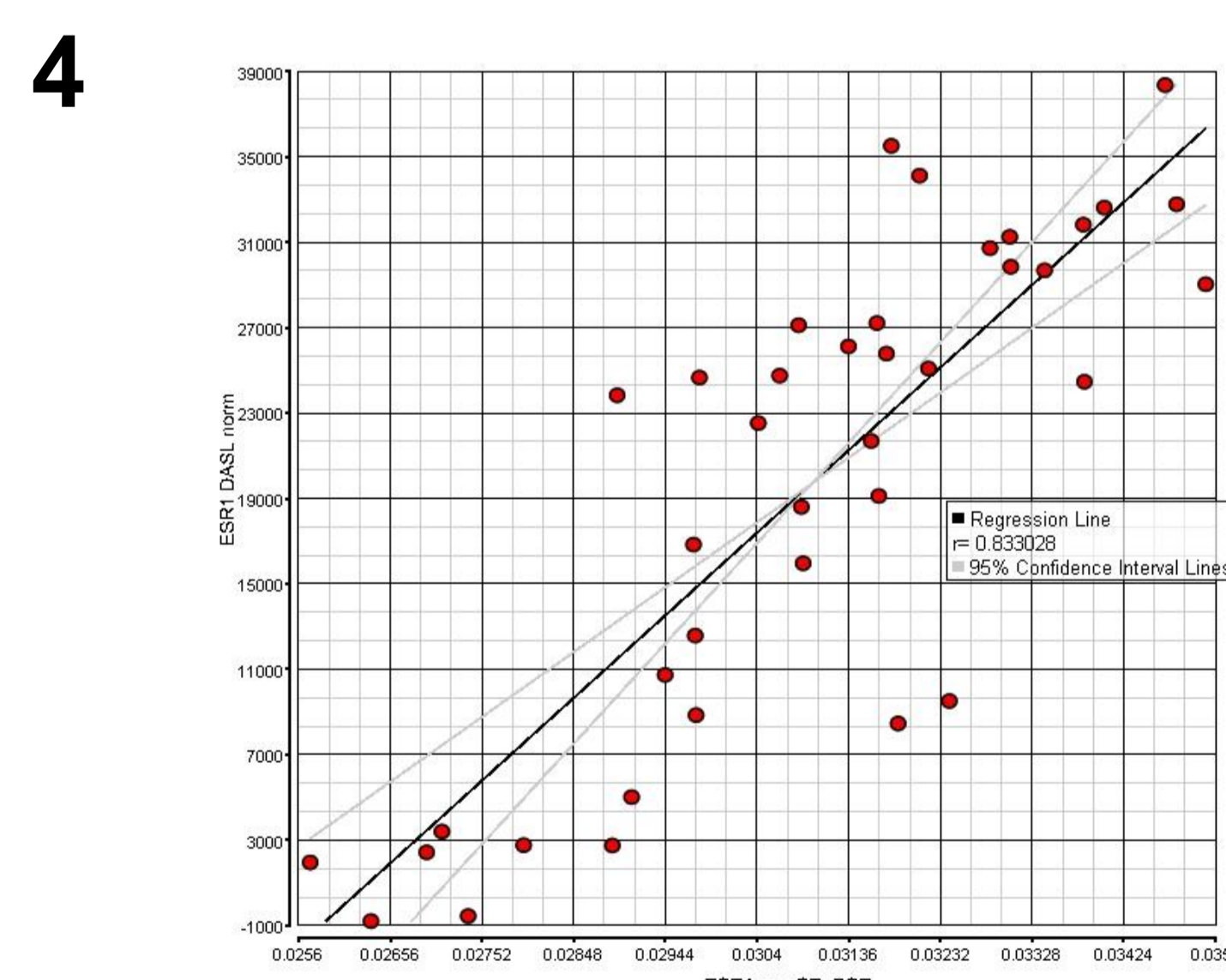
RT-PCR for RPL13A & 18S mRNA



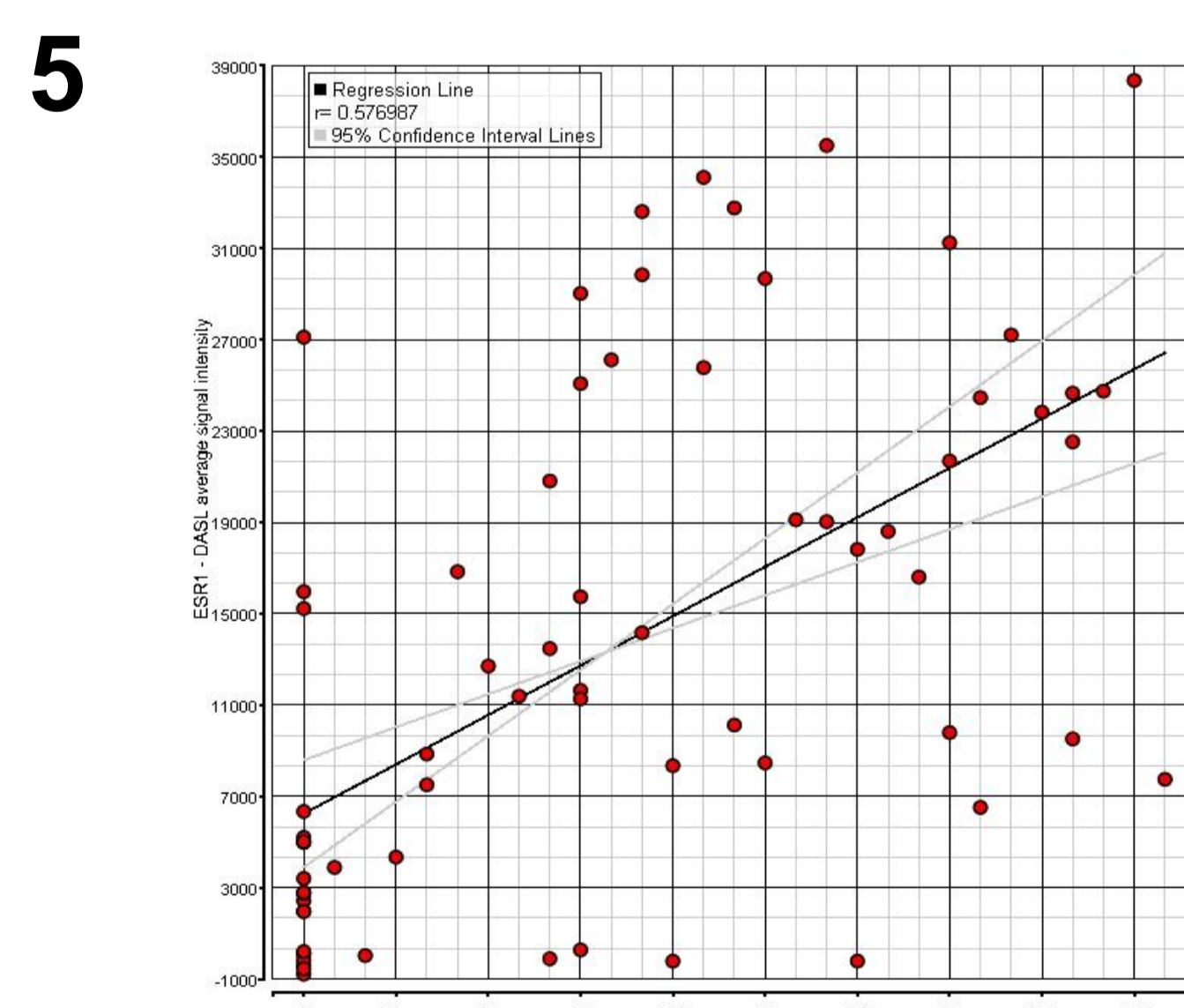
ESR1: DASL vs WG-6



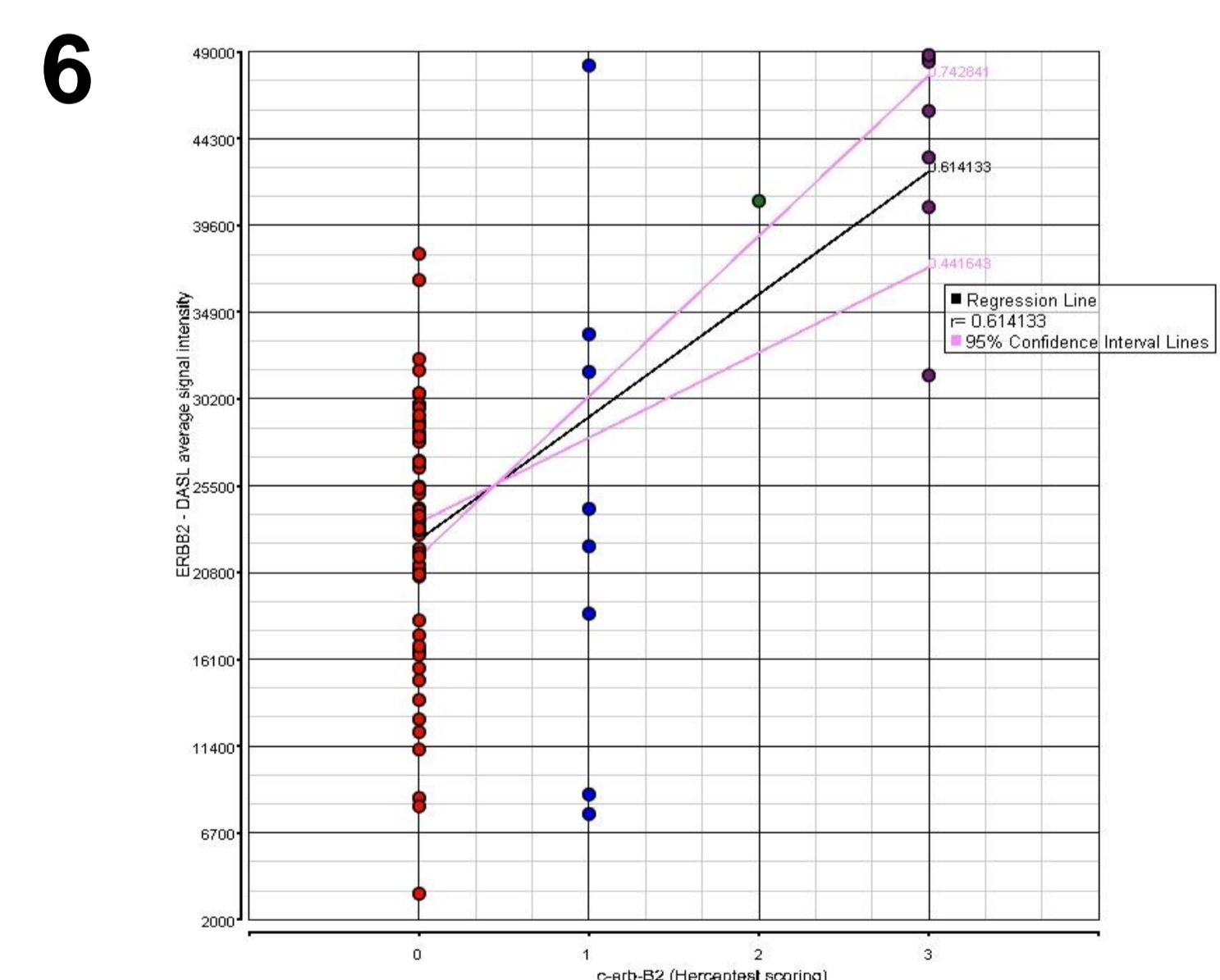
ErbB2: DASL vs WG-6



ESR1: RT-PCR vs DASL



ESR1: IHC (H-score) vs DASL



ErbB2: IHC (Herceptest) vs DASL

**Figure 1:** Real-time PCR for *RPL13A* and *18S* genes revealed a wide spread of sample quality in the 80 FFPE blocks. Of the 34 samples with detection levels of <300 genes, 74% had RPL13 Ct values above 27 (data not shown)

**Figure 2:** DASL was used to evaluate gene expression in 74 samples. Data were normalised with the average normalisation algorithm and genes were considered as detected if the detection P-value was lower than 0.01. For DASL, we set a 'pass' threshold of >300 genes detected to indicate a suitable sample for DASL. Of the 74 samples that were used on both DASL and WG-6 arrays, 43 samples exhibited  $\geq 300$  genes detected on the DASL arrays; 31 were therefore considered inadequate

**Figure 3:** *ERBB2* gene expression: Correlation between DASL and RT-PCR (TaqMan) in 43 ( $\geq 300$  genes detected on the DASL arrays samples) ( $R=0.83$ )

**Figure 4:** *ESR1* gene expression: DASL values for 39 samples filtered as indicated above showed good correlation with real-time PCR results ( $R=0.83$ )

**Figure 5:** *ESR1* gene expression: Correlation between DASL and IHC (H-score) in 71 samples ( $R=0.58$ )

**Figure 6:** *ERBB2* gene expression: Correlation between DASL and IHC (Herceptest score) in 69 samples ( $R=0.61$ )