

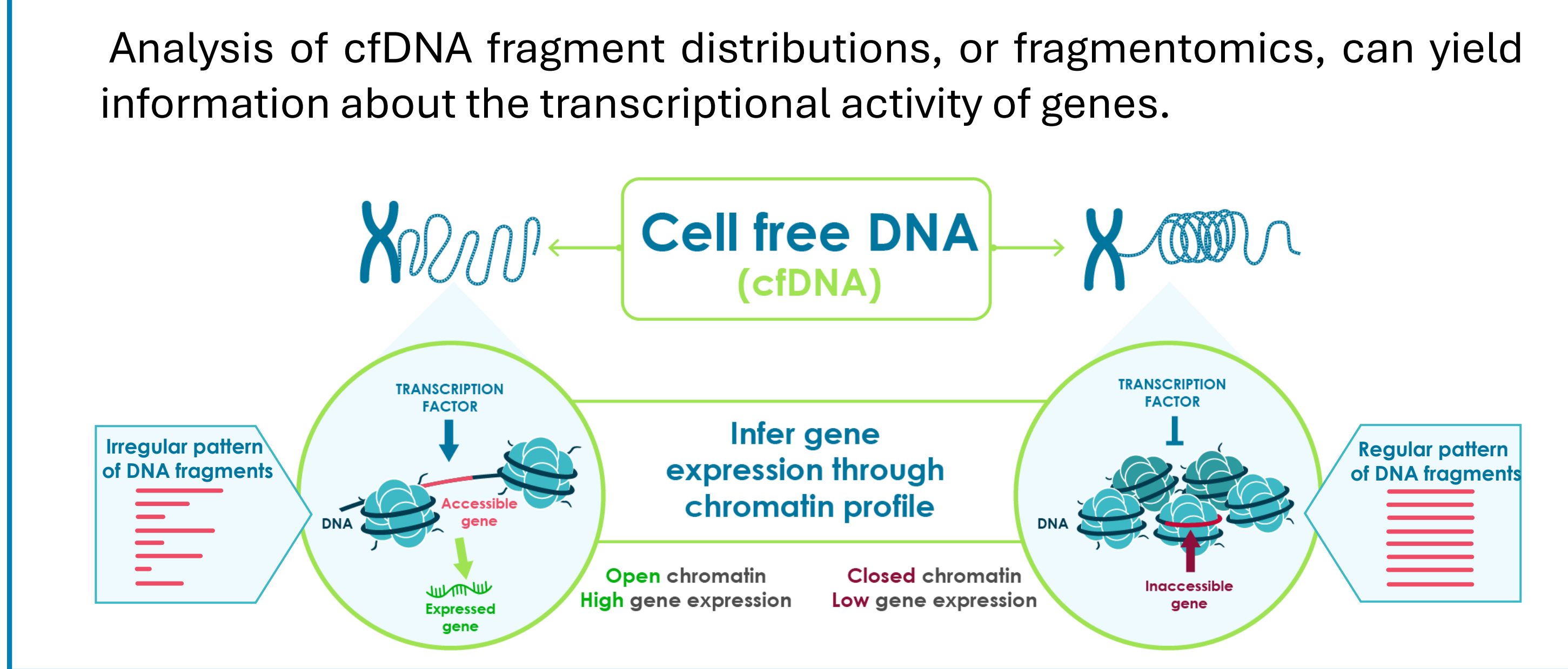
Fragmentomic analysis of cfDNA WGS at regulatory regions generates gene-level expression-like traits for subtype analysis in breast cancer

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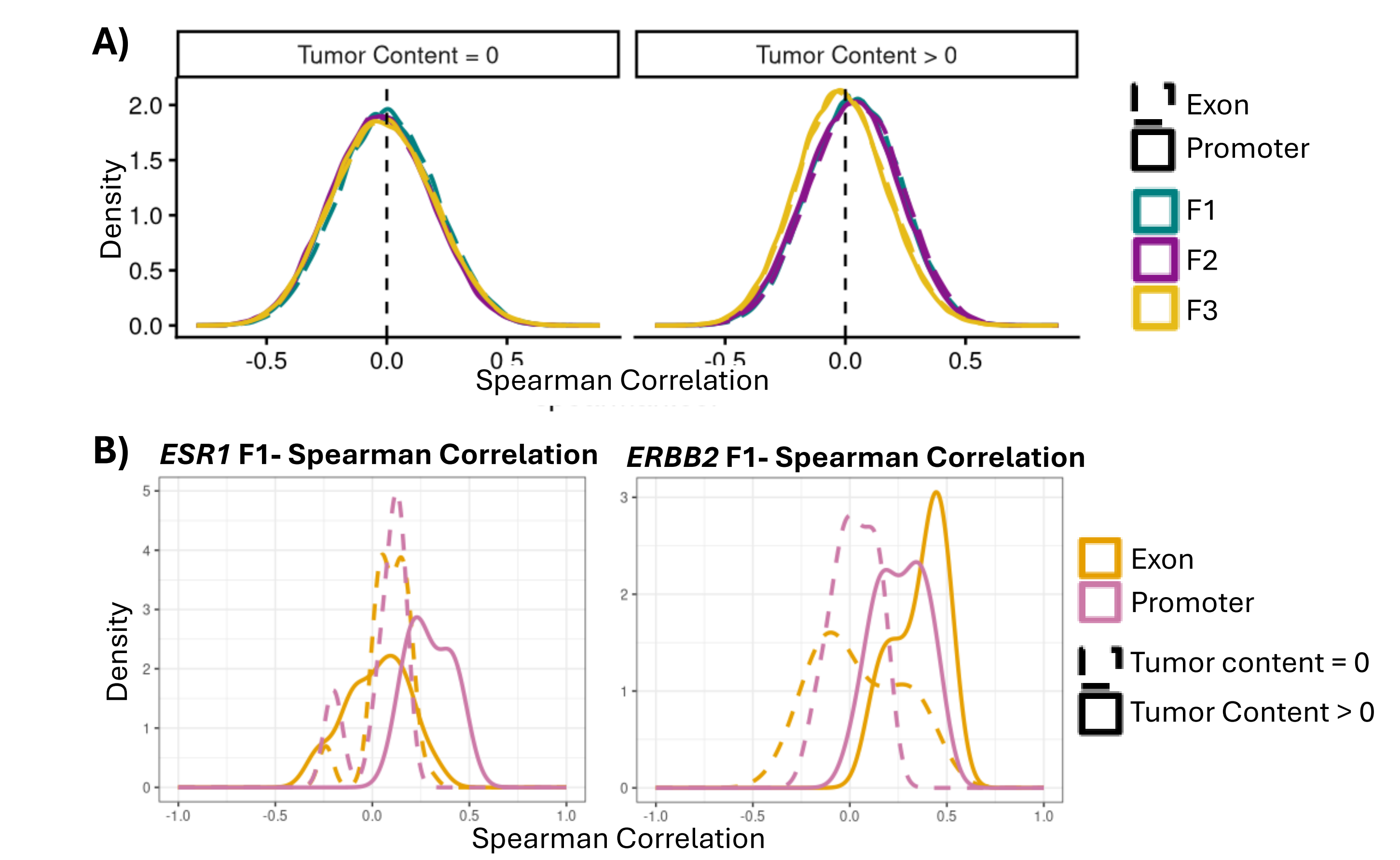
Background



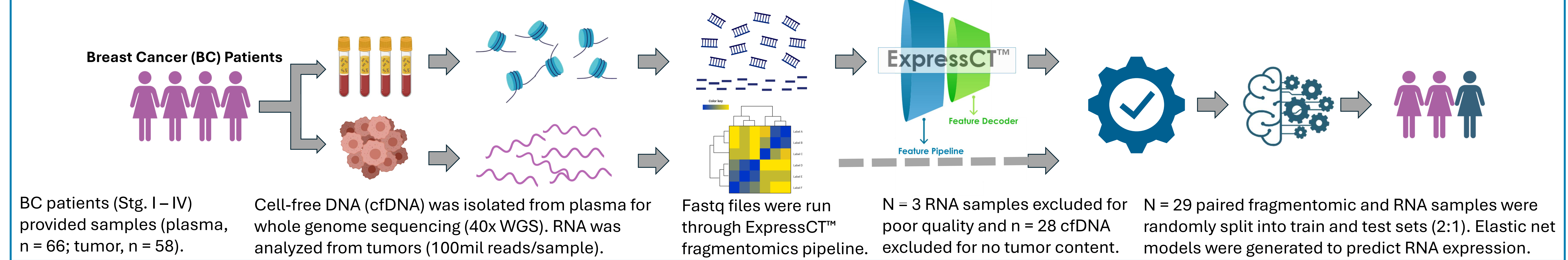
- Fragmentomic features may enable the development of classifiers reflective of discrete tissue expression markers such as hormone receptor and HER2.

Correlation of Fragmentomic Features & RNA

Genome-wide cumulative comparisons of the correlation between fragmentomic features (F1, F2, and F3) and RNA expression of associated genes (A) show only a small positive shift. However, closer inspection of specific, relevant genes (B), such as *ESR1* and *ERBB2*, show modest correlation of features and RNA at both promoter and exonic regions.



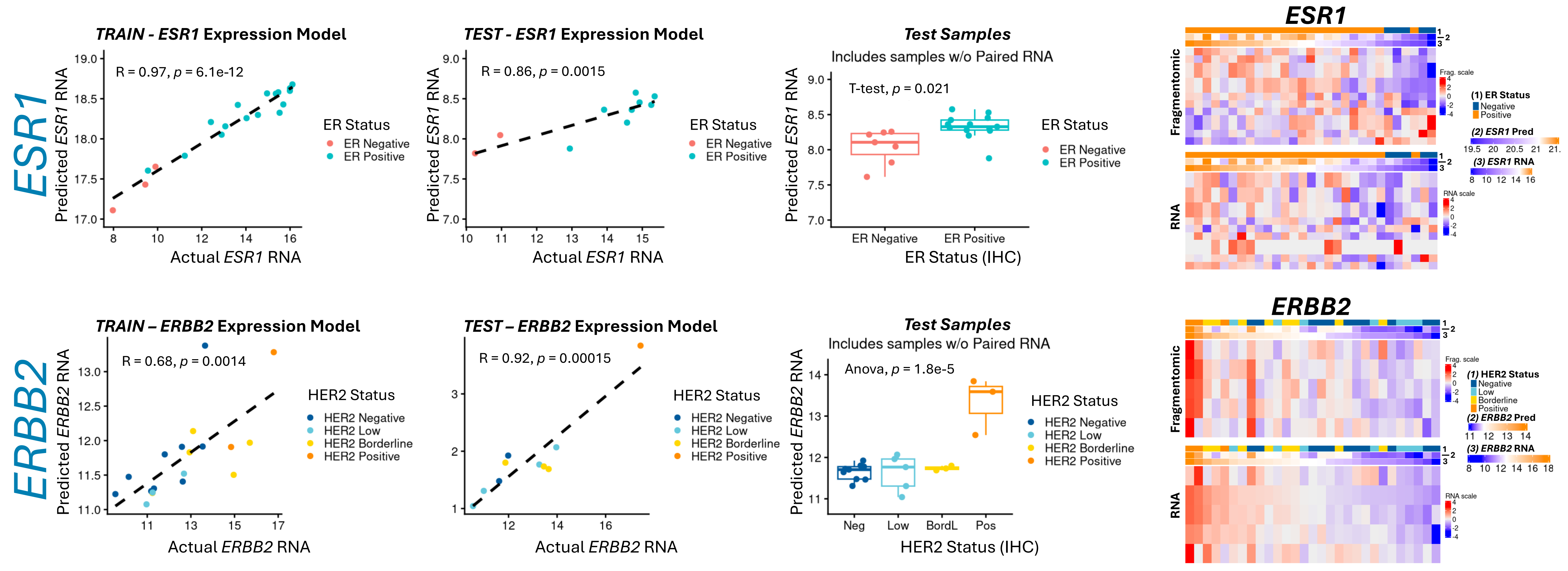
Study Design and Procedures



- WGS library prep was performed from two different library prep kits (IDT or Twist).
- Train and test sets were assessed for balanced ER/HER2 status, *ESR1/ERBB2* RNA expression, and cfDNA estimated tumor content. Six of eight cfDNA samples without paired RNA passed tumor content threshold.

Multi-Gene Fragmentomic Model of *ESR1* and *ERBB2* RNA Expression

RNA expression of individual genes can be more accurately modeled using fragmentomics from multiple, biologically relevant features.



Conclusions

- ExpressCT fragmentomics resulted in classifiers for ER and HER2 status with strong concordance to tissue-based IHC and RNA sequencing supporting cfDNA fragmentomics as a viable, noninvasive surrogate for breast cancer subtyping.
- The predictive fragmentomic features underlying these classifiers and their association with promoter, *cis*- or exonic regions are likely to vary by gene, reflecting the distinct regulatory and transcriptional architectures that govern individual loci.
- These findings support the potential for cfDNA fragmentomic classifiers as practical, scalable tools for both baseline breast cancer stratification and longitudinal therapeutic monitoring.