

# Exosomal liquid biopsy reveals mRNA and lincRNA biomarkers in early stage breast cancer patient plasma

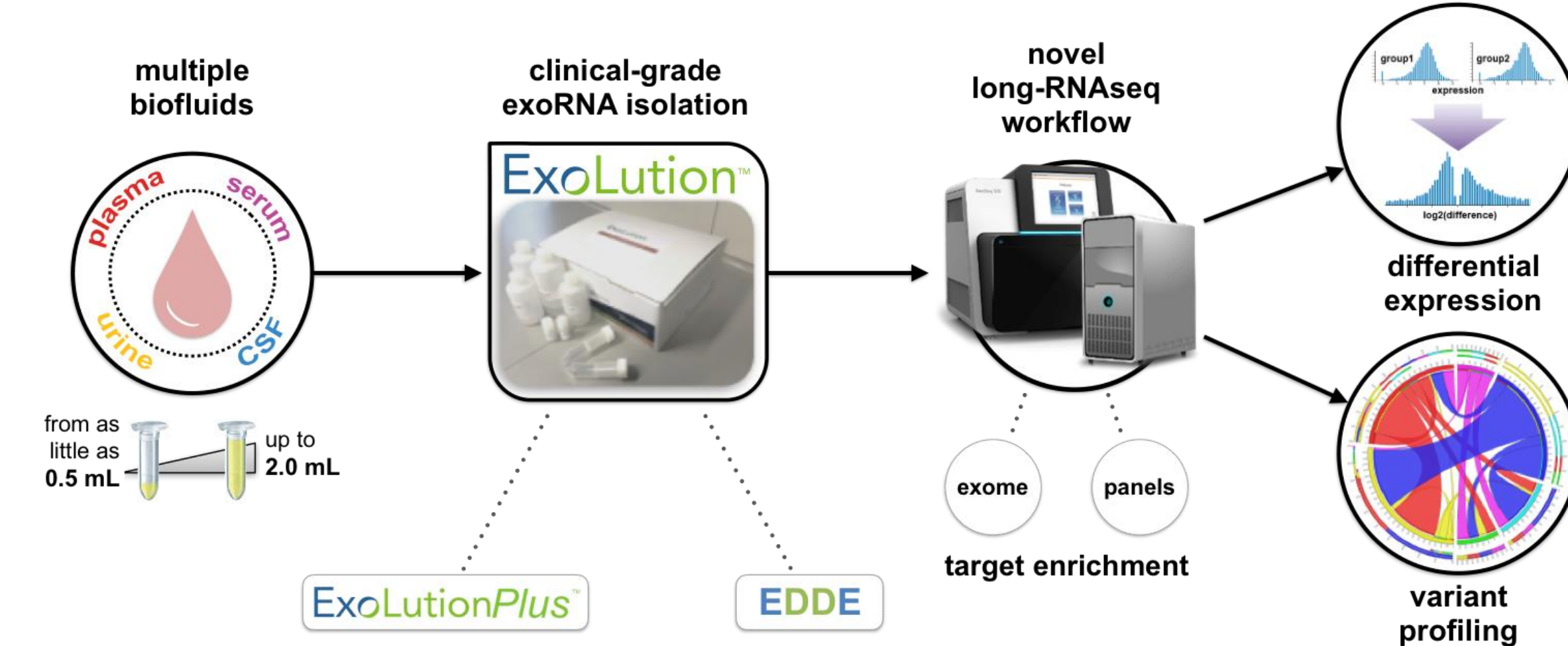
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- Breast cancer is the most prevalent cancer in women: ~250,000 diagnoses per year in the US
- Early detection is critical but current liquid biopsy approaches such as CTCs and cfDNA struggle to detect rare markers of disease
- Exosomes reflect active cellular processes thus enabling early cancer detection
- Exosome-based liquid biopsy is minimally invasive and highly sensitive, especially using techniques to enhance signal-to-noise:
  - Able to enrich specific exosomal subpopulations originating from a tumor or any tissue of interest
  - Able to deplete non-relevant, non-cancerous exosomes originating from abundant cell-types such as blood cells

## Technology

### ExoDx Long RNASeq analysis suite



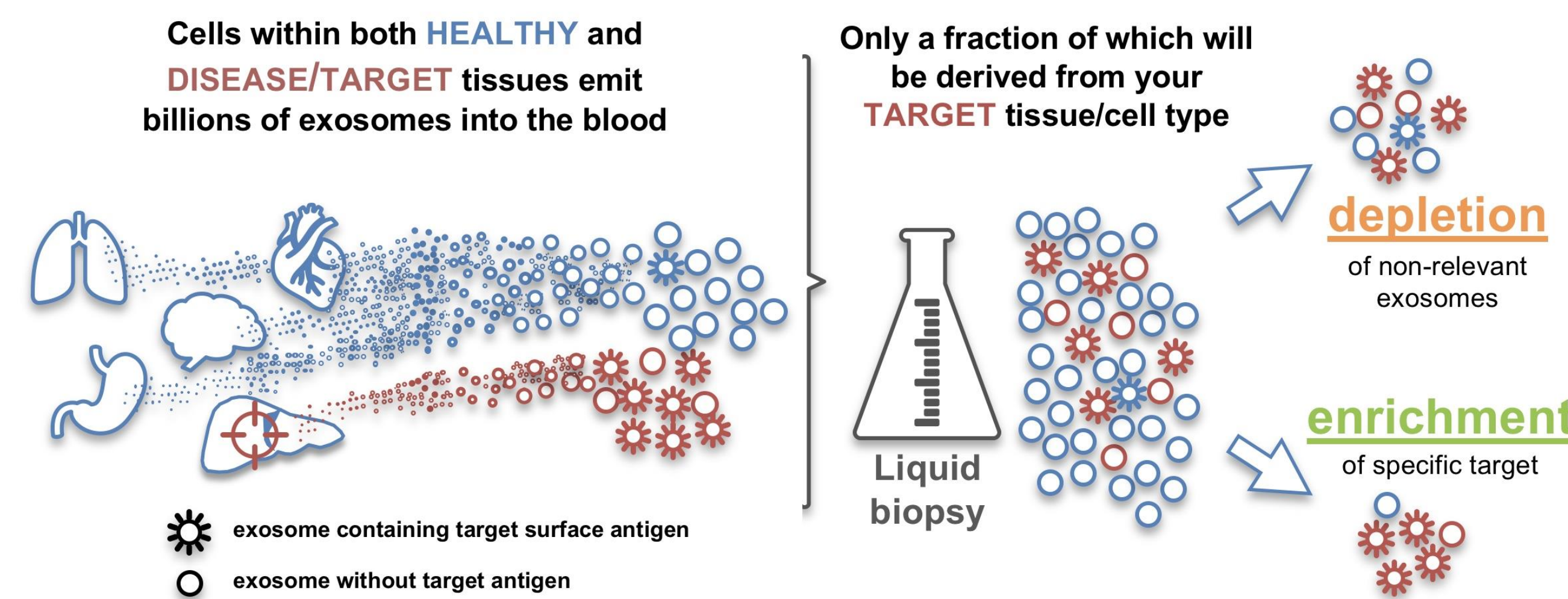
- A novel platform for enrichment or depletion of subpopulations of exosomes: **EDDE (ExoDx Depletion and Enrichment)**

- A novel platform for performing long RNASeq of exosomes
  - Highly reproducible workflow (Average R<sup>2</sup> = 0.99)
  - Wide dynamic range: 10 – 1.8 Million molecules of RNA
  - Full transcript coverage of exosomal RNA

- Applied EDDE to Stage I & II ER+/Her2- breast cancer and matched healthy controls using both:
  - Plasma depleted of non-relevant blood cell-derived exosomes
  - Exosomes enriched for those derived from the breast tumor

- We performed long-RNA sequencing on RNA obtained from matched breast cancer formalin-fixed paraffin-embedded (FFPE) tissue and plasma exosomes (both enriched and depleted)

### ExoDx Depletion and Enrichment (EDDE)



### RNAseq of plasma exosomes is comparable to FFPE tissue

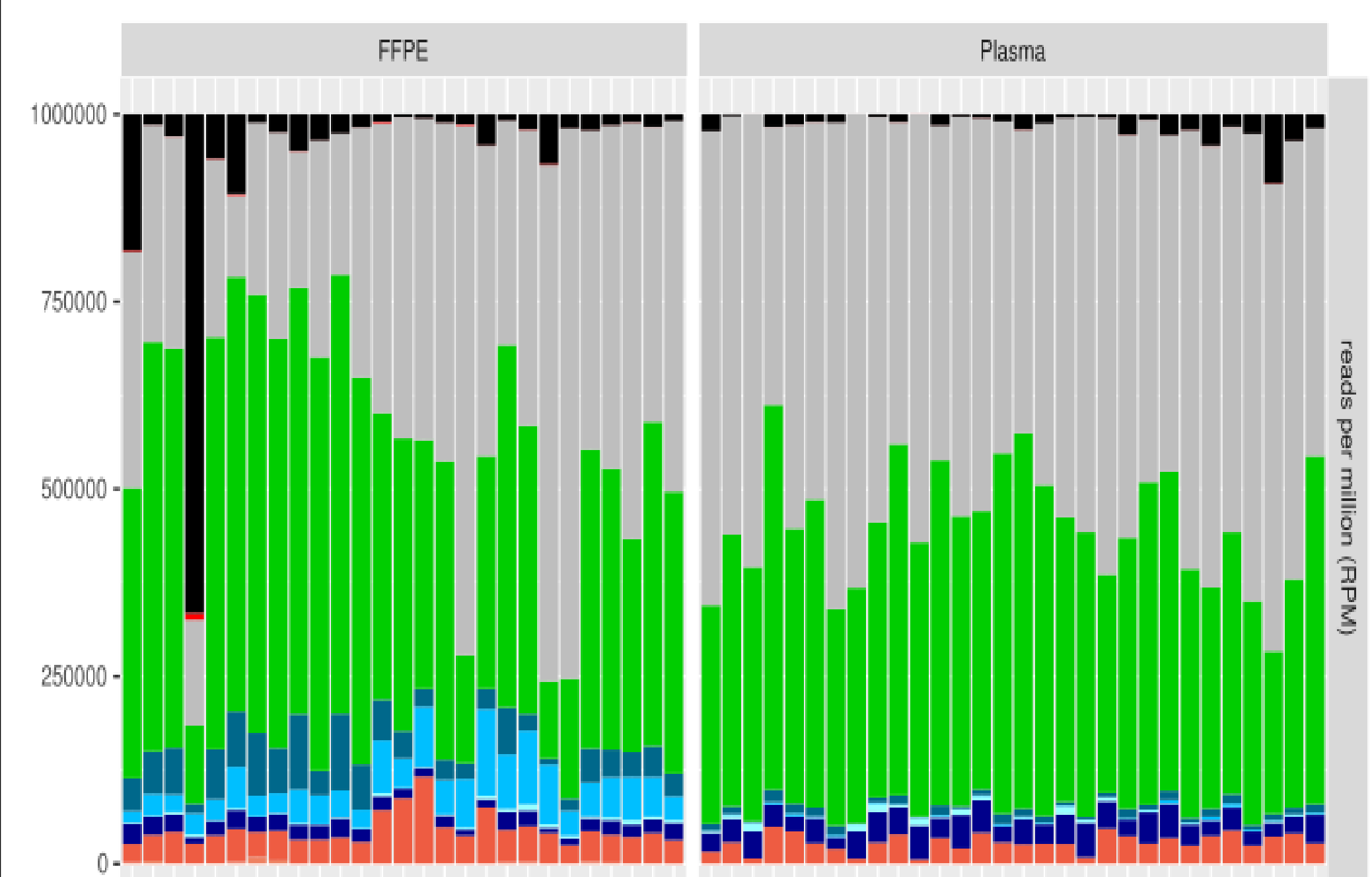


Fig. 1. Mapping statistics of matched plasma exosomes & FFPE tissue  
High rate of mRNA mapping in both FFPE and plasma, ~50% of transcriptome-mapped reads are mRNA

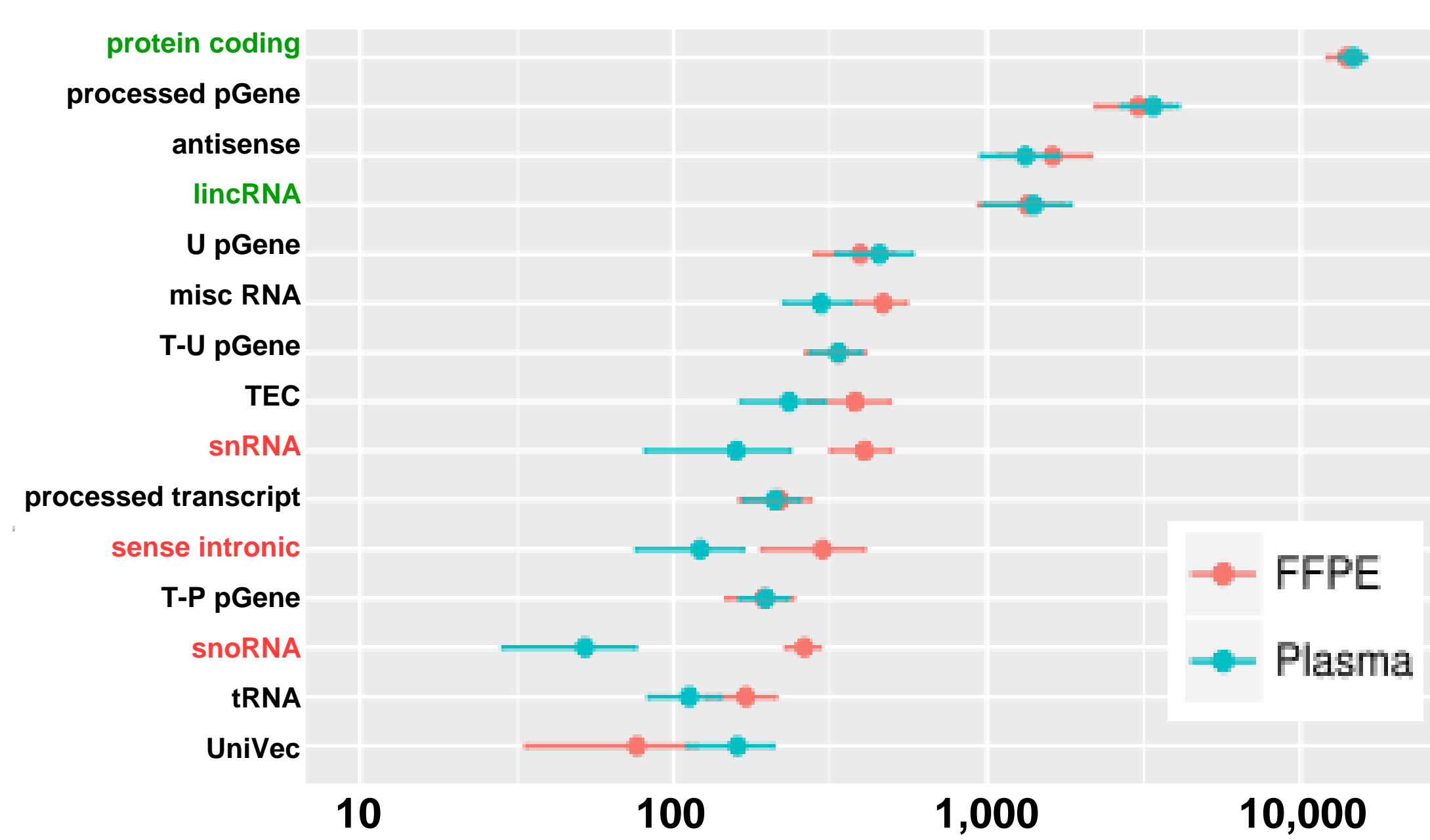


Fig. 2. Similar detection of gene biotypes in exosomal RNA and FFPE tissue.  
>12,000 mRNAs and >1,000 lincRNAs detected  
Same diversity seen in both plasma exosomes and FFPE

### EDDE depletion separates healthy individuals from breast cancer patients

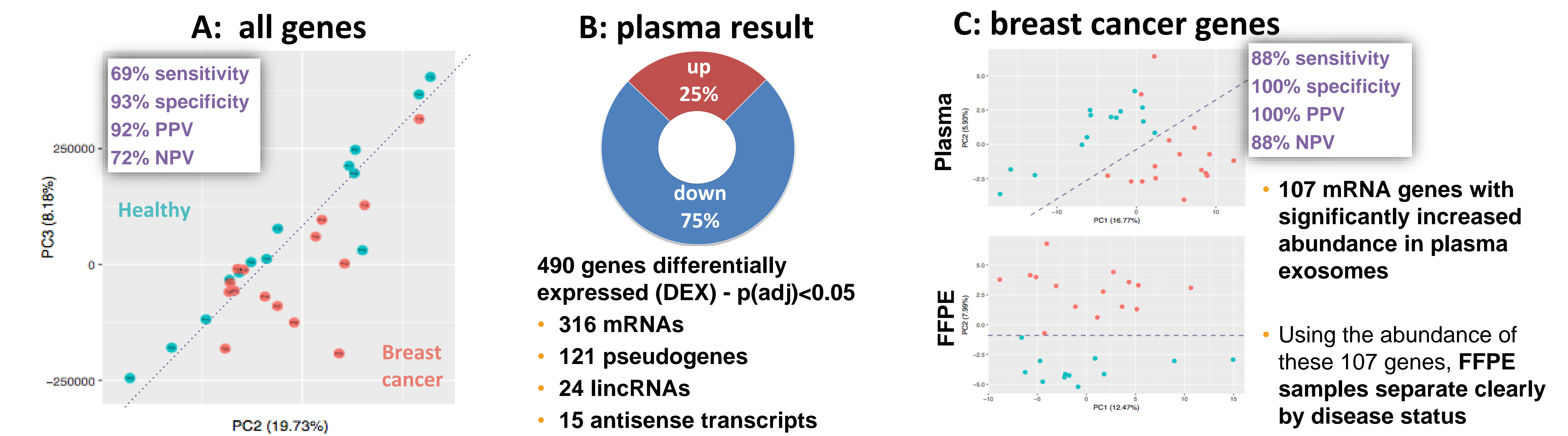


Fig. 3A. Global clustering by principle component analysis (PCA): samples cluster by disease status with EDDE depletion.  
3B. DEX analysis: 490 genes Differentially EXpressed (DEX) between breast cancer & healthy, including 316 mRNAs and 24 lincRNAs.  
3C. PCA using mRNAs with significantly increased abundance in plasma: these 107 mRNAs also separate breast cancer from healthy FFPE.

### EDDE breast cancer enriched plasma exosomes cluster with their matched FFPE tissue

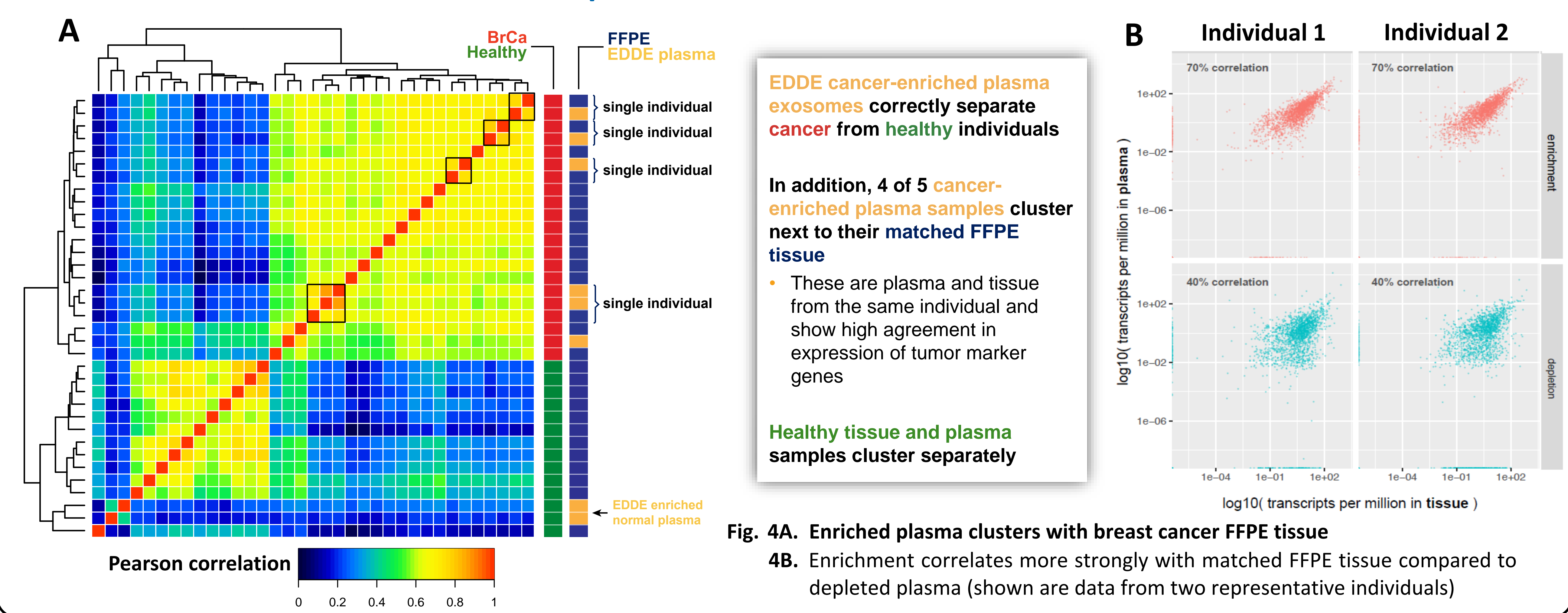


Fig. 4A. Enriched plasma clusters with breast cancer FFPE tissue  
4B. Enrichment correlates more strongly with matched FFPE tissue compared to depleted plasma (shown are data from two representative individuals)

### Enrichment vs Depletion results in different gene signatures & biological pathways

Gene set	fold enrichment	pValue (X <sup>2</sup> , Bonf)
Smirnov et al. - Circulating Endotheliocytes in Cancer Up	11.3	1.66E-11
Schuetz et al. - Breast Cancer Ductal Invasive Up	5.7	1.79E-05
SMID - Breast Cancer Luminal A Up	10.9	7.05E-05
GO - Regulation Of Immune System Process	3.04	4.31E-04
Reactome - Extracellular Matrix Organization	10.46	1.08E-04
Durand et al. - Stroma Up	4.65	9.43E-03
Mishra et al. - Carcinoma Associated Fibroblast Up	18.1	2.78E-03

EDDE depletion reveals components of tumor microenvironment

Gene set	fold enrichment	pValue (X <sup>2</sup> , Bonf)
Turashvili et al. - Breast Lobular Carcinoma vs Lobular Normal	17.61	8.73E-44
KEGG - ECM Receptor Interactions	16.33	2.12E-43
Hallmark - Estrogen response early	10.39	5.20E-41
PID - Integrin1 Pathway	17.66	6.62E-40
Anastassiou et al. - Multi-cancer Invasiveness Signature	17.42	4.33E-37
Hallmark - Epithelial to Mesenchymal Transition	10.09	1.35E-37
Charafe et al. - Breast Cancer Luminal vs Mesenchymal	6.35	1.85E-29

EDDE enrichment captures known breast cancer pathways from the tumor