E-biopsy: Electroporation-Based Molecular Sampling for *In Vivo* Research Applications

Technical Overview and Experimental Insights for Scientific Use

Abstract

Electroporation-based molecular sampling (E-biopsy) enables non-destructive extraction of intracellular contents from solid tissues in vivo. This document summarizes the methodology, reproducibility, and potential research applications based on preclinical evidence in murine 4T1 breast tumor models. The goal is to provide researchers with sufficient technical detail to replicate and adapt E-biopsy for proteomic, genomic, and metabolomic studies.

Methodology Overview

E-biopsy employs pulsed electric fields (PEF) to transiently permeabilize cell membranes, followed by aspiration of intracellular fluid. *In vivo* experiments used two 23G needles spaced ~1 cm apart: cathode and anode. Pulse regimen: 40 high-voltage short pulses (~1000 V, 40 µs, 4 Hz) and 40 low-voltage long pulses (~150 V/cm, 15 ms, 4 Hz). Extracted fluid was processed for LC-MS/MS label-free quantitation.

Experimental Reproducibility

Five Balb/c mice bearing 4T1 tumors were sampled at six positions per tumor (center, middle, periphery) plus healthy breast tissue. All animals survived without adverse effects. Correlation analysis confirmed high reproducibility between replicate samples.

Key Findings

- Proteomic profiles from E-biopsy distinguished tumor from healthy tissue.
- 13 proteins were consistently overexpressed in all tumor regions vs healthy breast ($p<0.01$).
- 242 proteins differentially expressed at $p<0.05$, supporting spatial heterogeneity mapping.
- GO analysis revealed biologically plausible patterns: decreased ribosomal activity toward tumor center; increased coagulation signals.

Potential Research Applications

- Spatial proteomics: map intra-tumor heterogeneity at macro-scale.
- Multi-omics integration: adapt protocol for RNA, DNA, and metabolite extraction.

- Tumor evolution studies: longitudinal sampling without excision.
- Drug delivery research: assess molecular gradients relevant to transport barriers.

Technical Considerations

- Electrode placement and pulse parameters critically affect yield and tissue integrity.
- Vacuum aspiration should be standardized to avoid variability.
- Downstream analysis compatible with LC-MS/MS; additional validation needed for nucleic acids.

Limitations and Future Directions

Current evidence is limited to murine models and proteomic profiling. Human feasibility studies and adaptation for other analytes are required. Further optimization of pulse regimens and sampling density could enhance molecular recovery and spatial resolution.

Primary Reference

Vitkin E., Singh A., Wise J., Ben-Elazar S., Yakhini Z., Golberg A. Nondestructive protein sampling with electroporation facilitates profiling of spatial differential protein expression in breast tumors in vivo. Scientific Reports (2022) 12:15835. doi:10.1038/s41598-022-19984-x.

Figures and Schematics

Figure 1: Workflow Diagram — E-biopsy process from electrode insertion to sample analysis.

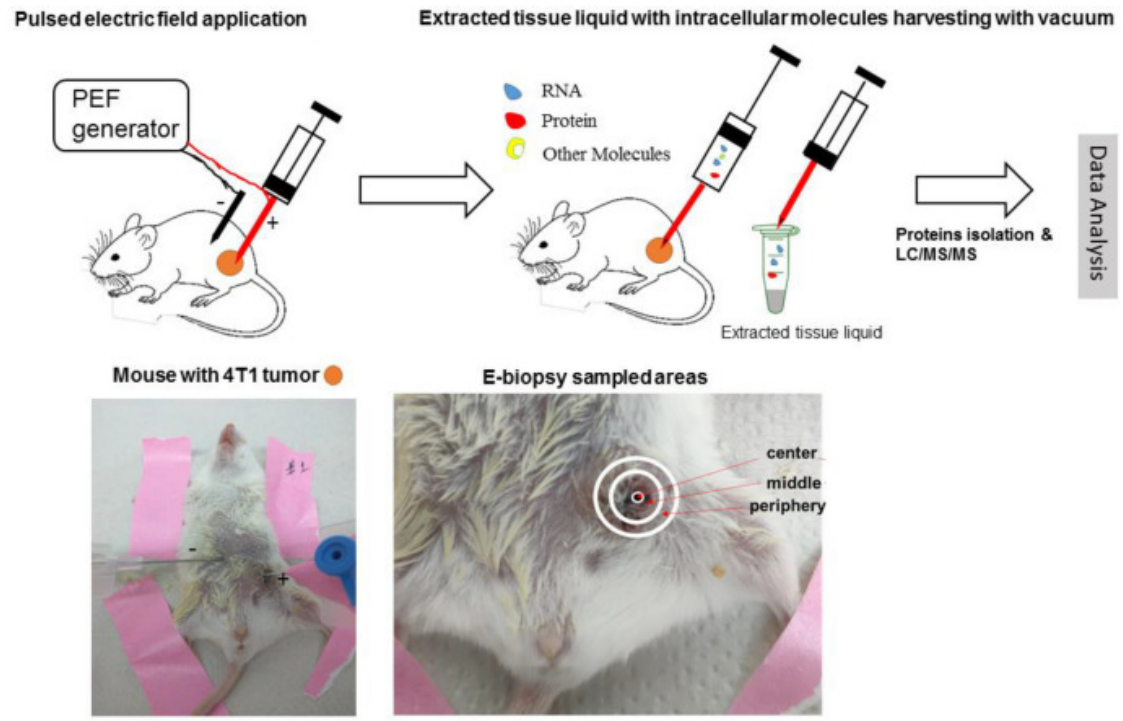
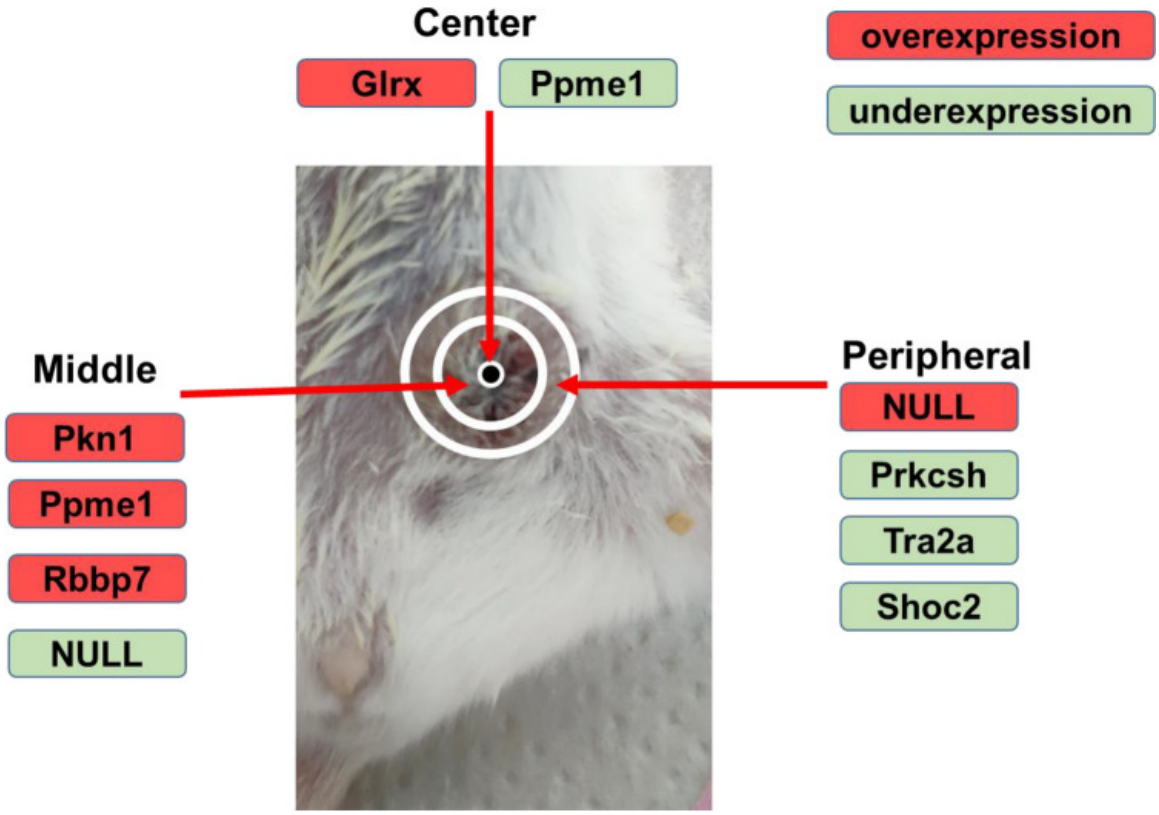


Figure 2: Sampling Map — Tumor regions (center, middle, periphery) and healthy tissue.



Detailed Protocol for Replication

1. Prepare animal model: Use Balb/c mice with 4T1 tumors (subcutaneous injection of 0.5 × 10^6 cells).

2. Anesthetize animal and expose tumor region.

3. Insert two 23G needles ~1 cm apart (cathode and anode).

4. Apply pulsed electric fields: 40 pulses at 1000 V, 40 μs, 4 Hz; then 40 pulses at 150 V/cm, 15 ms, 4 Hz.

5. Aspirate intracellular fluid using vacuum syringe immediately after pulses.

6. Transfer aspirate to tubes with 100 μL double-distilled water.

7. Process samples for proteomics: LC-MS/MS label-free quantitation.

8. Optional: Adapt for RNA/DNA extraction using phase separation reagents.

9. Perform statistical analysis: LFQ intensity normalization, t-tests, GO enrichment.