

## NEPA21 DevBio Quick Start Setup checklist

### Before you start

- Organoid stage appropriate (early cyst/spheroid preferred)
- ECM thinned/partially removed if needed (reduce barrier thickness)
- Cargo prepared at starting concentrations
- RNPs pre-complexed ( $\geq 10$  min at RT)
- Recovery media + ROCK inhibitor warmed and read

### Parameter selection (high-level)

- Cargo type:  Plasmid  mRNA  RNP
- Organoid type:  Gut  Brain (or other)
- Start conservative; optimize **access + stage** before pushing voltage
- Increase **transfer pulse number** before increasing **poring voltage**

### During electroporation

- Organoids oriented correctly between electrodes
- No air bubbles; avoid arcing
- Consider rotating organoids if re-pulsing (improve coverage)
- Use polarity exchange/rotation strategy when spatial bias is limiting

### Immediate recovery

- Transfer to fresh media immediately
- Add ROCK inhibitor (mandatory for many neural contexts; often helpful elsewhere)
- Minimize mechanical stress first 24 h

### 24–72 h check

- Viability acceptable (target  $\geq 60\%$ )
- Expression/editing detectable
- Decide:  Optimize  Repeat  Switch method (viral)