NEPA21 Decision Points Cheat-Sheet

Electroporation Decision Points for Disease-Modelling Organoid Labs (NEPA21)

Rule of thumb: NEPA21 to decide → Viral to standardize (when uniform, stable perturbation is the next requirement).

Choose NEPA21 when you need	Choose viral (AAV/Lenti/Retro) when you need
• speed (same-day delivery, results in days) • low biosafety overhead (non-viral prototyping) • viability in fragile primary/stem-cell systems • large or multi-component payloads (co-delivery) • minimal genomic footprint (RNP or transient expression)	• uniform perturbation (bulk -omics, stable pooled screens) • long-term expression across passages • inducible systems / lineage tracing • clonal reproducibility for definitive validation

1) Fast yes/no decisions: when to choose NEPA21

A. Go/no-go on constructs, variants & targets	**Decision:** Is this perturbation worth investing in as a stable model? **Why NEPA21:** Same-day delivery; results in days; no vector build/titer/selection to start.
B. Immediate gene-loss dependencies (acute RNP KO)	**Decision:** What happens right after gene loss—before adaptation? **Why NEPA21:** RNP knockout is fast, low footprint, avoids long-term Cas9 expression.
C. Cell-autonomous vs neighbour-driven effects (mosaicism)	**Decision:** Is the phenotype intrinsic to edited cells or driven by neighbours/context? **Why NEPA21:** Mosaic delivery creates within-organoid controls (edited vs unedited neighbours).
D. Scaling across patient-derived cohorts (PDO biobanks)	**Decision:** Is the effect consistent across many patients/genotypes? **Why NEPA21:** Low per-condition cost; no custom virus per construct for early testing.
E. Large or multi-component payload feasibility	**Decision:** Can a big construct or multi-plasmid system work at all? **Why NEPA21:** Easy co-delivery (reporter + effector + barcode) without packaging constraints.
F. Timing-sensitive interventions (stage windows)	**Decision:** Does perturbation timing matter (early vs late organoid stage)? **Why NEPA21:** Target specific stages without waiting for viral expression/selection.
G. Fast "perturb → treat" pharmacology loops	**Decision:** Does gene/pathway X modify therapy or stress response within a week? **Why NEPA21:** Acute perturbation pairs cleanly with drug exposure; mosaicism can reveal relative fitness.
H. Minimal-genomic-footprint needs	**Decision:** Do we need to avoid integration and persistent transgene expression? **Why NEPA21:** RNP/transient expression minimizes footprint and integration concerns.

Typical success criteria: viability preserved + measurable delivery + interpretable phenotype within days.

2) Integration map, payload selector & standardization triggers

Workflow integration point	What teams do	Decision enabled
Pre-organoid engineering iPSC / donor / primary cells	Introduce mutations, reporters, or isogenic controls before differentiation.	Commit to differentiation + model build?
Organoid editing dissociate → EP → reaggregate	Label lines, knock-in reporters, test pathway perturbations, prototype large editors.	Does it work in this organoid context?
Immune co-culture engineering T/NK/macrophage perturbation	Engineer immune components rapidly for co-culture disease models.	Does immune modulation change phenotype/killing?
In vivo / ex vivo tissue EP	Bridge organoid findings to tissue/animal context without switching platforms.	Does biology translate beyond in vitro?
Rapid screening loops	Test multiple targets/guides quickly before stable line investment.	Which hits deserve viral standardization?

Payload selector	When to switch to viral
• **CRISPR RNP (Cas9 + gRNA)**: acute knockout, early dependency tests, fast perturb → treat loops • **Plasmids**: feasibility of large systems, co-delivery (reporter + effector), complex reporters/editors • **siRNA/shRNA** (where appropriate): quick pathway suppression screens	Switch to AAV/lenti/retro when you need: • uniform perturbation (bulk -omics, stable pooled screens) • long-term expression across passages • inducible systems / lineage tracing • clonal reproducibility for definitive validation **Best practice:** NEPA21 (rapid decision + feasibility) → Viral (stable standardization + scale)

3) Disease-specific “most common wins”

CRC / PDAC: fast triage across heterogeneous PDOs; acute RNP KO + drug/stress loops; mosaic competition/fitness; large constructs before viral.
Brain: early timing windows; neighbour-dependent signalling; RNP often preferred early; viral later for stability.
Lung: fate regulators; mosaic epithelial state shifts; injury/inflammation perturb–treat loops.
Kidney: timing-sensitive injury–repair programs; acute perturbation under nephrotoxic/ischemic-like stress; intrinsic vs neighbour injury susceptibility.