Protocol Selector

Organoid type → payload type (RNP / plasmid) → typical stage (pre-organoid vs dissociated organoid)

Purpose: help disease-modelling teams pick a *starting* NEPA21 workflow pattern (not a fixed voltage recipe). Start gentle, confirm viability + delivery, then optimize.

How to use this selector

1) Choose your **organoid type**.
2) Choose your **payload type**: **CRISPR RNP** (fast KO, minimal footprint) or **Plasmid DNA** (reporters/editors, co-delivery).
3) Choose the **typical stage** you can access: **Pre-organoid** (iPSC/primary/donor cells) or **Dissociated organoid cells** (single-cell suspension before reaggregation).
Output: a recommended integration pattern + common use cases + QC checkpoints.

Selector matrix (common starting patterns)

Organoid type	**RNP** Pre-organoid	**RNP** Dissociated organoid	**Plasmid** Pre-organoid	**Plasmid** Dissociated organoid
CRC	Use for: rapid dependency KO in donor/ASC-like cells. **Best when:** isogenic panel build. **QC:** indel check 48-96h.	Use for: fast KO + drug response loop in PDOs. **Best when:** early go/no-go across cohort. **QC:** viability 24h; phenotype 3-7d.	Use for: reporter feasibility in progenitors before differentiation. **Best when:** large constructs. **QC:** reporter 48-96h.	Use for: label lines (GFP/RFP), pathway reporters, knock-in donor testing. **QC:** reporter + selection/FACS.
PDAC	Use for: acute KO in sensitive primary-like cells. **Best when:** fast mechanism check. **QC:** viability priority.	Use for: acute RNP KO + stress/therapy assays. **Best when:** hard-to-transfect PDOs; minimize footprint. **QC:** viability 24h; edit 72h.	Use for: feasibility of multi-plasmid systems before organoid build. **QC:** reporter + growth.	Use for: reporters/perturbations in dissociated PDO cells, then reaggregate. **QC:** reporter 3-5d; selection as needed.
Brain	Use for: early lineage/patterning regulators in iPSC stage. **Best when:** timing matters. **QC:** viability + early markers.	Use for: mosaic KO to test neighbour effects (Notch-like dynamics). **Best when:** within-organoid controls needed. **QC:** mosaic readout 3-10d.	Use for: complex reporters/editors before differentiation (large payloads). **QC:** reporter + differentiation potential.	Use for: mosaic reporter/effector co-delivery in dissociated cells. **QC:** reporter distribution + morphology.

Lung	Use for: fate regulator KO in progenitors/pre-diff cells. **QC:** lineage markers.	Use for: rapid epithelial state shift tests (basal/secretory). **Best when:** perturb-treat loops. **QC:** state markers 3-7d.	Use for: sensor/reporter feasibility pre-organoid. **QC:** expression + viability.	Use for: pathway reporters + perturbations in dissociated organoid cells. **QC:** reporter + functional readout.
Kidney	Use for: early segment identity regulators in iPSC/progenitors. **Best when:** timing windows. **QC:** segment markers.	Use for: acute KO under injury/toxin stress; mosaic susceptibility testing. **QC:** viability + injury markers.	Use for: large reporter/editor feasibility before differentiation. **QC:** reporter + differentiation.	Use for: reporters/perturbations to map injury-repair programs post-reaggregation. **QC:** phenotype 3-10d.

Notes: “Pre-organoid” = iPSC/primary/donor stage before organoid formation. “Dissociated organoid” = single-cell suspension electroporation prior to reaggregation. Use RNP for fastest KO/minimal footprint; use plasmids for reporters, editors, and co-delivery feasibility.

Protocol selector outputs

Pattern 1 — RNP + Pre-organoid stage Best for: early go/no-go on gene function, isogenic model foundations, timing-window edits. Typical workflow: engineer cells → expand/recover → differentiate → build organoids. QC gates: viability 24h; edit check 48-96h; differentiation markers at day 7-14.	**Pattern 2 — RNP + Dissociated organoid stage** Best for: rapid dependency tests, perturb → treat loops, mosaic mechanism checks. Typical workflow: dissociate → EP in suspension → recover → reaggregate → assay. QC gates: viability 24h; edit check 72h; phenotype window day 3-10.
Pattern 3 — Plasmid + Pre-organoid stage Best for: feasibility of large editors/reporters, multi-plasmid co-delivery, sensor prototypes. Typical workflow: transfect/EP cells → confirm expression → proceed to differentiation. QC gates: reporter 48-96h; growth/fitness; differentiation potential.	**Pattern 4 — Plasmid + Dissociated organoid stage** Best for: line labelling (GFP/RFP), pathway reporters, staged perturbations, KI donor testing. Typical workflow: dissociate → EP plasmid(s) → recover → reaggregate → select/FACS if needed. QC gates: reporter 3-5d; enrichment/selection; phenotype day 5-14.

Standardization triggers (when to switch to viral)

Switch to AAV/lenti/retro when you need: **uniform perturbation** (bulk -omics, stable pooled screens), **long-term expression across passages**, **inducible systems/lineage tracing**, or **clonal reproducibility** for definitive validation.

Best practice: NEPA21 (rapid decision + feasibility) → Viral (stable standardization + scale).

QC checklist (recommended minimum)

- **24h:** viability and recovery (do not optimize for efficiency if viability collapses).
- **48–96h:** delivery confirmation (reporter expression or edit check/amplicon).
- **Day 3–10:** phenotype window (markers, imaging, functional readout, drug response).
- **Decision gate:** optimize EP conditions OR repeat in additional lines OR standardize with viral.