# Mini Case Studies (Before/After)

**3–5 short examples showing time-to-result, throughput, and why viral came next**

**Interpretation:** Each case contrasts two *starting paths* for the same decision point: **Path A (viral-first)** vs **Path B (NEPA21-first)**.

## Examples

### Case Study 1 — CRC PDO go/no-go across a cohort

**Context:** CRC patient-derived organoids (PDOs) in a small biobank panel; evaluating candidate dependencies + drug-response modifiers.
**Decision point:** Early go/no-go on targets before committing to stable lines.

| Path A — Viral-first (traditional starting approach) | Path B — NEPA21-first (rapid decision-first approach) |
|---|---|
| Lenti cloning + packaging + transduction + selection per construct; limited capacity per week; slower iteration when targets fail. | Dissociate PDOs → electroporate CRISPR RNP for acute KO → reaggregate → run a 3–7 day perturb→treat loop; expand only the hits. |

**Time/throughput impact:** Typical iteration compresses from ~2–4 weeks per construct (vector + titer + selection) to ~3–7 days for an initial readout; enables parallel testing across more PDO lines per week (dependent on culture capacity).
**Why viral came next:** After a hit is confirmed across multiple PDOs, teams often move to viral for uniform perturbation and longitudinal passaging / bulk -omics reproducibility.

### Case Study 2 — PDAC stress/therapy interaction assay

**Context:** PDAC organoids with high sensitivity to manipulation; testing whether pathway X drives hypoxia/nutrient-stress survival under therapy pressure.
**Decision point:** Fast “perturb → treat” loop with minimal genomic footprint.

| Path A — Viral-first (traditional starting approach) | Path B — NEPA21-first (rapid decision-first approach) |
|---|---|
| Viral transduction required optimization for each PDO line; variable transduction efficiency and viability; longer ramp to stable expression/selection. | Acute CRISPR RNP delivery by NEPA21 → rapid recovery → immediate stress + therapy exposure; focus on early phenotype before adaptation. |

**Time/throughput impact:** Turns a multi-week optimization/selection cycle into a ~1-week loop for mechanistic signal detection; reduces per-construct setup overhead for difficult PDAC lines.
**Why viral came next:** Viral used when the next experiments require stable expression across passages, uniform perturbation for bulk assays, or inducible systems for timing control.

## Case Study 3 — Brain organoids: neighbour effects & timing windows

**Context:** Developmental-stage brain organoids; probing fate decisions and neighbour-dependent signalling (mosaic analysis).
**Decision point:** Cell-autonomous vs non-autonomous mechanism check + timing-sensitive intervention.

| Path A — Viral-first (traditional starting approach) | Path B — NEPA21-first (rapid decision-first approach) |
|---|---|
| Viral approaches often aim for broad transduction; uniformity can obscure neighbour contrasts; waiting for expression/selection blurs early timing windows. | Mosaic delivery via NEPA21 (RNP or reporter plasmid) at a chosen developmental stage → within-organoid comparison of edited vs unedited neighbours. |

**Time/throughput impact:** Enables stage-targeted intervention without waiting for viral expression; supports within-organoid controls and faster mechanistic triage before committing to stable lines.
**Why viral came next:** Once timing/mechanism is resolved, viral is used to standardize for long-term lineage tracing, inducible expression, or reproducible -omics across batches.

## Case Study 4 — Lung organoids: rapid fate regulator screen

**Context:** Lung epithelial organoids; testing differentiation/fate regulators (basal ↔ secretory decisions) and injury/inflammation response modifiers.
**Decision point:** Throughput scaling + rapid differentiation-stage perturbation.

| Path A — Viral-first (traditional starting approach) | Path B — NEPA21-first (rapid decision-first approach) |
|---|---|
| Viral build/transduction per construct; slower cadence for testing multiple regulators; timing mismatches with differentiation windows. | Electroporate RNP or plasmid reporters into dissociated organoid cells → reaggregate → read fate markers within 3–7 days; iterate fast on top candidates. |

**Time/throughput impact:** Increases the number of regulators testable per month by avoiding repeated viral prep; aligns perturbation timing tightly with differentiation windows.
**Why viral came next:** For definitive validation (stable expression, inducible control, or uniform perturbation for bulk readouts), teams standardize top regulators with viral systems.

### Case Study 5 — Kidney organoids: injury–repair programs under stress

**Context:** Kidney organoids; modelling nephrotoxic or ischemic-like injury and recovery programs; separating intrinsic susceptibility from neighbour effects.
**Decision point:** Acute perturbation under stress + mosaic susceptibility testing.

| Path A — Viral-first (traditional starting approach) | Path B — NEPA21-first (rapid decision-first approach) |
|---|---|
| Viral integration and selection add time and can shift baseline stress responses; difficult to preserve a clean ‘acute injury’ window. | Deliver RNP acutely via NEPA21 immediately before injury induction; use mosaic delivery to compare edited vs unedited neighbours within the same organoid. |

**Time/throughput impact:** Supports near-immediate perturbation prior to injury (hours–days) rather than waiting weeks; enables faster cycling across conditions and organoid batches.
**Why viral came next:** Once a robust injury-response modifier is identified, viral models help maintain consistent perturbation across passages for reproducible longitudinal studies.

## How to use these mini case studies

Use these as short, client-facing examples that reinforce the positioning: **NEPA21 to decide** → **Viral to standardize**. Replace the timing ranges with your validated internal benchmarks if you have them, and optionally add one published citation per disease area for proof.