Organoid-on-chip pipeline

Step	Pipeline stage	What it means (step-by-step)
1	**NEPA21 perturbation before chip loading**	This is the "genetic engineering step." • You electroporate (RNP / plasmid / etc.) into organoid cells or organoids off-chip. This avoids putting viral particles into microchannels/tubing and avoids dealing with diffusion/adsorption issues on-chip. **Think of it as: do the edit in a dish → then move edited material into the device.**
2	**recover → load into chip → perfuse → readout**	This is the "chip experiment step." • Recover: give the organoids time to survive the electroporation and stabilise. • Load into chip: seed into the device. • Perfuse: start flow/gradients/drug perfusion/hypoxia gradients/co-culture. • Readout: measure whatever the chip is built to measure (barrier function, polarity, morphology, secretomics, single-cell, imaging, etc.). **Think of it as: edited organoids experience the engineered microenvironment, then you measure the response.**

3	**decision/QC outputs**	This is the "what did we learn quickly?" step — the outputs that tell you whether to proceed.
		• It's not a separate experiment; it's the conclusion from step 2.
		• Typical "decision/QC outputs" in chip language are things like:
		  o Compatibility: did the perturbation keep viability, architecture, flow response intact?
		  o Timing: do you see a change at 24–72h (true acute response) vs only after adaptation?
		  o Spatial contrast: does mosaicism create informative neighbor comparisons under gradients?
		  o Signal attribution: does the phenotype look biological vs "delivery background"?
		  o Shortlist: which perturbations are worth repeating/standardizing?
		
		So "decision/QC outputs" really means: a fast go/no-go and a shortlist.

4	**viral approval gate**	This is simply a rule: only use viral once you've proven the biology and now need stability/uniformity.
		• So the "gate" question is:
		  o Do we now need viral because the next decision requires uniformity or long-term stability?
		  ▪ If no, stay with NEPA21 and iterate (different guide, timing, chip condition, matrix, flow rate, etc.).
		  ▪ If yes, you "graduate" to viral.

5	**viral transduction later**	This is the "standardize and scale" step.
		• You do viral only when you need:
		  o long-term uniform reporters across prolonged experiments
		  o lineage tracing across extended perfusion
		  o stable inducible systems
		  o uniform perturbation across all cells for bulk assays
		
		So viral is not competing with NEPA21 at the beginning.
		It's what you adopt once the NEPA21-based experiment has shown something worth stabilising