

Lentiviral Vector Development Assessment

What to Review Before Scaling Your Process

A practical technical framework for evaluating process performance, vector quality, analytical strategy, reproducibility, and scale-up readiness before committing additional resources.

PROCESS → PRODUCT → ANALYTICS → DATA → DECISION

Independent technical expertise for lentiviral vector development and process strategy.

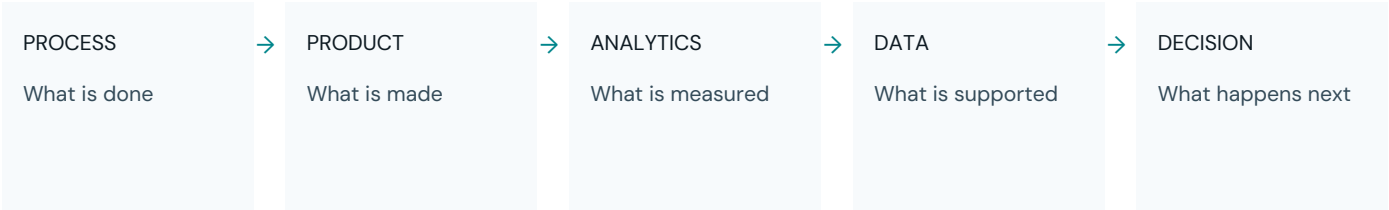
Technical development framework. Not regulatory advice, a validation protocol, or a determination of clinical suitability.

Before You Scale, Make Sure You Understand What You Are Scaling

Scale can amplify process variability, recovery losses and analytical uncertainty. Equipment effects, raw-material variability and poorly understood process dependencies can turn a promising small-scale result into an unreliable larger-scale process. More output is useful only if its quality and reproducibility remain interpretable.

FIVE QUESTIONS TO RESOLVE

- 01 What does “good” look like?
Define intended use, vector identity, relevant quality attributes and provisional decision criteria.
- 02 Which variables actually drive vector performance?
Separate parameters that are convenient to control from those supported by evidence.
- 03 Can the process be reproduced?
Review independent batches, cell and material history, operators and the sources of spread.
- 04 Do the analytical methods tell a consistent story?
Reconcile physical and functional measurements before interpreting a process improvement.
- 05 What could change during scale-up or technology transfer?
Identify equipment, timing, sampling and material differences before they become deviations.



A decision is only as sound as the links connecting it to the process and product. An analytical result without context is not yet a development conclusion.

Development Readiness Scorecard

Choose one status per area. Record the evidence or missing evidence, not just your confidence. The PDF fields are fillable in compatible readers; this page also works as a printed worksheet.

Program / vector:

Date:

GREEN / READY

Evidence supports the next step.

YELLOW / ATTENTION

Evidence is limited or inconsistent.

RED / GAP

Key evidence is absent or unresolved.

AREA	GREEN	YELLOW	RED	EVIDENCE / NEXT ACTION
Product Definition	☐	☐	☐	
Upstream Process	☐	☐	☐	
Downstream Process	☐	☐	☐	
Analytical Strategy	☐	☐	☐	
Data & Process Understanding	☐	☐	☐	
Scale-Up / Tech Transfer Readiness	☐	☐	☐	

Prioritize the weakest link—not an average score.

A green upstream assessment does not offset an unresolved functional assay or downstream loss. A red area is a development priority, not an automatic program failure.

Highest-priority uncertainty / owner / next review:

03 / PRODUCT & PROCESS

Product & Process Assessment

Assess the biological objective and the production system together. A parameter is meaningful only in the context of the vector design, cell platform and intended use.

PRODUCT DEFINITION

- ☐ Intended use. Define the target cells and required biological outcome; these determine what performance matters.
- ☐ Vector identity. Confirm construct, transgene, packaging configuration and envelope; changes can alter production and function.
- ☐ Functional requirements. Specify delivery and expression requirements, not merely an amount of vector.
- ☐ CQAs. Identify candidate critical quality attributes: identity, functional activity, purity and relevant impurities. Link each to product risk.
- ☐ Provisional acceptance criteria. Set development-stage decision boundaries, state their rationale and revise them as evidence matures.
- ☐ Physical vs. functional measures. Define what each result represents and what it cannot establish about biological activity.
- ☐ Known product risks. Record construct- or envelope-dependent limitations, instability and impurity concerns instead of assuming platform equivalence.

UPSTREAM PROCESS

- ☐ Cell health & history. Track viability, growth, passage and adaptation history; similar density can conceal different cell states.
- ☐ Plasmid quality. Review identity, integrity, purity and lot history; material changes can masquerade as process effects.
- ☐ Plasmid ratios. Document the ratio basis and rationale; the balance among components can affect particle production and function.
- ☐ Transfection conditions. Control preparation, addition and timing; consistent nominal inputs do not ensure consistent delivery.
- ☐ Culture conditions. Evaluate media, cell density, pH, gas transfer and metabolite trends in relation to performance.
- ☐ Harvest timing. Balance accumulated vector against deterioration and impurity burden; assess holds as well as collection time.
- ☐ Batch variability. Trend independent runs with material and operator metadata before attributing spread to biology.
- ☐ Scale-dependent effects. Consider mixing, delivery distribution and timing; matching concentrations alone is insufficient.

What to take away

A working product definition, traceable cell and material history, and a short list of process hypotheses supported by batch evidence.

Where Is the Vector Being Lost?

Map recovery in total functional units—not concentration alone. Concentration can increase TU/mL while the total amount of functional vector decreases.

PROCESS STEP	OBJECTIVE & LOSS HYPOTHESIS
Production	Generate vector Cell state, transfection and release; define the measurable starting point.
↓	
Harvest	Collect vector Collection timing, temperature and hold duration; distinguish continued release from loss.
↓	
Clarification	Remove cells / debris Adsorption, filter loading and retained liquid; separate partitioning from inactivation.
↓	
Concentration	Reduce volume / exchange buffer Membrane interactions, recirculation exposure and concentration-dependent behavior.
↓	
Purification	Separate vector / impurities Binding, elution and pool selection; recovery is meaningful only alongside impurity removal.
↓	
Formulation / Storage	Preserve function Buffer compatibility, contact surfaces, holds and freeze–thaw history.

Ask the same six questions at every stage.

What is the objective? What recovery is expected? What is measured? What could cause loss? Which variable is most likely to matter? What evidence supports the operating range?

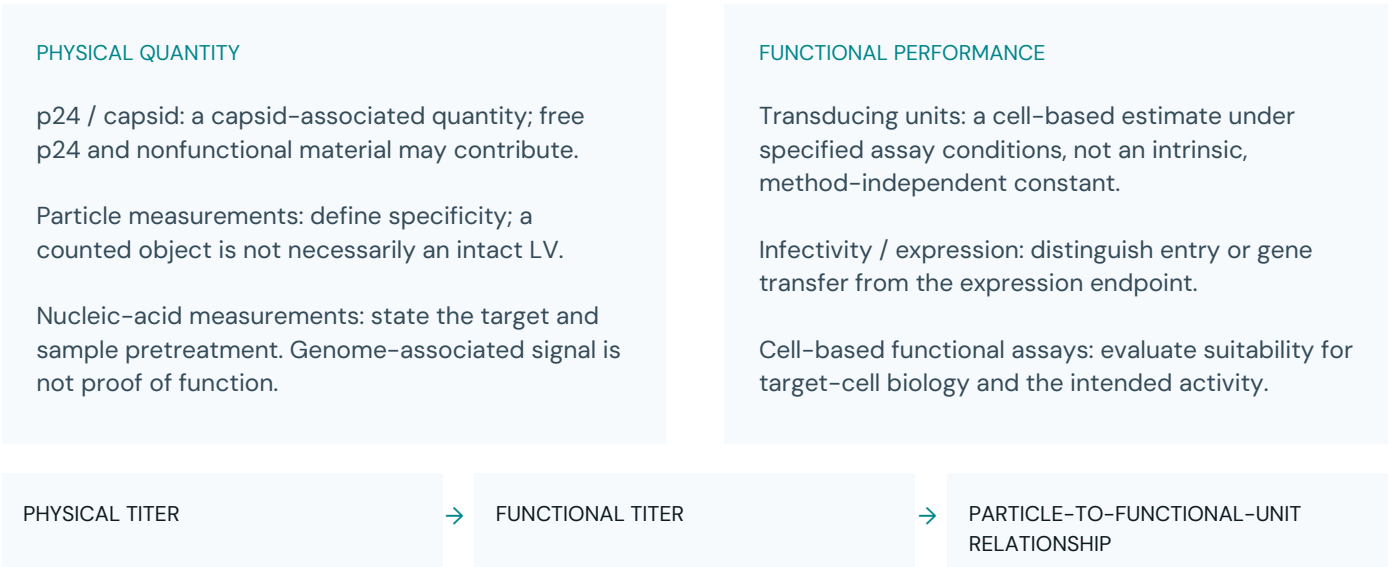
Step Recovery × Product Quality × Process Consistency

A decision lens, not a numerical score. Compare input and output TU/mL × volume using comparable assays and matched handling. Account for samples, discarded pools and hold losses; check matrix effects before concluding inactivation.

Evidence context: membrane design and module geometry can affect recovery [2, 3]. Selected references are on page 9.

A High Particle Count Does Not Automatically Mean a High-Performing Vector

Quantity and function answer different questions. No single assay establishes identity, activity, purity and suitability for intended use. [1]



Define the relationship before trending it. Specify numerator, denominator, units and method. p24 mass/TU is not particles/TU; genome/TU and particle/TU ratios are not interchangeable. There is no universal acceptance threshold.

Investigate both product and assay when results disagree. Stable physical signal with declining TU may indicate lost function, a changed population, matrix inhibition or altered assay conditions. A mismatch is a development signal—not automatically an assay failure.

REVIEW BEFORE COMPARING RESULTS

Assay variability & suitability	Precision, working range, reference material and cell-system responsiveness.
Handling, dilution & storage	Matched aliquots, timestamps, matrix, dilution behavior and freeze-thaw history.
Orthogonal methods & trends	Use complementary measurements; trend by method version and sample context.

Next step: compare matched physical and functional results across process stages, with controls and volume accounting. Evidence: [1]; references on page 9.

06 / DATA & EXPERIMENTS

Turn Data Into Development Decisions

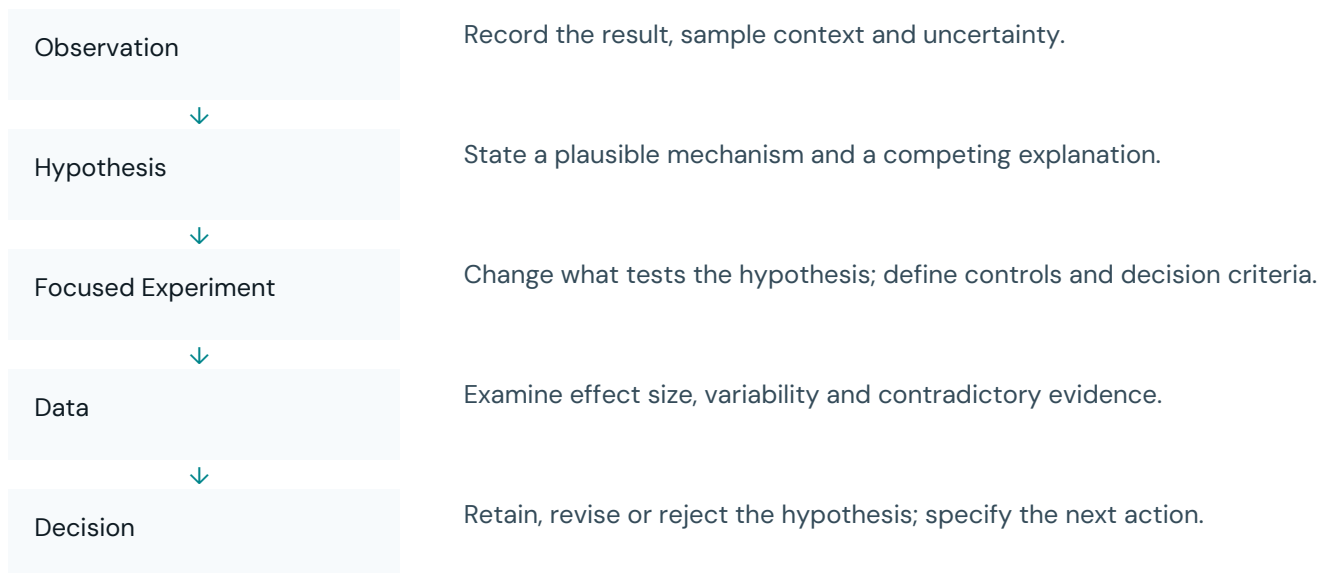
The most useful data are not always the largest dataset. They are the data that change a technical decision and make the next experiment more informative.

DESCRIPTIVE DATA

“Functional titer varied between batches.”
Describes the outcome but does not isolate its cause.

DECISION-MAKING DATA

“Does the observed shift exceed assay spread, and does it track a controlled process change?”
Defines a test and the decision it informs.



Trend batches with context. Link outcomes to cell history, raw-material lots, operators and equipment. Correlation generates hypotheses; it does not establish causation. Investigate outliers before excluding them.

Design experiments that can discriminate. Use controlled variables, suitable controls, independent process replication and randomized or blocked runs where feasible. DOE can expose interactions; it cannot rescue poor measurements or confounded runs. Technical assay replicates do not substitute for independent production batches.

What experiment would reduce the most uncertainty?

Before spending on another broad optimization campaign, identify the result that would change your next development decision.

07 / SCALE-UP & TRANSFER

Scaling Is Not Simply Making More Vector

Choose scale-up criteria by mechanism. Different operations may need different scaling bases; one equipment ratio cannot preserve every relevant condition. [2]

Mixing / mass transfer	Review mixing distribution, gas transfer, addition strategy and equipment geometry together.
Surfaces / equipment	Compare contact materials, surface-to-volume effects, flow paths and consumable configuration.
Timing / hold steps	Larger volumes can change addition, harvest, transfer and processing duration; evaluate time-temperature exposure.
Sampling / execution	Define representative sampling, material equivalence, operator actions and equipment differences.

SCALE-UP RISK MAP

LOW RISK	Well characterized / robust / reproducible Confirm that evidence applies to the proposed scale and equipment.
MODERATE RISK	Limited data / scale dependence possible Run a focused bridging study; define what would increase concern.
HIGH RISK	Poorly understood / highly variable / not demonstrated Resolve the mechanism or test the critical assumption before committing.

CDMO OR INTERNAL TRANSFER READINESS

- ☐ Process description & ranges. Versioned steps, parameter rationale and hold limits; proposed CPPs supported by product-impact evidence.
- ☐ Materials & equipment mapping. Specifications, qualified equivalence questions, contact materials and scale-dependent differences.
- ☐ Analytical handoff. Methods, sample handling, controls, reporting units and cross-site comparison plan.
- ☐ Knowledge transfer & ownership. Batch data, deviations, loss maps, training, responsibilities and a risk-based demonstration plan.

Readiness means the receiving team can execute, measure and explain the process—not merely receive a protocol.

08 / RED FLAGS & DECISIONS

10 Signs Your Process May Not Be Ready to Scale

Use these signals to prioritize investigation. Their presence does not establish that a process has failed; it identifies where a scale-up decision is not yet well supported.

- 01 Physical titer is the primary indicator of product performance without a justified functional link.
- 02 Batch-to-batch variability has not been investigated using comparable measurements.
- 03 Critical process parameters have been selected without evidence of product impact.
- 04 Assay variability is not understood well enough to interpret process differences.
- 05 A single successful batch is being treated as process validation.
- 06 Recovery losses have not been mapped across downstream steps using total units and volumes.
- 07 Scale-dependent risks, equipment differences and hold times have not been evaluated.
- 08 Experimental changes are being made without a clear hypothesis or decision criterion.
- 09 Increasing complexity is being used instead of improving process understanding.
- 10 The team cannot clearly explain what would make the next batch better—or how to test it.

Before scaling, identify what you know, what you think you know, and what you still need to prove.

Proceed when key evidence supports the next step. Bridge a bounded uncertainty with a focused study. Pause a scale commitment when unresolved risks could invalidate the conclusion.

SELECTED PRIMARY EVIDENCE / NOT A UNIVERSAL PROCESS RECIPE

[1] Geraerts et al. Comparison of lentiviral vector titration methods. BMC Biotechnology (2006). [doi:10.1186/1472-6750-6-34](https://doi.org/10.1186/1472-6750-6-34)

[2] Labisch et al. Scaling Up of Steric Exclusion Membrane Chromatography for Lentiviral Vector Purification. Membranes (2023). [doi:10.3390/membranes13020149](https://doi.org/10.3390/membranes13020149)

[3] Pamenter et al. Membrane adsorber design for lentiviral vector recovery. Molecular Therapy Methods & Clinical Development (2025). [doi:10.1016/j.omtm.2025.101533](https://doi.org/10.1016/j.omtm.2025.101533)

Where LentiWorks Can Help

Independent technical expertise for lentiviral vector development and process strategy. LentiWorks helps biotechnology companies, academic research groups and early-stage teams make better technical decisions.

Lentiviral Vector Development

Process development, optimization, troubleshooting and scale-up strategy.

Vector Characterization

Physical and functional characterization, analytical strategy, quality attributes and data interpretation.

Process & Experimental Strategy

DOE, process characterization, root-cause analysis and reproducibility assessment.

Technology Transfer & Development Readiness

CDMO technical evaluation, process-transfer preparation, scale-up risk assessment and development roadmap.

Fractional Technical Leadership

Experienced LV technical leadership for early-stage companies that need senior scientific judgment without immediately hiring a full-time senior executive.

LentiWorks is not a CDMO and does not manufacture clinical-grade vector. This technical development framework is not regulatory advice or a determination of clinical or regulatory qualification.

Have an LV development problem you need to pressure-test?

LentiWorks can review your process, data, analytical strategy, and development objectives to identify the highest-value technical questions and next steps.

[Discuss Your Program →](#)www.lentiworks.com