CELLECTA

USER MANUAL

CloneTracker XP™ Lentiviral Barcode Libraries

v1b — Last update: 15 July 2025

Cellecta, Inc.

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Table of Contents

1. CloneTracker XP™ Lentiviral Barcode Libraries 3
1.1. Detection of Red-Shifted Luciferase 4

2. Required Materials 6
2.1. Materials Provided 6
2.2. Additional Materials for Production of Lentivirus 8
2.3. Additional Materials for Transduction of Cells 9
2.4. Additional Materials for Genomic DNA Extraction 9
2.5. Additional Materials for NGS of sgRNA or Barcodes 9
2.6. Related Products and Services from Cellecta 10

3. Recommended Pilot Experiments 12
3.1. Cell Doubling Time 12
3.2. Calculating a Kill Curve 13
3.3. Check Toxicity of LentiTrans Transduction Reagent 13

4. Transduction of Founder Cells 14
4.1. General Lentiviral Packaging Protocol 14
4.2. General Lentiviral Transduction Protocol 18
4.3. Assessing Transduction Efficiency by Flow Cytometry or Antibiotic Selection 19
4.4. Lentiviral Titer Calculation 20

5. Extraction and Sequencing of sgRNA or Barcodes from Genomic DNA 23
5.1. Genomic DNA Extraction 23
5.1.1. Large-Scale Genomic DNA Extraction Protocol 23
5.2. Amplification of sgRNA or Barcode Sequences from Genomic DNA 24
5.3. NGS Sample Purification 28
5.4. Sequencing sgRNA Inserts or Barcodes 28
5.5. Antibiotic Selection of Library Transduced Cells 30

6. Detection of Expressed Barcodes or sgRNA with scRNA-Seq 32
6.1. 10X Chromium 3’ Detection of Barcodes/sgRNA in pScribe Libraries 33

7. Technical Support 35

8. Safety Guidelines 36

9. Contact Us 37

1. CloneTracker XP™ Lentiviral Barcode Libraries

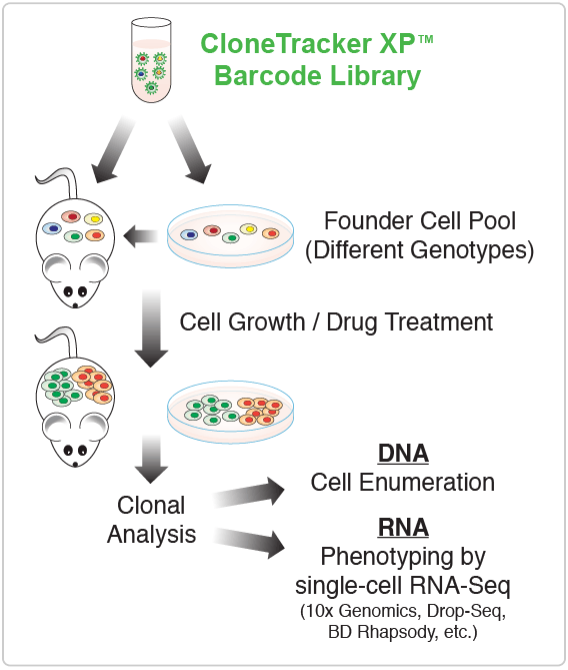
Cellecta's CloneTracker XP™ Lentiviral Barcode Libraries are pooled expressed barcode libraries that enable the tracking and profiling of individual clones derived from a population of cells using either PCR/NGS of barcodes integrated in genomic DNA or RNA-Seq of transcribed barcodes. Pre-made pooled libraries consisting of either 1 million or 10 million barcoded constructs are available, with each construct expressing a unique barcode sequence.

When a cell population is transduced with a CloneTracker XP Barcode Library, the barcodes integrate into the genomic DNA of the cells. The result is a starter founder population where almost every cell has a different barcode integrated into its DNA. Since the barcode is stably integrated, it is passed onto any cell progeny when genomic DNA is replicated. This feature enables identification of all the progeny derived from each individual cell.

When used in conjunction with single-cell RNA-Seq, the expressed barcode may be used to identify expression profiles and activated genes in different cells so that distinct clonal populations of cells from a single progenitor, as well as sub-populations of cells with distinct pathway activations or expression profiles, can be readily identified.

Additionally, CloneTracker XP barcode libraries, similarly to the standard CloneTracker (CellTracker) 50M Barcode Library (discontinued as of Dec. 2023), allow researchers to track cell proliferation over the course of an experiment. For instance, it is possible to:

- Track the fate of cell clones over a course of drug treatment.
- Study the effect of differentiation on hematopoietic cells.
- Assess the effect of treatment on implanted tumor cell populations.



Cellecta's CloneTracker XP Barcode Library allows the tracking and profiling of founder cell clones

The CloneTracker XP Barcode Libraries are constructed in Cellecta third-generation lentiviral vectors. The specially-designed, optimized barcodes facilitate Next-Generation Sequencing (NGS) data analysis and barcode identification. Using an Illumina NGS instrument, barcode sequences are identified and converted to lists with enumerated data.

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The protocols included provide guidelines for packaging the CloneTracker XP Barcode Library into VSV-G pseudotyped lentiviral particles, transduction of target cells, and the preparation of barcodes derived from transduced cells for NGS, enumeration, and analysis.

References and Product Citations for all Cellecta products can be found on the Cellecta website: https://cellecta.com/pages/citations-publications.

Please read the entire user manual before proceeding with your experiment. Also, please note that, when working with pseudoviral particles, you should follow the recommended guidelines for working with Biosafety Level 2 (BSL-2) materials.

Click the "Download as PDF" link located at the bottom of the left menu to download the PDF version of this user manual.

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1.1. Detection of Red-Shifted Luciferase

**Some Cellecta CloneTracker XP libraries incorporate a red-shifted luciferase (rLuc) derived from *Photinus pyralis* (Ppy RE9 mutant) as a reporter for bioluminescent imaging (BLI). The CloneTracker XP libraries with the Ppy RE9 rLuc were designed for this purpose. Luciferase reporters provide a useful tool for indirect cell labelling and tracking of cell fates *in vivo*. While the BLI technique has been successfully used for monitoring of migration, growth, and viability associated with drug treatment of transplanted cancer, stem, or immune cells in mouse models, standard luciferase assays in animal models are particularly sensitive because they are not subject to high background as a result of tissue autofluorescence. Far-red emitting enzymes offer enhanced sensitivity and resolution in deep tissue.

CloneTracker XP barcoded libraries with rLuc employ a highly sensitive, codon-optimized rLuc that emits 50-100-fold better light intensity than the click beetle (*Renilla*) enzyme. Cancer cells transduced with CloneTracker XP libraries that have the rLuc reporter can be used for monitoring tumor and metastasis growth in drug-treated mouse models. BLI of rLuc-tagged cells *in vivo* requires the use of an appropriate imager for detecting photons generated from D-Luciferin substrate by CCD cameras able to sample the entire visible and near infrared spectrum. For details on *in vivo* imaging, please refer to the standard protocols provided with your instrument, for example, In vivo Bioluminescence Imaging of Luciferase-labeled Cancer Cells protocol for BLI using the IVIS Spectrum imager from PerkinElmer.

Additional information, protocols, and applications can also be found in the following references:

> Branchini BR, Ablamsky DM, et al. Red-emmiting luciferases for bioluminescence reporter and imaging applications. Anal. Biochem. (2010) 396:290-297.
>
> Kim JE, Kalimthu S, Ahn BC. In vivo cell tracking with bioluminescense imaging. Nucl. Med. Mol. Imaging (2015) 49:3-10.

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Kosher B, Piwnica-Worms D. Illuminating cancer systems with genetically engineered mouse models and coupled luciferase reporters in vivo. Cancer Discov. (2013) 3:616-629.

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2. Required Materials

The following sections detail the materials provided with Cellecta's CloneTracker Lentiviral Barcode Libraries and the additional materials required to carry out the protocols associated with using this library.

Last modified: 29 December 2023

2.1. Materials Provided

Cellecta's CloneTracker XP Lentiviral Barcode Libraries are provided as plasmid DNA or as packaged VSV-G pseudotyped viral particles.

CloneTracker XP Barcode-3' Libraries with RFP-Puro

Cat.#	Description	Quantity	Storage
BCXP50M3RP-P	CloneTracker XP™ 50M Barcode-3' Library in pScribe4M-RFP-Puro (plasmid)	200 µg	-20°C
BCXP5M3RP-XS-P	CloneTracker XP™ 5M Barcode-3' Library in pScribe4M-RFP-Puro (plasmid) —10 different uniquely barcoded libraries available—discount for purchase or 2+	200 µg	-20°C
BCXP50M3RP-V	CloneTracker XP™ 50M Barcode-3' Library in pScribe4M-RFP-Puro (virus)	1×10^8 TU	-80°C
BCXP5M3RP-XS-V	CloneTracker XP™ 5M Barcode-3' Library in pScribe4M-RFP-Puro (virus) —10 different uniquely barcoded libraries available—discount for purchase or 2+	1×10^8 TU	-80°C

CloneTracker XP Barcode-3' Libraries with RFP-Thy1.1

Cat.#	Description	Quantity	Storage
BCXP50M3RT-P	CloneTracker XP™ 50M Barcode-3' Library in pScribe4ThyM-RFP-Thy1.1 (plasmid)	200 µg	-20°C
BCXP50M3RT-V	CloneTracker XP™ 50M Barcode-3' Library in pScribe4ThyM-RFP-Thy1.1 (virus)	1×10^8 TU	-80°C

CloneTracker XP Barcode-3' Libraries with Venus-Puro

Cat.#	Description	Quantity	Storage
BCXP10M3VP-P	CloneTracker XP™ 10M Barcode-3' Library in pScribe5-Venus-Puro (plasmid)	200 µg	-20°C
BCXP10M3VP-	CloneTracker XP™ 10M Barcode-3' Library in pScribe5-Venus-Puro (virus)	1×10^8	-80°C

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CloneTracker XP Barcode-3’ Libraries with rLuc-Puro

Cat.#	Description	Quantity	Storage
BCXP10M3LP-P	CloneTracker™ XP-rLuc 10M Barcode-3’ Library in pScribe6-rLuc-Puro (plasmid)	200 µg	-20°C
BCXP10M3LP-V	CloneTracker™ XP-rLuc 10M Barcode-3’ Library in pScribe6-rLuc-Puro (virus)	1×10^8 TU	-80°C

CloneTracker XP Barcode-5’ Libraries with Venus-Puro

Cat.#	Description	Quantity	Storage
BCXP10M5VP-P	CloneTracker XP™ 10M Barcode-5’ Library in pScribe5.2-Venus-Puro (plasmid)	200 µg	-20°C
BCXP10M5VP-V	CloneTracker XP™ 10M Barcode-5’ Library in pScribe5.2-Venus-Puro (virus)	1×10^8 TU	-80°C

Product Information

For information on the specific CloneTracker XP Barcode Library that you have purchased, please review the product information you received by email upon delivery (either by link or attachment):

Product Certificate (PC or PAC)

The Product Certificate contains important information such as the viral titer and volume (for libraries provided as viral particles), complexity or number of independent clones, mutation rate, clone sequencing Q.C. data, NGS QC statistics and graphs, and Lot Number. Product Certificates for pre-made CloneTracker XP Libraries may be also downloaded from the Cellecta Product Certificates web page and are organized by Lot Number.

Barcode Sequence Files

Provided as Microsoft Excel files upon purchase, the sequence files contains barcode sequences and NGS QC data including read counts and distribution. If you have not received it, please contact us at tech@cellecta.com.

NGS Cassette Diagram

Available in PDF format, the NGS cassette diagram shows the barcode sequence structure, design of the barcode expression cassette including location of PCR and NGS primers, and primer sequences. The NGS cassettes of Cellecta’s standard CloneTracker XP pScribe and pScribeM vectors are available on the Cloning and Expression Vector Information web page.

Vector Map and Sequence

CloneTracker XP pScribe and pScribeM vector sequences are available in SnapGene, GenBank/VectorNTI (.gb), and FASTA formats. The sequences are available on the Cloning and Expression Vector Information web page.

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Alignment Software Supporting Files

The Cellecta LNGS Prep Kits include the Alignment Software which is used to demultiplex and deconvolute Illumina NGS data. Supporting files such as the Library Configuration Files (.json format) and Sample Description Form are available on the respective CloneTracker XP Product Pages.

Additional information on Cellecta's pre-made libraries can be found on the following web pages:

- CloneTracker XP™ Lentiviral Expressed Barcode Libraries
- CloneTracker™ XP-rLuc Lentiviral Expressed Barcode Libraries

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2.2. Additional Materials for Production of Lentivirus

- 293T Cell Line (e.g. 293T/17, ATCC, Cat.# CRL-11268™ or 293FT, Thermo Fisher, Cat.# R70007)
- Ready-to-use Lentiviral Packaging Plasmid Mix (Cellecta, Cat.# CPCP-K2A). Libraries can be packaged into lentiviral particles with nearly any 2nd or 3rd generation HIV-based lentiviral packaging mix. Cellecta's 2nd generation lentiviral packaging mix contains two plasmids: psPAX2 and pMD2.G, pre-mixed in a ratio optimized for production of lentivirus.
- Dulbecco's Modified Eagle Medium (D-MEM) (1X) (Corning cellgro™, Cat.# 15-013-CV)

> **NOTE:** ADD FRESH GLUTAMINE (1X) at the time a sealed bottle of D-MEM is opened, even if the label indicates glutamine has already been added. Glutamine in solution at 4°C has a half-life of 1-2 months, so glutamine(+) D-MEM purchased "off-the-shelf" from a supplier is to be regarded as glutamine(-). In our experience, the addition of glutamine increases titer approximately 2-fold. If D-MEM comes supplemented with stable L-Alanyl-L-Glutamine dipeptide, addition of fresh glutamine is not necessary.

- Glutamine (L-Alanyl-L-Glutamine, Dipeptide L-glutamine) (Corning glutagro™, Cat.# 25-015-CI)
- Fetal Bovine Serum (Recommended: Corning, Cat.# 35-010-CV)
- Trypsin-EDTA, 1X (Corning, Cat.# 25-052-CI)
- Tissue Culture Plates
  - 150 × 25mm (15-cm) Tissue Culture Plates (Corning, Cat.# 430599)
  - 100 × 20mm (10-cm) Tissue Culture Plates (Corning, Cat.# 430167)
- Related Tissue Culture Supplies
- Lipofectamine® Transfection Reagent (Thermo Fisher, Cat.# 18324020)
- PLUS Reagent (Thermo Fisher, Cat.# 11514015)
- DNase I, RNase-free (Epicentre-Illumina, Cat.# D9905K)
- HEPES pH 7.2-7.6, 1M solution (Corning, Cat.# 25-060-CI)
- $MgCl_2$, 1M solution
- Nalgene™ Rapid-Flow™ Sterile 500 ml, 0.2 µm PES filter units (Thermo Fisher Cat.# 569-0020)
- LentiFuge™ Viral Concentration Reagent (Cellecta, Cat.# LFVC1) *(Optional, for concentration of virus)*
- D-PBS, 1X (Corning, Cat.# 21-031-CV)

Other than the specific reagents and instruments listed above, the protocols assume the user has access to standard materials (e.g., polypropylene tubes, pipette tips), equipment (table top centrifuges, pipettes, scales), and common reagents (e.g., TE buffer, ethanol) and buffers used in a typical life science laboratory.

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2.3. Additional Materials for Transduction of Cells

The materials below are based on the transduction protocol in this manual which has been optimized for HEK293 and K-562 cells. Depending on the characteristics of your specific cells, the choice of media or other cell culture specifics may be different.

- LentiTrans™ Transduction Reagent (Cellecta, Cat.# LTDR1)
- Puromycin, 10 mg/ml (Sigma-Aldrich, Cat.# P9620-10ML)
- Dulbecco's Modified Eagle Medium (D-MEM) (1X) (Corning cellgro™, Cat.# 15-013-CV)
- Glutamine (L-Alanyl-L-Glutamine, Dipeptide L-glutamine) (Corning glutagro™, Cat.# 25-015-CI)
- Fetal Bovine Serum (Recommended: Corning, Cat.# 35-010-CV)
- Trypsin-EDTA, 1X (Corning, Cat.# 25-052-CI)
- Tissue Culture Plates and Related Tissue Culture Supplies
- HEPES pH 7.2-7.6, 1M solution (Corning, Cat.# 25-060-CI)
- $MgCl_2$, 1M solution
- Doxycycline, Ready Made Solution, 100 mg/ml (Sigma-Aldrich, Cat.# D3072-1ML) *(Optional, for tet-inducible vectors)*

Other than the specific reagents and instruments listed above, the protocols assume the user has access to standard materials (e.g., polypropylene tubes, pipette tips), equipment (table top centrifuges, pipettes, scales), and common reagents (e.g., TE buffer, ethanol) and buffers used in a typical life science laboratory.

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2.4. Additional Materials for Genomic DNA Extraction

- QIAamp DNA Micro Kit (QIAGEN, Cat.# 56304) — **for <1M cells**
- DNeasy Blood and Tissue Kit (QIAGEN, Cat.# 69504) — **for 1M-10M cells**
- Falcon® 15ml PP Screw-Cap Centrifuge Tubes (12,000 RCF rated, P:CHCl3-resistant) (Corning, Cat.# 352196)
- Buffer P1 (50mM Tris-HCl pH 8.0, 10mM EDTA) (QIAGEN, Cat.# 19051)
- Sodium Dodecyl Sulfate (SDS), 10% solution (Thermo Fisher, Cat.# 24730020)
- RNase A (QIAGEN, Cat.# 19101)
- Sonicator, for shearing Genomic DNA
- Proteinase K from Tritirachium album (Sigma-Aldrich, Cat.# P4850)
- Phenol:Chloroform:Isoamyl Alcohol 25:24:1 pH 8.0 (Sigma-Aldrich, Cat.# P3803)
- Sodium Acetate, 3M solution (Sigma-Aldrich, Cat.# 71196- 100ML)
- Isopropanol (2-Propanol) (Sigma-Aldrich, Cat.# I9516-500ML)
- Linear Acrylamide (5 mg/ml) (Thermo Fisher, Cat.# AM9520) — **for <5M cells**

Other than the specific reagents and instruments listed above, the protocols assume the user has access to standard materials (e.g., polypropylene tubes, pipette tips), equipment (table top centrifuges, pipettes, scales), and common reagents (e.g., TE buffer, ethanol) and buffers used in a typical life science laboratory.

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2.5. Additional Materials for NGS of sgRNA or Barcodes

- PCR and NGS primers for sgRNA/barcode amplification and sequencing (IDT): See NGS Cassette Diagram, provided as a link or attachment in email upon shipment of product or in Product Certificate of Library NGS Prep Kit.

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> For your convenience, we also offer **Library NGS Prep Kits** which provide primers and reagents to prepare samples screened with your library. The NGS Prep Kit and Supplementary Primer Set each contain 12 unique indexing primers that can be used to multiplex up to 24 screening samples on a flow cell or lane.

- Titanium Taq DNA Polymerase with PCR buffer (Clontech-Takara, Cat.# 639242)
- dNTP Mix (10 mM each) (Thermo Fisher, Cat.# 10297018)
- QIAquick PCR Purification Kit (QIAGEN, Cat.# 28106)
- QIAquick Gel Extraction Kit (QIAGEN, Cat.# 28706)
- PhiX Control (Illumina, Cat.# FC-110-3001)
- NGS Reagent Kits, for NextSeq and HiSeq (Illumina):

Platform	Kit Type	Illumina Cat.#	Description
NextSeq® Single Read (SR) or Paired End (PE)	Sequencing & Cluster Gen.	20024906	NextSeq 500 v2.5 Kit (75 cycles, High-Output)
HiSeq® 2000/2500 **Single Read (SR)**	Sequencing	GD-401-3001	TruSeq SBS Kit v3 – HS (50 cycles)
	Cluster Gen.	FC-401-3002	TruSeq SR Cluster Kit v3-cBot-HS
HiSeq® 2000/2500 **Paired End (PE)**	Sequencing	GD-401-3001	TruSeq SBS Kit v3 – HS (50 cycles)
	Cluster Gen.	PE-401-3001	TruSeq PE Cluster Kit v3-cBot-HS
HiSeq® 3000/4000 **Single Read (SR)**	Sequencing	FC-410-1001	HiSeq 3000/4000 SBS Kit (50 cycles)
	Cluster Gen.	GD-410-1001	HiSeq 3000/4000 SR Cluster Kit
HiSeq® 3000/4000 **Paired End (PE)**	Sequencing	FC-410-1001	HiSeq 3000/4000 SBS Kit (50 cycles)
	Cluster Gen.	PE-410-1001	HiSeq 3000/4000 PE Cluster Kit

> **NOTE:** See Illumina website for information on kits for other Illumina instruments (e.g. NovaSeq, NextSeq 1000/2000, MiSeq).

Other than the specific reagents and instruments listed above, the protocols assume the user has access to standard materials (e.g., polypropylene tubes, pipette tips), equipment (table top centrifuges, pipettes, scales), and common reagents (e.g., TE buffer, ethanol) and buffers used in a typical life science laboratory.

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2.6. Related Products and Services from Cellecta

Related Products

Description	Cat.#	Quantity
NGS Prep Kit for Barcode Libraries in pScribe (CloneTracker XP™)	LNGS-300	Kit for 6-48 samples (48 preps of 50 µg each)
Supplementary Primer Set for LNGS-300 (12 Additional Index Primers)	LNGS-300-SP	48 Reactions (12 Index Primers x 4 reactions each)
Ready-to-Use Lentiviral Packaging Plasmid Mix	CPCP-K2A	250 µg (25 transfections in 10-cm plates)
LentiFuge™ Viral Concentration Reagent	LFVC1	1 ml of 1000X solution
LentiTrans™ Transduction Reagent (Polybrene for Transduction)	LTDR1	1 ml of 1000x

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LentiPrep™ Lentiviral Reagent Set with GFP Control for Packaging and Transduction	LTSET-G	CPCP-K2A x 1 LFVC1 × 1 LTDR1 × 1 GFP Ctrl
LentiPrep™ Lentiviral Reagent Set with RFP Control for Packaging and Transduction	LTSET-R	CPCP-K2A x 1 LFVC1 × 1 LTDR1 × 1 RFP Ctrl

Related Services

- Lentiviral Library Packaging Services
- NGS of Samples from Genetic Screens
- Custom Pooled Barcode Libraries

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3. Recommended Pilot Experiments

In order to obtain reliable data from your library screen or individual construct experiment, we suggest appropriate planning beforehand. We recommend the pilot studies below in your cell system of choice (the cell system that will be used in your experiment). Cell-type specific data from these pilot experiments will provide you more confidence in your results.

Using the following procedures, confirm the cell doubling time, antibiotic efficacy on the target cells, and sensitivity of the target cells to the LentiTrans Transduction Reagent.

- Cell Doubling Time
- Calculating a Kill Curve
- Check Toxicity of LentiTrans Transduction Reagent

Visit the Cellecta Vector Information web page for details on our most common sgRNA, shRNA, and barcode lentiviral vectors.

Last modified: 7 May 2024

3.1. Cell Doubling Time

The doubling time is the time it takes your cells to double in number. It is useful to know the doubling time of your cells so that you can plate the appropriate number for transduction with a lentiviral library or construct.

1. Start with cells that have already been growing for a few weeks, rather than using cells that have just been thawed from a frozen state. To calculate the doubling time, trypsinize your cells as if you were going to split them.

2. Count them using a hemacytometer or cell counter, and keep track of the number that you replate onto the cell culture plates. The starting number of cells (at the beginning) is ***Xb***.

3. Propagate the cells as you normally do, replacing media as necessary.

4. The next time they are ready to be split, trypsinize them as usual and count them again using a hemacytometer or cell counter. The number of cells at the end is referred to as ***Xe***.

> **NOTE:** The cells should be in the log phase of growth to calculate doubling time properly, so it is important to not let the cells become confluent.

To calculate the doubling time, use the following formula:

> *Doubling Time* = [***T*** × (ln2)] / [ln (***Xe*** / ***Xb***)]
> where ***T*** = Time in any units

Example

> Let's say that on Day 0, you count 2 × 10^6 cells. Three (3) days later, you count 16 × 10^6 cells.
>
> ***Xb*** = 2 × 10^6

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> ***T*** = 3 days
> ***Xe*** = 16×10^6
>
> Doubling Time = [3 × (ln2)] / [ln(16,000,000 / 2,000,000)]
> = [3 × (0.69)] / [ln(8)]
> = 2.08 / 2.08 = **1 day**

Last modified: 29 December 2023

3.2. Calculating a Kill Curve

Most of the lentiviral vectors used in making Cellecta's libraries express an antibiotic resistance marker. Regardless of the antibiotic selection marker of the library, you need to know the concentration of antibiotic that kills non-transduced cells within a given amount of time in order to successfully select transduced cells. In order to avoid the enrichment of double integrants and loss of single-integrants, we recommend avoiding over-selection: calculate an antibiotic kill curve in your cell line beforehand and choose the lowest antibiotic concentration which gives 90%-95% killing. For fine-tuning of the antibiotic concentration, if the lentivirus carries both an antibiotic selection marker and a fluorescence marker, follow the procedure described in the Antibiotic Selection of Library Transduced Cells section.

Last modified: 15 July 2025

3.3. Check Toxicity of LentiTrans Transduction Reagent

The LentiTrans™ Transduction Reagent used during the transduction of target cells with lentivirus contains Polybrene, a transduction enhancement reagent. Some cell lines may be sensitive to this reagent. Before doing large-scale transduction, we recommend checking the toxicity of the LentiTrans Reagent using the procedure below:

1. In six wells of a 12-well plate, grow cells for 24 hours in complete culture medium without the LentiTrans Reagent, and with LentiTrans added at 0.25 µl/ml, 0.5 µl/ml and 1:1000 (1 µl/ml).

2. Replace old medium with LentiTrans-free complete culture medium and grow cells for an additional 72 hours.

3. Check for toxicity by counting viable cells.

For your experiments, use the highest concentration of LentiTrans Reagent that results in less than 10% cell toxicity compared to the no-LentiTrans culture (typically, a 1:1000 is recommended). For a few cell types, you may not be able to use LentiTrans Reagent.

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4. Transduction of Founder Cells

By transducing the CloneTracker or CloneTracker XP Barcode Library into a large pooled cell population, you can create a founder population in which each cell contains a unique integrated barcode. During transduction, the library of lentiviral constructs carrying each barcode enter the cells and stably integrate into the genomic DNA. Each lentiviral construct also has an RFP marker and puromycin selection to help maintain the barcode cassette.

Notes on Transducing Founder Cells

- Cell transduction is a random process following a statistical distribution. Therefore, if too high an MOI is used, many cells will take up more than one barcode. Cells with more than one barcode show up as more than one population in the final analysis. For example, if two barcodes integrate into one founder cell, and the cell produces 50 progeny, the data (after harvesting the cells and Next-Gen Sequencing of the genomic DNA) will show two clonal populations with two different barcodes, each having the same number of cells. It will not be obvious that these two populations are from the same founder cell. For this reason, we typically recommend using low MOIs of <0.2 so that >90% of the transduced cells only contain one barcode.

- Transducing larger populations of cells increases the frequency of having more than one founder cell with the same barcode. If you want to minimize having multiple founder cells transduced with the same barcode, we recommend transducing a cell number that is less than ~7% of the barcodes in the library. In other words, for 50M barcodes libraries, transduce a maximum of 3.5M cells, for libraries with 10M barcodes, a maximum transduction of 700,000 cells, etc. For more details on the complexity and representation of barcodes in the library, please refer to the QC information available on the PAC or described in the Quality Control section.

- Since the purpose of using the CloneTracker Barcode Library is to track the fate of cells from a founder population, we do not recommend splitting and discarding any cells during the course of an experiment:
    - Discarding any portion of cells will eliminate some of the barcodes unless the cell population has expanded to several orders of magnitude over the founder population size.
    - Even if the population has expanded many fold over, splitting or otherwise discarding cells may skew the clonal population sizes for different barcodes (i.e., clones that grow slowly versus cells that grow quickly may be differentially affected).
    - If you must discard cells, it is crucial to design the experiment to minimize the impact on the barcode representation in your samples.

- Following transduction, you may want to select cells with barcodes using the appropriate antibiotic for the library vector (typically puromycin). To empirically determine the concentration of antibiotic that kills untransduced cells within a given amount of time, you should calculate a Kill Curve as described in the **Recommended Pilot Experiments** section.

To transduce your target cells, follow the General Lentiviral Transduction Protocol.

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4.1. General Lentiviral Packaging Protocol

The following protocol describes the general procedure for generation of pseudoviral packaged lentiviral constructs using ThermoFisher's Invitrogen Lipofectamine™ and PLUS Reagent (see **Additional Materials for Production of Lentivirus**). Other transfection reagents may be used, but the protocol should be adjusted to fit the manufacturer's protocol. This protocol can be used to package individual lentiviral plasmid constructs expressing shRNA, sgRNA, Cas9/dCas9, barcodes, cDNA,

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promoter reporters, and sgRNA, shRNA, or barcode libraries in 3rd generation lentiviral vectors.

- The yield of recombinant lentiviral particles produced under these optimized conditions is typically between 1 × 10^6 TU/ml and 5 × 10^6 TU/ml for individual lentiviral constructs with a viral transcript less than 7kb (i.e. the region from the beginning of 5'LTR to the end of 3'LTR).

Plate Size	Surface Area	Volume of Media	Approximate Virus Yield *
10 cm	55 cm^2	10 ml	2 × 10^7 TU
15 cm	150 cm^2	30 ml	6 × 10^7 TU

> * **NOTE: The yield of lentiviral particles will be *significantly lower* for lentiviral constructs and libraries with viral transcripts larger than 7kb (e.g., approximately 1 × 10^5 TU/ml for Cas9 constructs with viral transcripts around 8kb).**

- If using tissue culture plates or flasks of other sizes, please scale amounts in the protocol based on surface area.

Cellecta offers Lentiviral packaging services. Please contact us at sales@cellecta.com or visit Cellecta's Lentiviral Packaging Services web page.

2-3 Days Prior to Packaging

1. Start growing 293T cells in D-MEM medium plus glutamine* supplemented with 10% FBS without antibiotics, and expand until you have sufficient cells to package at the scale desired.

> * **NOTE:** ADD FRESH GLUTAMINE (1X) to Dulbecco's Modified Eagle Medium (D-MEM) at the time a sealed bottle of D-MEM is opened, even if the label indicates glutamine has already been added. Glutamine in solution at 4°C has a half-life of 1-2 months, so glutamine(+) D-MEM purchased "off-the-shelf" from a supplier is to be regarded as glutamine(-). If D-MEM comes supplemented with stable L-Alanyl-L-Glutamine dipeptide, addition of fresh glutamine is not necessary. In our experience, the addition of glutamine increases titer approximately 2-fold.

Day 0 – Plate Cells

1. Twenty four (24) hours prior to transfection, plate 12.5 × 10^6 293T cells per **15-cm plate** (or 150 cm^2 flask) and use 30 ml of media per plate. If you are using **10-cm plates**, plate 4 × 10^6 cells and use 10 ml of media per plate. Disperse the cells and ensure even distribution.

2. Incubate at 37°C in a CO_2 incubator for 24 hours.

> **NOTE:** The goal is to have the 293T cells reach ~80% confluency by Day 1. You may want to calculate the number of cells seeded empirically since cell counts can vary.

Day 1 – Transfection into 293T Cells

1. Using the volumes in the table below, mix Ready-to-use Lentiviral Packaging Plasmid Mix and your Plasmid Lentiviral

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construct in a sterile, appropriately-sized polypropylene tube. Add the D-MEM medium without serum or antibiotics to the plasmid mixture, then mix. Add the PLUS Reagent, mix, and incubate at room temperature for 15 minutes.

1 × 10-cm plate	1 × 15-cm plate	4 × 15-cm plates	8 × 15-cm plates	16 × 15-cm plates	24 × 15-cm plates	Component
20 µl	60 µl	240 µl	480 µl	960 µl	1440 µl	Packaging Plasmid Mix (0.5 µg/µl)
2 µl	6 µl	24 µl	48 µl	96 µl	144 µl	Plasmid / Library (1 µg/µl)* *see **NOTE***
1,000 µl	1,200 µl	4,800 µl	9,600 µl	19,200 µl	28,800 µl	D-MEM, no FBS, no antibiotics
20 µl	60 µl	240 µl	480 µl	960 µl	1,440 µl	PLUS Reagent
1,042 µl	**1,326 µl**	**5,304 µl**	**10,608 µl**	**21,216 µl**	**31,824 µl**	**Total volume**

> **NOTE:** The volume of plasmid DNA assumes the DNA is suspended at a 1 µg/µl concentration. For plasmid DNA at other concentrations, adjust the volume accordingly (e.g., for 0.5 µg/µl, use twice the indicated µl).

2. Add Lipofectamine Reagent to the D-MEM medium without serum or antibiotics in order to make a master mix according to the table below. Mix gently.

1 × 10-cm plate	1 × 15-cm plate	4 × 15-cm plates	8 × 15-cm plates	16 × 15-cm plates	24 × 15-cm plates	Component
1,000 µl	1,200 µl	4,800 µl	9,600 µl	19,200 µl	28,800 µl	D-MEM, no FBS, no antibiotics
30 µl	90 µl	360 µl	720 µl	1,440 µl	2,160 µl	Lipofectamine Reagent
1,030 µl	**1,290 µl**	**5,160 µl**	**10,320 µl**	**20,640 µl**	**30,960 µl**	**Total volume**

3. Add the diluted Lipofectamine (from Step 2) to the DNA / PLUS Reagent complex (from Step 1 above), mix gently by flicking the tube or vortexing and incubate at room temperature for 15 minutes.

4. Add 2.5 ml of the resulting DNA / PLUS Reagent / Lipofectamine complex to each plate (from the previous step **Day 0 – Plate Cells**), and mix complexes into the medium with gentle rotation. Take care not to dislodge cells from the plate.

5. Incubate at 37°C in the CO_2 incubator for 24 hours.

Day 2 – DNase I Treatment

1. At 24 hours post-transfection, replace the medium containing complexes with 30 ml (for 15-cm plates) or 10 ml (for 10-cm plates) of fresh D-MEM medium supplemented with 10% FBS, DNase I (1 U/ml), $MgCl_2$ (5 mM), and 20mM HEPES, pH 7.4.

2. Continue incubation in the CO_2 incubator at 37°C overnight.

> **NOTE:** Overnight DNase I treatment before harvesting virus does not negatively affect lentiviral titer or infectivity and helps prevent undesirable carryover of plasmid DNA into the virus prep.

> ***IMPORTANT:*** Failure to change the media the day after transfection results in large carryover of plasmid (free and/or Lipofectamine-bound) into your lentiviral prep. This may cause problems with most downstream molecular biology

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> applications, especially whenever there is a PCR step involved such as during NGS sample preparation after a pooled library screen.

Day 3 – Collect Lentiviral Supernatant

1. At 48 hours post-transfection, collect the entire virus-containing medium from each plate and filter the supernatant (~30 ml per 15-cm plate or ~10 ml per 10-cm plate) through a Nalgene 0.2 µm PES filter (a low protein-binding filter) to remove debris and floating packaging cells. Failure to filter supernatant could result in carry-over of cells into your lentiviral prep.

> **NOTE:** Usually, the peak of virus production is achieved at 48 hours post-transfection. Supernatant can also be collected again at 72 hours post-transfection—replace the collected 48-hour supernatant with 30 ml (for 15-cm plates) or 10 ml (for 10-cm plates) of fresh D-MEM medium supplemented with 10% FBS and 20mM HEPES pH 7.4, and continue incubation in the CO_2 incubator at 37°C for 24 hours. Then, repeat Step 1 above for 72 hours post-transfection.

NOTE: Freezing and thawing lentivirus results in some loss of titer with each cycle.

Concentration of Lentivirus (Optional)

The following procedure was optimized to concentrate virus harvested at 48 hours with high recovery. Although concentrating virus is optional, it is recommended if any of the following conditions applies:

- Very high titer virus stock is needed to achieve desired MOI in hard-to-transduce target cells.
- The virus needs to be suspended in a different media (besides D-MEM/10%FBS) that is optimal for sensitive target cells.
- If packaging an shRNA, sgRNA, or barcode library for screening, because the concentration minimizes problems that might arise from genomic DNA carryover.

Virus Concentration Protocol

1. Aliquot lentiviral supernatant in clear, sterile centrifuge tubes.
2. *(Recommended)* Add Cellecta's LentiFuge™ Viral Concentration Reagent (see **Additional Materials for Production of Lentivirus**) according to the protocol described in the LentiFuge User Manual.
3. Centrifuge at 15,000 × g for at least 1 hour at 4°C. Mark the tubes to identify the location where the pellet will be. At the end of centrifugation, you may or may not be able to see a pellet—assume it is at the location of the mark.
4. Immediately discard the supernatant by aspirating.
5. Place the tubes on ice, resuspend the pellet (which may not be visible) in PBS, PBS/10%FBS, or PBS/1%BSA, make aliquots, and freeze at -80°C. 100-fold concentration is recommended (e.g., resuspend in 1 ml PBS if starting from 100 ml supernatant).

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4.2. General Lentiviral Transduction Protocol

This section provides the general protocol for transduction of mammalian cells with VSV-G packaged lentiviral particles. This protocol was developed and optimized using HEK293 and K-562 cells, and has been successfully used with many other common cell types. However, each cell is different and, depending on the characteristics of your specific cells, some optimization may be necessary.

Lentiviral transductions are performed by mixing cells and virus in culture media. For both adherent and suspension cells, transductions are initiated in suspension and carried out overnight. Adherent cells are allowed to adhere to substrate during transduction and are transduced at a cell density that allows for 2-3 population doublings before reaching confluence. Suspension cells are typically transduced at a higher density than standard growth density, and then they are diluted to standard growth density 18-24 hours after transduction. Do not let cells become too dense or let the medium become yellow at any point.

Before transduction, seed and expand cells from frozen stocks. Cells should be actively growing.

> ! **CAUTION:** Only open the tube containing the lentiviral particles in the laminar flow hood. Please refer to the **Safety Guidelines** and check with your institution regarding the use of lentiviruses.

Day 0—Inoculate Cells

1. Quickly thaw the lentiviral vector particles in a water bath at 37°C. Transfer the thawed particles to a laminar flow hood, gently mix by rotation, inversion, or gentle vortexing, and keep on ice. Unused viral particles can be aliquoted, refrozen at -80°C, and used again for subsequent experiments. There will be some loss of viral activity (usually 10-20%) with each refreeze.

2. Suspend sufficient cells for transduction in appropriate complete media supplemented with 1 µl/ml LentiTrans™ Transduction Reagent—if cells are sensitive to the LentiTrans Reagent, use a lower concentration or omit it. For HEK293 cells, we usually suspend at a density of 1×10^5 cells/ml in D-MEM supplemented with 10% FBS and LentiTrans Reagent. For K-562 cells, we usually suspend at a density 2×10^6 cells per ml in RPMI/10%FBS supplemented with 20mM HEPES, pH 7.4 and LentiTrans Reagent.

3. Aliquot cells into wells or plates. For small scale transductions or titering assays, cells may be plated into multiwell microtiter plates (e.g., 0.5 ml/well for 24-well plates or 1 ml/well for 12-well plates). To transduce larger numbers of cells, use larger plates and scale up the volume accordingly

4. To each plate, add an appropriate amount of lentivirus. The amount of virus will depend on your viral titer and your experiment. Refer to the Transduction Guidelines or Assay Procedures section of the product manual for application specific recommendations.

5. Close the plate and mix by gentle agitation:
    - For adherent cells, place the plate into the CO_2 incubator and grow cells under standard conditions for 16-24 hours.
    - For suspension cells only, “spinoculate” by wrapping the perimeter with parafilm, placing the plate into the centrifuge with an appropriate balance, and spin the cultures at 1,200 × g at +25°C for 2 hours. Following centrifugation, remove plate(s) from centrifuge, carefully remove parafilm, and place in incubator. After 3

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hours, “feed” cells with 0.5 ml additional complete medium per well (no LentiTrans Reagent).

Day 1—Change Media

Between 16 to 24 hours post-transduction, remove media and replace with fresh complete media without LentiTrans Reagent. For suspension cells, spin down and resuspend cells in complete media at 1-5 × 10^5 cells/ml. Place in incubator and grow for an additional 24-48 hours. Avoid confluency or too high a density of cells during and after transduction. If necessary, replate.

Day 3 or 4—Harvest or Split Cells

At about 72 hours after adding virus, you may expand cells as normal or harvest cells for an assay. To continue growing cells, split the cells 1:4 to 1:8 (or as appropriate, depending on the type of cells) as the culture approaches confluence, and add complete medium. As required by your experiments add antibiotics, other factors (tetracycline), etc., and expand as normal.

NOTE: Typical doxycycline concentration for induction is 0.5 ug/ml. However, some cell lines can be more sensitive than others, so you may want to optimize the concentration.

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4.3. Assessing Transduction Efficiency by Flow Cytometry or Antibiotic Selection

For lentiviral constructs with a fluorescent marker or antibiotic resistance marker, transduction efficiency (i.e., % infected cells) can be determined from the fraction of fluorescent or antibiotic resistant cells in the population. To calculate the viral titer, it is also important to know the total number of cells at the time of transduction.

To determine viral titers, transductions should be set up as a serial dilution range of different amounts of virus in 12-well plates, 100,000 cells/well. For example, when titering standard virus from Cellecta, we recommend aliquoting 0, 0.1, 0.3, 1, 3.3 of concentrated stock to 5 different wells (or, for non-concentrated virus, scale up volumes ca. 100-fold). If you are using cells that transduce with low efficiency (e.g., primary cells, some suspension cell lines, etc.) you may need to use 10 µl, or even 30ul, of concentrated virus stock for titering. Always be sure to include the initial well of cells only (no virus) as a negative control.

Option 1: Use flow cytometry with a fluorescent protein marker to determine % infected cells:

> **IMPORTANT:** Do not use fluorescence microscopy to assess the percentage of transduced cells. A significant number of transduced cells will be missed, so transduction efficiency will be underestimated.

1. About 72 hours after adding virus to the cells, spin down and resuspend cells in plate in 1X D-PBS.
2. If trypsin is used, block it with FBS/media, then centrifuge.
3. Determine the percentage of transduced (RFP/GFP/BFP-positive) cells by flow cytometry. For detection of Cellecta’s

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RFP-, GFP-, and BFP-positive cells use the following settings:

Flow Cytometry Settings for TagRFP	
Excitation:	561nm (yellow laser) [530nm laser is still acceptable]
Emission:	590/20nm band-pass filter, or similar
Flow Cytometry Settings for TagGFP2	
Excitation:	488nm (blue laser)
Emission:	530/20nm band-pass filter, or similar
Flow Cytometry Settings for TagBFP	
Excitation:	405nm (violet laser)
Emission:	470/20nm band-pass filter, or similar

4. Use the formula below to calculate the percentage of transduced cells:

> (Fluorescent cells / Total number of cells) x 100 = **% infected cells** (use this for the titer calculation)

Option 2: Use antibiotic selection to determine % infected cells:

1. About 72 hours after adding virus to cells following the standard transduction protocol, split each transduction dilution (including the no-virus control) into 2 separate twin wells using 1:8 split ratio.

2. Add an appropriate amount of antibiotic to one twin well for each viral dilution (including the no-virus control).

3. Incubate all cultures for 2-5 additional days to allow antibiotic time to kill >99% cells in the no-virus control. Do not let the cultures reach confluence (split if needed).

4. After the antibiotic has had time to kill the cells, remove media from cells and replace with fresh media containing a 1/10 total volume alamarBlue stock solution. Include a media-only sample (no cells) for a background alamarBlue reading.

5. Incubate 1 hour at 37°C. Read the fluorescence intensity of the alamarBlue staining using a plate reader. Use the alamarBlue fluorescence values in the following calculation to determine the percent transduced cells in each antibiotic/non-antibiotic pair:

> ([antibiotic-selected cells – media-only] / [non-selected {no antibiotic} cells – media-only]) x 100 = **% infected cells** (use for titer calculation)

Last modified: 18 January 2024

4.4. Lentiviral Titer Calculation

This section describes how to calculate the titer of packaged lentiviral particles. To calculate the number of transducible viral particles in the viral stock, you need to know the number of cells transduced at a specific dilution factor of the stock virus. This can be assessed by counting RFP-positive cells on a flow cytometer or using staining to assess cells with antibiotic resistance (e.g., puromycin).

- It is important to use an amount of virus sufficient to only transduce a fraction of the target cells so that you can accurately assess the number of transductions occuring. You cannot accurately assess the number of transductions if the whole population is transduced (i.e., fluorescent, or die out due to antibiotic selection). Ideally, it is best to aim for

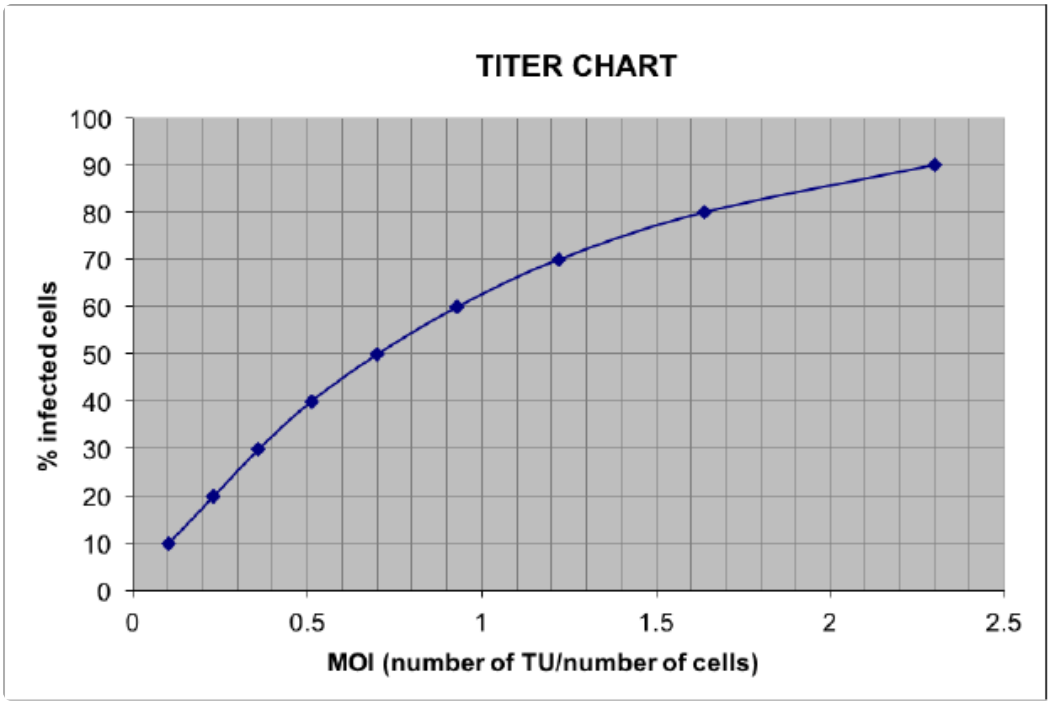
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transducing less than half the cells so that most of the cells have been transduced with a single viral particle. For this reason, it is typical to titer several dilutions of the viral stock to obtain a culture with enough transduced cells for the calculation but not to the point where there are more viral particles than cells.

- To calculate the titer of a viral stock, it is necessary to have the following information:
  - The number of cells at transduction.
  - The percent of transduced cells after transduction (i.e., the Transduction Efficiency).
  - The volume of the viral stock used to transduce the cells.

Calculate the Transduction Units at Infection.

Lentiviral titer is measured as Transduction Units per ml (TU/ml). One TU produces one integration event in target cells. To calculate the viral titer, it is first necessary to determine the number of Transduction Units (TU) used to infect the cells. When the percentage of infected cells is at or below 20%, the number of integrations is approximately equal to the number of transduced cells. However, at higher transduction levels, the fraction of transduced cells with multiple integrations increases, so that the percentage of transduced cells relative to integration events per cell is no longer linear. Using the chart below, the number of integrations per cell, or **MOI** (Multiplicity Of Infection), can be accurately estimated for cultures with up to 75% transduced cells (i.e., MOIs in the range of ~0.2-1.5).



% Infected Cells	MOI
10	0.10
20	0.23
30	0.36
40	0.51
50	0.70
60	0.93
70	1.22
80	1.64
90	2.30

Estimation of percentage of cells infected based on Multiplicity Of Infection (MOI).

Calculate the Stock Titer

To calculate the titer of the original viral stock, apply the formula below:

> TU/ml = (# of cells at Transduction) × [MOI / (ml of Lentiviral Stock used at Transduction)]

- # of cells at Transduction = Total number of cells in the culture when viral particles were added
- MOI = Derived from the chart above based on the percentage of transduced cells.
- ml of Lentiviral Stock used for Transduction = The volume in ml of the virus added to the cells. Include any dilution of

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the viral stock.

Example Calculations:

> **IF:**
> The original # of cells at Transduction was 100,000, and
> The volume of virus stock used was 10 µl, and
> The observed % of transduced cells (RFP+ or antibiotic resistant) is 25%,
>
> **THEN:**
> The calculated MOI is 0.3 (from the chart), and
>
> The **TITER** is:
> 100,000 × 0.3 / 0.01 = 3,000,000 TU/ml

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5. Extraction and Sequencing of sgRNA or Barcodes from Genomic DNA

Identification of sgRNA, shRNA, or barcode sequences in experimental cell or tissue samples from screens with pooled libraries requires (1) isolation and amplification of the target sgRNA or barcode inserts from the genomic DNA, and (2) Next-Generation Sequencing (NGS) of amplified sgRNA or barcode sequences using an Illumina NGS instrument. Following amplification, representation levels of each of the sgRNA or barcode sequences are then quantified to assess enrichment or depletion relative to the starting library or relevant control samples, depending on the type of screen.

Last modified: 21 September 2023

5.1. Genomic DNA Extraction

There are several protocols that can be used for DNA isolation. However, to accurately measure the relative fraction of each sgRNA or barcode present in a specific cell population, it is important to isolate the whole amount of genomic DNA from the cells derived from a genetic screen or barcoded sample. Purification of genomic DNA from just a fraction of cells at a particular time point or treatment condition in a screen, may not provide full representation the guides, hairpins, or barcodes present in the screened sample. As a result, the choice of DNA isolation protocol should be based on the number of cells in your samples.

We recommend the following protocols:

- For genomic DNA isolated from 10 million or more cells—which are typical from standard “dropout viability” CRISPR and RNAi screens with larger libraries—we highly recommend using the Large-Scale Genomic DNA Extraction Protocol. With this procedure, you usually obtain 50-100 µg of genomic DNA per 10 million cells. This amount of genomic DNA will overload most column-based DNA isolation kits and compromise yields. Conventional Genomic DNA Extraction is not column based and avoids DNA loss which can distort representation of the guides or barcodes in the population.

- For small and medium-sized populations of cells (from small libraries screens, positive selection screens where most of the cells are killed off, or FACS-based enrichment screens), we recommend using the following QIAGEN kits:
    - From 1 million to 10 million cells: Use the QIAGEN DNeasy Blood and Tissue Kit (QIAGEN, Cat.# 69504)
    - For fewer than 1 million cells: Use the QIAGEN QIAamp DNA Micro Kit (QIAGEN, Cat.# 56304)

- After purification, you should resuspend your DNA at a concentration of ca. 1-2 µg/µl in water or Tris buffer (**Important:** Do not resuspend DNA in a buffer that contains EDTA. It will inhibit the PCR reaction.). DNA samples can be stored at +4°C for a few weeks or at -20°C for an extended period of time.

Last modified: 3 April 2025

5.1.1. Large-Scale Genomic DNA Extraction Protocol

> **NOTE:** Use of disposable tubes is highly recommended in order to avoid contamination.

1. Resuspend cell pellet in 5 ml QIAGEN Buffer P1 (with RNase A) in a 15 ml polypropylene (phenol:chloroform resistant), Falcon screw-cap centrifuge tube (12,000 RCF rated).

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2. Add 0.25 ml 10% SDS, mix, and incubate 5 minutes at room temperature.

3. Using an ultrasonic homogenizer, sonicate to shear DNA into 10-100 kb sized fragments. To prevent cross-contamination, thoroughly wash the ultrasound head with running water and dry with clean paper towels between samples.

4. Add 10 µl of Proteinase K, mix, and incubate 15 minutes at room temperature.

5. Add 5 ml Phenol:Chloroform:Isoamyl Alcohol solution, vortex hard, and spin down 60 min, 20°C at 8,000 rpm in JA-14 or equivalent rotor (Beckman).

6. At this point, there should be approximately 5 ml of clear upper phase. Transfer 4 ml of upper phase to a new 15 ml disposable screw cap tube (same as in Step 1).

7. Add 0.5 ml of 3M Sodium Acetate and 4 ml isopropanol, mix well, then spin down 30 min, 20°C at 8,000 rpm in a JA-14 or equivalent rotor.

> **NOTE:** To produce a more visible pellet that is compacted at the bottom of the tube, it is recommended to incubate overnight at room temperature before centrifugation.

8. Discard supernatant, add 10 ml of 70% ethanol, and spin down 5 min, 20°C at 8,000 rpm in a JA-14 or equivalent rotor.

9. Discard supernatant and air-dry pellet.

10. Dissolve DNA pellet in an appropriate volume of dH_2O to a concentration of ~2 mg/ml.

11. Incubate 30 minutes at 80°C before spectrophotometer reading.

> **NOTE:** Expected yield is about 10 µg genomic DNA per 1 million cells.

Last modified: 29 December 2023

5.2. Amplification of sgRNA or Barcode Sequences from Genomic DNA

The protocol is based on two rounds of PCR to amplify the integrated sgRNA, shRNA barcode sequences, or clonal barcodes from the genomic DNA. Also, the second round of PCR includes primers that add the sample-specific indexes and sequences (P5 and P7) complementary to primers immobilized in Illumina flow cells. With the PCR reactions, the optimization of cycles is required to avoid over-cycling. Using an optimal cycle number is necessary to generate a sufficient amount of amplicons to sequence while maintaining the relative representation of each sgRNA or barcode in the cell population.

- As an amplification/sequencing positive control, we recommend that you use the original shRNA/sgRNA/barcode plasmid library (1 ng) used in the screen. This positive control can be used as a baseline in NGS data analysis to measure the enrichment or depletion of effector constructs in the experimental samples. If you do not have the plasmid library, we suggest either using any construct in the same vector to serve as a positive control for PCR or the packaged library to serve as an amplification/sequencing positive control as described in the following bullet.

- Although it is typically not necessary, you may use the packaged effector library as a positive control. In order to use

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the packaged library as a positive control, you can start with a volume containing sufficient viral particles equal to at least a few hundred-fold the complexity of the library. For example, for a 50,000-construct library, you should use a volume containing 10-25 million transduction units (TU). Also, before starting PCR, you will need to reverse transcribe the viral RNA sequence using a reverse primer that targets the sense (upper) strand of the insert site at a location where it will generate a full transcript of the target sgRNA/barcode region, including the PCR primer sites. For most Cellecta libraries containing the UbiC promoter, the RevUbiC1 primer (5'-AGGCAGCGCTCGCCGTGAGGA-3') adjacent to the cloning site can be synthesized and used for the reverse transcription step.

First Round PCR

The goal of the first PCR is to amplify shRNA barcodes, sgRNA inserts, or barcode library sequences from genomic DNA isolated from each experimental cell population. The amount of starting DNA can vary depending on the type of screen. For samples with up to 50 µg of genomic DNA, run a single 100 µl reaction for the each sample. For samples with more than 50 µg of DNA, we recommend dividing up the run into multiple 100 µl PCR reactions, each with a maximum of 50 µg DNA.

1. For each sample, prepare a PCR mix according to the table below, where ***x*** is the volume of genomic DNA (typically 50 µl at 1 µg/µl). Many samples will have more than 50 µg of DNA. For these samples, you will need to scale up the master reaction below as needed for each sample, then divide the reaction into multiple tubes with 100 µl in each. For example, if you have 180 µg of DNA in one sample, you should prepare a 400 µl master mix, then aliquot 100 µl in four PCR tubes for amplification (180 µg / 50 µg = 3.6, so round to 4 reactions).

Volume	Component
x µl	Genomic DNA (up to 50 µg)
3 µl	Forward 1st Round PCR Primer
3 µl	Reverse 1st Round PCR Primer
2 µl	dNTP Mix
10 µl	Taq Polymerase Buffer
80 – ***x*** µl	PCR-Grade Water
2 µl	Taq Polymerase
100 µl	**Total volume**

2. Mix gently, centrifuge briefly to collect droplets, divide samples with more than 50 µg of genomic DNA into 100 µl PCR reactions as necessary.

3. Perform PCR using the following cycling conditions.

95°C, 2 minutes	1 cycle
95°C, 30 seconds, 65°C, 30 seconds, 68°C, 2 minutes	16-18 cycles*
68°C, 2 minutes	1 cycle

* **Note:** Depending on your library, the specific number of cycles may need some optimization. For barcode libraries (and shRNA libraries with barcodes), 16 cycles is typically sufficient. For sgRNA libraries and dual-sgRNA libraries, 18 cycles is recommended. If you are using less than 50 µg of genomic DNA in a 100 µl PCR reaction, increase the number of cycles (e.g., for 25 µg, use one extra cycle). However, please note that we do not recommend analyzing the First Round PCR product by gel or equivalent (Bioanalyzer, etc.). You may not see a band at all or just a light band at most. You should evaluate the PCR product only after running both First and Second Round PCR.

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Second Round PCR

The Second Round of PCR is required in order to significantly reduce genomic DNA carryover into the NGS step. Additionally, the Second Round PCR primers add the P5 and P7 sequences that are complementary to the immobilized primers in the NGS Illumina Flow Cells.

- For Cellecta NGS Prep Kits and Supplementary Index Primer Sets, the NRev Index Primers (up to 24) each contain a different index sequence which will be used to deconvolute the sequencing results for each sample mixed together in the same Flow Cell or sequencing lane (i.e., multiplex sequencing). It is important, therefore, to use a different NRev Index Primer (each signified by a different letter) to amplify each sample that will be run together in the same Flow Cell or lane.

For the Second Round of PCR, start with an aliquot of 5 µl of the First-Round PCR reaction for each sample.

1. If you are starting from more than 50 µg of genomic DNA, it is important to combine all individual reactions for each sample together into a single tube.

2. Perform the Second Round of nested PCR using a 5 µl aliquot of the First Round PCR product for each sample:

Volume	Component
5 µl	First Round PCR Product
5 µl	NFwd Primer
5 µl	NRev (Index) Primer*
2 µl	dNTP Mix
10 µl	Taq Polymerase Buffer
71 µl	PCR-Grade Water
2 µl	Taq Polymerase
100 µl	**Total volume**

> * **Note:** Each sample to be sequenced together in a single Flow Cell or lane needs to be amplified with a different NRev Index Primer in the Cellecta NGS Prep Kit. Be sure to use a differently lettered NRev Index for each sample that will be loaded on the same Flow Cell or lane.

3. Perform PCR under the following cycling conditions.

95°C, 2 minutes	1 cycle
95°C, 30 seconds, 65°C, 30 seconds, 68°C, 2 minutes	12 -16 cycles**
68°C, 2 minutes	1 cycle

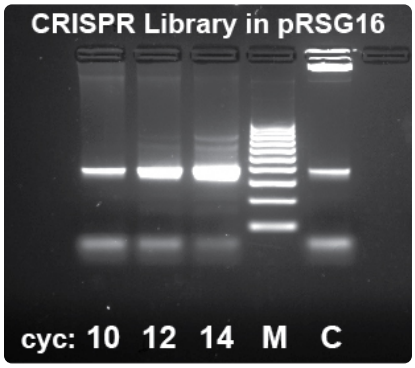
> ** **Note:** Additional cycles may be needed for samples as described below.

4. Analyze the amplified products from the Second Round of PCR. This analysis may be done on an Agilent® 2100 Bioanalyzer® instrument with the Agilent® High Sensitivity DNA Kit (Cat.# 5067-4626) or a Fragment Analyzer (Advanced Analytical) instrument using Standard Sensitivity NGS Analysis Kit (Cat.# DNF-473-1000) using the manufacturer's protocol, if you have access to this equipment. If not, then we recommend the following procedure using agarose gel electrophoresis to analyze the samples:
    - Run all samples including positive control on 3.5% agarose-1xTAE gel. Mix 5 µl of aliquot from the each

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sample with 2x loading buffer (e.g. 10% sucrose with Bromophenol Blue tracking dye).

- Analyze the intensity of the bands after electrophoresis (i.e., Bromophenol Blue tracking dye about 80% down the gel). The typical results of agarose gel electrophoresis analysis are shown in the figure below. The expected size of amplified barcode, shRNA barcode, and sgRNA products is typically between 125 to 400 bp or dual-sgRNA product around 750bp and is provided in the NGS Cassette Design information specific for your library or NGS Prep Kit Product Certificate. If you did not receive Cellecta's Order Information email with links to this information, please contact us at tech@cellecta.com.



CRISPR Human Genome 80K Knockout Library NGS PCR product analysis by gel electrophoresis. In this example, 10 cycles is the recommended cycle number for the Second Round PCR step.



DECIPHER Library NGS PCR product analysis by gel electrophoresis

5. Quantify the amount of the amplified product for each sample using software tools provided with instrument or gel imager. If all samples show similar yield of amplified products (+/- 2-fold) no addition cycling is required and you should proceed to NGS Samples Purification. If there is a greater than 2-fold variance, you should put the samples with very weak or missing bands back into the PCR instrument for additional cycles. For example, if the amount of PCR product in the sample is 4, 8, or 16-fold less than in other samples, run that sample for an additional 2, 3, or 4 PCR cycles, respectively. For samples in the +/- 5-cycle range, don't run additional cycles.
    - If you run additional cycles on any samples, quantify the reaction products again by using one of the instruments mentioned above or gel electrophoresis to ensure that all samples have correctly amplified products.
    - Avoid overcycling! Try to use the minimum number of cycles which allows quantitation of the bands. Importantly, for dual-sgRNA libraries, overcycling induces a significant level of recombination between different dual-sgRNA molecules. Overcycling also often generates additional higher molecular weight bands. These bands do not normally interfere with NGS unless they are very strong. Also, overcycling has the potential to compress the representation of the targeted sequences if the reaction exceeds the exponential phase. Therefore, it is desirable to minimize the cycle number. If there are excessive higher molecular weight bands, you can rerun Second Round PCR for that sample using another 2 µl of DNA from the First Round PCR and a fewer number of cycles.

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DNA samples can be stored for several days at +4°C at this stage or move directly onto NGS Sample Purification

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5.3. NGS Sample Purification

To maximize sequencing of the targeted sgRNA or barcodes and minimize background sequences, we recommend purifying the amplified PCR products by preparative gel electrophoresis.

1. Based on quantitative analysis of PCR product yield for each of the samples, combine the PCR products from each sample at the same amount. For example, for samples with yields of correct PCR product of 20 ng/µl and 60 ng/µl, combine 6 µl and 2 µl of PCR reaction, respectively.

Purify and concentrate the PCR products from the combined sample using the QIAGEN QIAquick PCR Purification Kit, following the manufacturer's protocol. The combined sample should be eluted in a volume of approximately 30 µl.

2. After purifying and concentrating, run each purified combined PCR product with 10X loading buffer on a 3.5% agarose-1XTAE gel with well sizes that accommodate at least 50 µl.

3. Using a scalpel and a 365 nm (UV-A) lamp, excise the narrow band that corresponds to the correct target sgRNA or barcode amplicon size. DO NOT use a 302/312 nm (UV-B) lamp!

CAUTION! Be sure to use UV safety glasses to protect your eyes when viewing and excising the DNA from the gel on the transilluminator.

4. After excision, purify the combined PCR product from the gel fragment using a QIAquick Gel Extraction kit following the manufacturer's protocol. Elute the purified PCR product in 20 µl of elution buffer.

> **Note:** Be sure to centrifuge QIAquick columns at maximum speed for at least 3 minutes before eluting DNA to avoid ethanol contamination in the purified PCR product.

5. Quantify extracted DNA in the combined sample by A260nm OD measurement using a NanoDrop spectrophotometer (or equivalent), and then adjust the concentrations in all samples to 10 nM. For example, if the amplicon size is 200bp, the 10 nM concentration corresponds to 1.32 ng/µl based on A260 OD measurement. For an amplicon size of 750bp (dual-sgRNA product), a 10 nM concentration corresponds to 4.95 ng/µl.

DNA samples can be stored at -20°C at this stage.

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5.4. Sequencing sgRNA Inserts or Barcodes

The Second Round nested PCR primers contain sequences (P5 and P7) complementary to the sequences of the immobilized primers necessary for generating amplification clusters on any of Illumina's NGS Instruments. In addition, if you are using a Cellecta NGS Prep Kit, the NRev Index Primers add sample-specific index sequences which allow you to combine and multiplex up to 24 samples on a single flow cell or lane. The primers were designed with the NextSeq instrument in mind, but they are compatible with other Illumina instruments as well.

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IMPORTANT: For certain libraries, such as sgRNA and dual-sgRNA, the Cellecta 6-nt Index should be read using Read 2, **not** in the Illumina Index 1. Please check the Product Certificate for the appropriate LNGS Kit for more information.

The number of sequencing cycles (read length) required depends on the sgRNA or barcode length. Please refer to your library information (NGS Cassette Diagram for the specific Cellecta library or the Product Certificate for the NGS Prep Kit).

Guidelines for preparing samples for sequencing:

- Combine together equal amounts (each adjusted to 10 nM) of experimental and positive control samples to be sequenced together. Importantly, each sample in the combined pool needs to have a unique index sequence.

We recommend that you DO NOT combine Cellecta samples with samples of different origin on a single flow cell or lane. It may cause sequencing errors.

> **Note:** The number of samples which can be sequenced in one lane or flow cell is based on the complexity of the pooled library and expected total number of reads generated with the Illumina instrument. You will need to determine how many target reads you want per sample based on your experiment.

- Prepare the samples for NGS by following the Illumina “Denature and Dilute Libraries Guide” specific for your system.
  - NextSeq 500: https://support.illumina.com/downloads/nextseq-500-denaturing-diluting-libraries-15048776.html
  - HiSeq: https://support.illumina.com/downloads/hiseq-denature-dilute-libraries-guide-15050107.html

- Spike in 5-15% of the PhiX Control to the pooled indexed sample to increase the nucleotide diversity.

- Mix Cellecta’s **Seq NGS Primer** with the Illumina primer mix (for the NextSeq, spike in the primer at the appropriate cartridge position—usually at Read 1). For indexing multiple samples in a flow cell or lane using a Cellecta NGS Prep Kit, also add the **Index NGS Primer** directly to the appropriate well (i.e., spike in either with the Illumina index primer or the reverse sequencing primer; see Note below). Each primer should have a final concentration of 300 nM for the NextSeq, or 500 nM for the HiSeq. We recommend measuring the final volume of the primer mix in each well to ensure accurate spiked-in primer concentrations. For primer spike-in information on other Illumina instruments, please refer to this Illumina guide: https://knowledge.illumina.com/library-preparation/general/library-preparation-general-reference_material-list/000001542

> **Note:** Depending on the way the amplification and sequencing primers were designed, the position of the Seq NGS and Index NGS primers may vary. Refer to the supplementary information (Product Certificate or NGS Cassette Diagram file) provided for the specifics on how to set up the NGS Primers for your specific library.

- Specify the appropriate number of cycles to sequence the complete sgRNA or barcode region (please refer to Product Certificate or NGS Cassette Diagram file), and specify the indicated number of cycles to read the 6-base Index sequence if using a Cellecta NGS Prep Kit.

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5.5. Antibiotic Selection of Library Transduced Cells

Antibiotic selection of transduced cells must be carried out if depletion of non-transduced cells is needed in your experiment. However, it is important to avoid using too high an antibiotic concentration for two reasons: (1) you want to avoid the killing of a sizable fraction of transduced cells, and (2) you do not want to enrich for cells with multiple viral integrants. The general procedure for the selection of the appropriate antibiotic concentration is described in “section 3.2 Calculating a Kill Curve”. If the lentivector carries a fluorescent marker as well (e.g. RFP), the procedure below is recommended:

Determine the optimal concentration of antibiotic by flow cytometry.

1. Based on the titer efficiency of your cells (see Assessing Transduction Efficiency by Flow Cytometry or Antibiotic Selection section), set up transduction cell pools using the amount of viral particles that resulted in 20%-50% transduced cells.

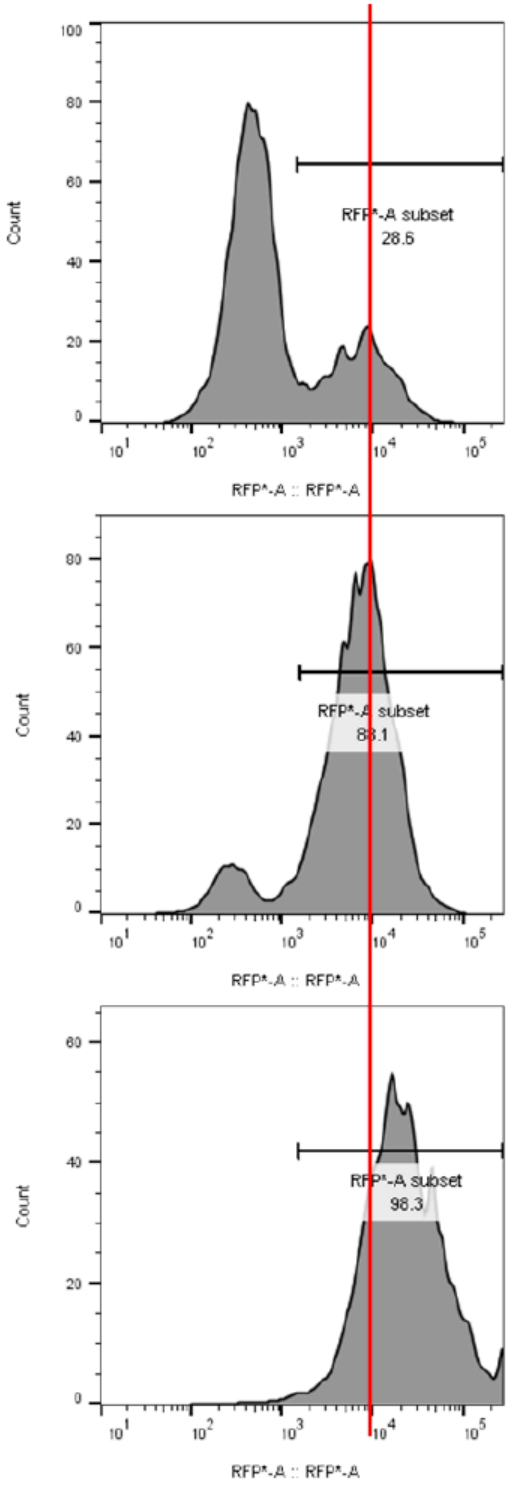
2. Aliquot same amount of cells in six 12-well wells (~50,000 cells /well).

3. Add increasing amount of antibiotic to each well (for puromycin selection, you would typically use a concentration range between 0.1 ug/ml – 10 ug/ml in 2-fold increments). Include a no-antibiotic control well.

4. Select the lowest antibiotic concentration that resulted in >90% transduced cells selection, while maintaining the fluorescence intensity of selected and unselected transduced unchanged.

In the example below, the top histogram is the cells transduced at MOI ~0.3, unselected; the middle histogram is the transduced cells selected with an antibiotic concentration which resulted in the enrichment of transduced cells to ~90%, without affecting RFP intensity; the bottom histogram is the transduced cells selected with an antibiotic concentration which resulted in the enrichment of transduced cells to ~99%, but also increased RFP intensity (at the expense of library representation).

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Distribution of RFP+ Cells

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6. Detection of Expressed Barcodes or sgRNA with scRNA-Seq

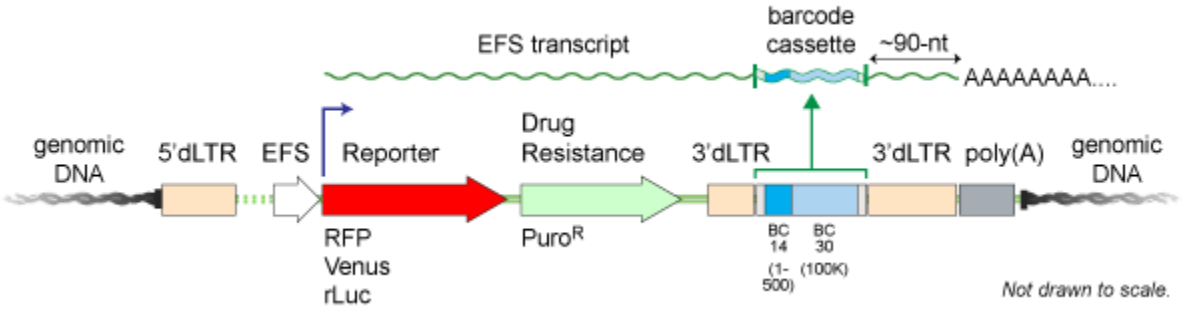
CloneTracker XP™ Barcode Libraries and CRISPR sgRNA libraries cloned into Cellecta pScribe vectors (e.g. pRSGScribe1) express the barcode and/or the sgRNA guide on an RNA transcript (i.e., the selection marker gene) so it is readable in single cell RNA-Seq (scRNA-Seq) applications. As a result, the barcodes and/or guide sequences can be detected when running scRNA-Seq. It is only necessary to amplify the barcode and/or sgRNA sequence after making the cDNA.

The general approach to extract the barcode or guide RNA sequence is described below using the CloneTracker XP barcode as an example. The approach with the sgRNA libraries in Cellecta pScribe vectors is very similar. The specific protocol for both types of libraries can be found in the next Section: 10X Chromium 3' Detection of Barcodes/sgRNA in pScribe Libraries.

The lentiviral-based CloneTracker XP Barcode Libraries have two main functional elements—the reporter (RFP, Venus, or rLuc) and drug resistance marker (PuroR)—both expressed from one promoter on a single transcript. The barcode cassette in the Barcode-3' Libraries is located in the 3'-dLTR (3'-UTR) region of the reporter/drug resistance marker transcript. In the Barcode-5' Libraries, the cassette is located immediately downstream of the promoter driving the reporter and marker expression.

The barcode has a composite structure built by positioning of multiple 14-18-nt sequences with multiple 30-nt sequences separated by a short spacer (4nt spacer in the 1M/10M libraries and 21nt spacer in the 5M/50M libraries). With the Barcode-3' libraries, the barcode cassette is positioned within approximately 90 nt of the polyA start of the transcript while the barcode is approximately 150-200 nt from the polyA start (depending on the particular vector). As a result, the transcribed barcodes are captured by cDNA first strand synthesis using barcoded oligo-dT primers, which is the typical first step of most scRNA-Seq procedures (e.g. Drop-Seq, 10x Genomics, BD Rhapsody, Bio-Rad ddSeq, Takara SMARTer ICELL8 system, etc).

Each composite barcode is a unique and highly diverse sequence that tolerates up to 5 mutations that help ensure accurate identification of barcodes even in the most demanding applications (e.g., cell lineage tracing *in vivo*). A link to the list of barcode sequences for your library is provided with the Product Information emailed to you upon shipment of the library. Please contact Cellecta if you did not receive this email.



Linear map of pScribe-EFS-TagRFP-2A-Puro-BC14-BC30-3LTR

For details on how to detect barcodes and/or guide sequence when using the 10X Chromium 3'-Protocol, see Section **10X Chromium 3' Detection of Barcodes/sgRNA in pScribe Libraries**.

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6.1. 10X Chromium 3’ Detection of Barcodes/sgRNA in pScribe Libraries

The 10x Chromium 3’ Reagent Kit was developed for amplification of 3’-ends of polyA+ RNAs and based on ligation of an adaptor upstream of cDNA at a variable distance from the poly-A+ priming site. The ligated adaptor is used as a binding site for a primer to read into the mRNA coding sequence. The sequence read from this upstream adaptor primer is typically only just 100 bases or so. The CloneTracker XP BC14-spacer-BC30 barcode is located at a fixed ~150-200nt distance upstream from the poly-A+ site, depending on the particular vector.

To effectively read the barcode sequence in the CloneTracker XP Barcode-3’ Library when using the 10x Genomics Chromium 3’ Kit, you need to include an additional PCR amplification step with a barcode-specific primer that is located just upstream of the CloneTracker XP barcode. This will ensure amplification of the segment of cDNA that contains both the CloneTracker XP barcode and the cell barcode associated with the poly-A+ sequence in the scRNA-Seq protocol (e.g., the “bead-barcode” with bead-based scRNA systems). The general procedure for how to integrate this additional PCR with the 10x Chromium 3’ Kit (Version 3.1) procedure is shown below.

Please note that the procedure below describes barcode/sgRNA detection when using the Chromium 3’-Kits from 10x Genomics. Depending on the specific scRNA-Seq protocol you are using, some modifications and optimization will be required.

Amplification of Barcodes and/or sgRNA for NGS when Using 10x Single Cell Analysis.

After the cDNA amplification/cleaning/quantitation step (Step 2.4 in Chromium Next GEM Single Cell 3′ v3.1, User Guide, Rev E), retrieve a ~10 ul (~25%) aliquot of 10x cDNA and set up a separate PCR reaction just before the cDNA fragmentation step.

1. Amplify the barcodes/sgRNA by running a PCR reaction with the same cycling conditions as the previous cDNA amplification (Section 2.2 of the Chromium Next GEM Manual) and the following PCR primer below for your library:

   (A) The Partial Read 1 primer (5’-CTACACGACGCTCTTCCGATCT-3’ — see 10x Chromium 3’ Kit User Guide) for one PCR primer,

   and

   (B) 1 uM of either of the following primers for the second PCR primer (depending on the library you are using):

   Adaptor-FBP1 primer — For CloneTracker XP Barcode-3’ Libraries in pScribe vectors:

> **Adaptor (Read 2)** **FBP1 primer**
> 5’-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCGACCACCGAACGCAACGCACGCA-3’

   Adaptor-U6 primer — For sgRNA libraries in pRSGScribe1 and pRSGScribe1cb:

> **Adaptor (Read 2)** **U6 primer**

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> 5’-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACC-3’

- The standard Illumina adaptor sequence is located in the first 34 nucleotides of the 5’ end (underlined).

> * **Note:** scRNA-Seq platforms other than 10x Chromium may use a different adaptor, in which case you should modify the primer accordingly.

> * **Note:** The above PCR takes the place of the adaptor ligation step in the standard 10x protocol. Do ***not*** perform the *Fragmentation*, *Adaptor Ligation*, or post-Ligation *SPRI Bead clean up* for the amplified barcodes. Jump directly to the Sample Index PCR (Section 3.5 of the Chromium Next GEM Manual) and amplify the adaptor-ligated cDNA and barcodes using different i7 and i5 index primers as described in Step 2 below.

2. Amplify the barcode and adaptor-ligated cDNA that was made in Step 3.3 of the 10x of the Chromium Next GEM Manual. Use 5 µl of the Barcode amplification mix from Step 1 above as the template to set up the Index Primer PCR using the of the reagents from 10x kit and the program described in Step 3.5 of the 10x Chromium Next GEM Manual.

   Use a different set of i5 and i7 index primers for the Barcode Sample than used for the cDNA sample and, also, add an additional 6 PCR cycles for the barcode sample. The 6 additional PCR cycles will increase the yield of the CloneTracker XP barcoded cDNA sequences to approximately 64-fold higher levels than other transcripts in the cDNA reaction. This helps ensure you recover the majority of the CloneTracker XP barcodes.

3. Run a Bioanalyzer analysis after amplification. You should be able to see a band of ~350-375nt (depending on the particular library), which corresponds to the barcode product amplified from lentiviral barcoded transcript.

   The amplified product will have the following structure—Read2(Adaptor)-FBP1-**Barcode**-UMI-10xBarcode-Read1—which is the same structure as PCR products generated from amplified adaptor-ligated cDNA, except it has the CloneTracker barcode sequence instead of the cDNA—Read2(Adaptor)-**cDNA**-UMI-10xBarcode-Read1. If you can’t see the barcode-specific product, add an additional 2-4 extra cycles and repeat Bioanalyzer analysis. Then, quantitate them, purify using SPRI beads, and mix at the desired ratios for the follow-up NGS step.

4. Purify the Indexed Barcode Amplicon as described in *Post Sample Index PCR Double Sided Size Selection* (Section 3.6 of the Chromium Next GEM Manual) of the 10x protocol. However, increase the amount of the SPRIselect Reagent added in Step e from 20 µl to 80 µl (1.4x). In other words, change Step e to the following:

   *e. Vortex to resuspend the SPRIselect reagent. Add **80 µl** SPRIselect Reagent (1.4X) to each transferred supernatant. Pipette mix 15x (pipette set to 150 µl).*

5. If you are mixing the Barcode sample with the cDNA to make the NGS Library, we recommend mixing cDNA samples with the Barcode samples at a ~9:1 ratio (~9 parts cDNA to 1 part barcode/sgRNA reaction).

Processing Cellecta CloneTracker Barcodes (or Guides) Single-Cell Data

For processing Cellecta CloneTracker Barcode single-cell data from the 10x system and assigning CloneTracker barcodes, please use the step-by-step workflow guide on GitHub: https://github.com/Cellecta/CloneTracker_single_cell.

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7. Technical Support

Please provide answers to the questions below (where applicable) and then contact us for assistance.

Library Used:

1. Which library did you use?
2. What is the lot number?

Packaging the Library:

1. What was the lentiviral titer, and what was the total number of TU packaged?
2. How was the virus concentrated? (if applicable)

Transducing Target Cells:

1. What MOI did you use to transduce your target cells?
2. What target cells did you use?
3. How many replicates did you use? (i.e., duplicate, triplicate, etc.)
4. Did you use puromycin after transduction, and at what concentration?
5. For how long did you use puromycin on the cells?

Library Screen:

1. Could you briefly explain your experiment?
2. How many infected cells were used?

Sample Preparation & NGS:

1. Describe the protocol you used to amplify the barcodes.
2. What NGS system and which Illumina Kit did you use?
3. How much PCR product was used for NGS?
4. How many sequences were read per sample?

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For the latest technical news and updates, visit Cellecta's blog at: https://cellecta.com/blogs/news

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8. Safety Guidelines

The HIV-based lentivector system is designed to maximize its biosafety features, which include:

- A deletion in the enhancer of the U3 region of 3'ΔLTR ensures self-inactivation of the lentiviral construct after transduction and integration into genomic DNA of the target cells.
- The tat-transactivator domain has been removed from the upstream 5'-LTR in the lentivector to disable transcription from the 5'-LTR.
- The three proteins (gag, pol, env) are necessary for replication and packaging of the lentiviral vector. When packaging lentiviral particles, the genes encoding these three packaging proteins are separately introduced and expressed in the packaging cells using separate ordinary plasmids that lack packaging signals and share no significant homology to the lentivector. As a result, none of the HIV-1 genes (gag, pol, env) will be present in the packaged lentiviral genome.
- None of the HIV-1 genes (gag, pol, env) will be present in the packaged lentiviral genome, as they are expressed from packaging plasmids lacking packaging signal—therefore, the lentiviral particles generated are replication-incompetent.
- Lentiviral particles will carry only a copy of your expression construct.

Despite the above safety features, use of replication-incompetent lentiviral particles falls within NIH Biosafety Level 2 criteria. For a description of laboratory biosafety level criteria, consult the Centers for Disease Control Office of Health and Safety Web site at:

> https://www.cdc.gov/labs/bmbl/

It is also important to check with the health and safety guidelines at your institution regarding the use of lentiviruses and follow standard microbiological practices, which include:

- Wear gloves and lab coat at all times when conducting the procedure.
- Always work with lentiviral particles in a Class II laminar flow hood.
- All procedures are performed carefully to minimize the creation of splashes or aerosols.
- Work surfaces are decontaminated at least once a day and after any spill of viable material.
- All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory area are to be placed in a durable, leakproof, properly marked (biohazard, infectious waste) container and sealed for transportation from the laboratory.

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