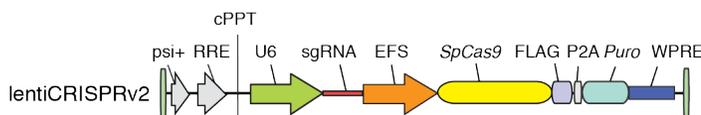GeCKO
Genome-scale
CRISPR Knock-Out

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## Genome-scale CRISPR Knock-Out (GeCKO) v2.0 pooled libraries

CRISPR (**C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats) is a microbial nuclease system containing a combination of CRISPR-associated (Cas) genes as well as non-coding RNA elements capable of programming the specificity of the CRISPR-mediated nucleic acid cleavage. Using lentivirus, we delivered the type II CRISPR nuclease system to facilitate genome editing in mammalian cells in a pooled library targeting early consecutive exons (Shalem*, Sanjana*, *et al.*, Science 2014). **Here we describe how to amplify GeCKO v2.0 DNA plasmids to have sufficient quantity to produce lentivirus, while maintaining full library representation**.

Library description: The GeCKO v2 libraries consist of specific sgRNA sequences for gene knock-out in either the human or mouse genome. Each species-specific library is delivered as two half-libraries (A and B). When used together, the A and B libraries contain 6 sgRNAs per gene (3 sgRNAs in each library). We recommend screening the entire library (A and B) when possible but if adequate representation cannot be obtained with the entire library, screening can be performed with one half-library. Since more cells are needed in the screen as number of constructs in the library increases, this design has the flexibility of screening with just the A library (3 sgRNAs per gene) at a smaller scale or screening the full library (6 sgRNAs per gene). Both A and B libraries contain 1000 control sgRNAs designed not to target in the genome. The A library also targets miRNAs (4 sgRNAs per miRNA). Each library is available in a 1 vector (lentiCRISPRv2) or 2 vector (lentiCas9-Blast and lentiGuide-Puro) format. The 2 vector format with the library in lentiGuide-Puro has the advantage of higher titer for the library virus but requires cells to already contain Cas9 (usually genomically integrated using lentiCas9-Blast). See p. 3 for full library specs.

One vector lentiviral GeCKO system

Two vector lentiviral GeCKO system
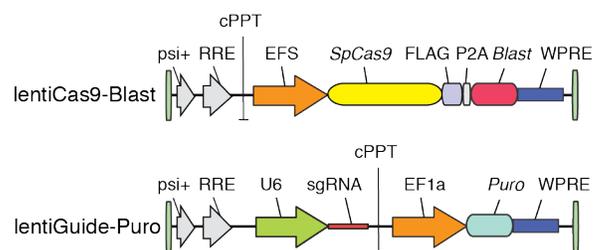
Lentiviral production: Since these vectors enable lentiviral delivery of both Cas9 and sgRNA for targeted gene knock out, it is important to perform these experiments in a lab with the appropriate biosafety level and controls, which can vary between different institutions. Before starting any lentiviral work, please ensure compliance with your Environmental Health and Safety office and government / organization / university. Briefly, to make lentivirus, lentiCRISPR (with sgRNA cloned) must be co-transfected into HEK293(F)T cells with the packaging plasmids pVSVg (AddGene 8454) and psPAX2 (AddGene 12260). As a positive control for viral production, we use a CMV-EGFP lentiviral transfer plasmid (eg. AddGene 19319).

Representation: Each GeCKO library is a pool of many different vectors mixed together. The libraries contain 122,417 (human) or 130,209 (mouse) unique sgRNAs. To ensure no loss of representation, it is important to amplify the library using the protocol given on the next page. **The GeCKO library should not be transformed using chemically competent cells or amplified in liquid cultures. Please read over the entire protocol before starting library amplification.**

Citation: Please reference the following publications for the use of this material.

Improved lentiviral vectors and genome-wide libraries for CRISPR screening. Sanjana NE*, Shalem O*, Zhang F. *Nature Methods* (2014).

Genome-scale CRISPR-Cas9 knockout screening in human cells. Shalem O*, Sanjana NE*, Hartenian E, Shi X, Scott DA, Mikkelsen T, Heckl D, Ebert BL, Root DE, Doench JG, Zhang F (2014). *Science*, 343, 83-7. DOI: 10.1126/science.1247005

Online resources: Please see *http://www.genome-engineering.org/gecko/* for GeCKO library sequences, vector maps, protocols, discussion forums and technology updates.

GeCKO pooled electroporation, plating, determination of transformation efficiency and maxi prep

Follow these instructions for each library (A and B). Amplify and prep half-libraries A and B **separately** as follows:

1. Dilute the GeCKO library to 50 ng/uL in water or TE (if not already diluted).

2. Electroporate the library
   a. Add 2 uL of 50 ng/uL GeCKO library to 25 uL of electrocompetent cells with an efficiency of $\geq 10^9$ cfu/ug. We have had success with many electrocompetent cells, including NEB DH5a cells, Invitrogen DH5a cells and Lucigen Endura. In our hands, the Lucigen Endura cells (cat # 60242) have yielded the highest efficiency with the GeCKO library.
   b. Electroporate using the manufacturer's suggested parameters/protocol.
   c. Recover in 975 uL recovery media (i.e. media provided with cells) and transfer to a loosely capped tube with an additional 1 mL of recovery media.
   d. Repeat for a total of 4 electroporations and rotate at 250 rpm for 1 hour at 37 C

3. Plate a dilution to calculate transformation efficiency
   Note the library plasmids have **ampicillin resistance** – *prepare all plates accordingly.*
   a. Pool all 8 mL of electroporated cells. Mix well.
   b. Remove 10 uL and add to 1 mL of recovery media, mix well, and plate 20uL onto a pre-warmed 10cm petri dish (ampicillin). This is a 40,000-fold dilution of the full transformation and will enable you to estimate transformation efficiency to ensure that full library representation is preserved.

4. Plate the transformations
   Follow Step a) if your lab has 24.5 cm^2 bioassay plates for large-scale bacteria culture; otherwise follow Step b), which substitutes 20 standard (10 cm round) petri dishes.
   a. Plate 4mL of transformation on each of 2 pre-warmed 24.5 cm^2 bioassay plates (ampicillin) using a spreader. Spread the liquid culture until it is largely absorbed into the agar and won't drip when turned upside down.
   b. Alternatively, spread 400 uL of transformation mix per petri onto 20 pre-warmed petri dishes.

5. Grow all plates inverted for **14 hours** at **32 C**. Growth at this lower temperature reduces recombination between the lentiviral long-terminal repeats. (Growth at 37 C is also acceptable if 32 C is not possible.)

6. Calculate transformation efficiency
   a. Count the number of colonies on the dilution plate.
   b. Multiple this number of colonies by 40,000 for the total number of colonies on all plates.
   c. Proceed if the total number of colonies is at least 3×10^6. This efficiency is equivalent to 50X colonies per construct in the GeCKO library (ie. 50X for library A or B individually).

7. Harvest colonies
   a. Pipette 10 mL of LB onto each 24.5 cm^2 bioassay plate (or, 500 uL per 10 cm petri dish)
   b. Scrape the colonies off with a cell spreader/scraper.
   c. Pipette off the liquid plus scraped colonies into a tube and repeat the procedure a second time on the same plate with additional 5-10 mL. *Note: Weigh this tube prior to adding any liquid to it.*

8. Weigh the bacterial pellet to determine the proper number of maxiprep columns to use
   a. Spin down all liquid to pellet the bacteria and then discard the supernatant.
   b. Weigh the bacterial pellet and subtract the weight of the tube

9. Maxi-prep for downstream virus production and future amplification
   a. Using a maxi scale plasmid prep, each column can handle approximately 0.45 g of bacterial pellet.
   b. Perform a sufficient number of maxi preps so as to not overload a column.

10. Proceed to transient transfection of HEK293(F)T cells with maxi-prepped GeCKO lentiCRISPR library and appropriate packaging plasmids. lentiCRISPRv2 and lentiGuide-Puro backbone vectors are compatible with both 2nd and 3rd generation lentiviral packaging plasmids (see www.addgene.org/lentiviral/packaging)
    a. If producing virus from the full library, transfect cells with both A and B half-library plasmids.
    b. Library plasmid should also be deep sequenced to verify representation and pool complexity.

GeCKO v2.0 library specifications

	GeCKO v2 human library	GeCKO v2 mouse library
Species	human	mouse
Number of genes targeted	19,050	20,611
Targeting constructs per gene	6 per gene (3 in Library A, 3 in Library B)	6 per gene (3 in Library A, 3 in Library B)
Number of miRNA targeted	1,864	1,175
Targeting constructs per miRNA	4 per miRNA	4 per miRNA
Control (non-targeting) sgRNAs	1,000	1,000
Total sgRNA constructs	122,411 (65,383 in Library A, 58,028 in Library B)	130,209 (67,405 in Library A, 62,804 in Library B)
Viral plasmid vector	Single and dual vector: lentiCRISPR v2 and lentiGuide-Puro	Single and dual vector: lentiCRISPR v2 and lentiGuide-Puro

All library sequences for screen readout are available for download at *http://www.genome-engineering.org/gecko/*