SIGMA-ALDRICH®

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Product Information

Sigma Whole Human Genome Lentiviral CRISPR Pool
Product Number **HWGCRISPR**

Gecko2 Human Whole Genome CRISPR Pool, gRNA Only Lenti Particles (Gecko2 vector)
Product Number **HGECKO2G**

Human Kinase Lentiviral CRISPR Pool
Product Number: **HKCRISPR**

Storage Temperature -70 °C

Pooled CRISPR Technical Bulletin

General Product Description

CRISPR technology has revolutionized genome editing by radically simplifying re-targeting methods for DNA binding proteins. Since CRISPR systems can be programmed using minimal genetic information (19-20 bp), they are compatible with high throughput cloning methods and have been rapidly adapted to drug screening workflows (Shalem et al., 2014; Wang et al., 2014; Koike-Yusa et al., 2014).

Sigma® has strong core scientific and production capabilities in both genome editing (Geurts et al., 2009; Chen et al., 2011; Ji et al., 2014; Sampson et al., 2015) and genetic screening using lentiviral shRNA libraries (Ji et al., 2007; Duan et al., 2010; Guin et al., 2014; Whitworth et al., 2012). These core capabilities have been combined to create new reagents, services, and expertise to support CRISPR-based genetic screening.

In contrast to arrayed gRNA libraries containing separate, individual clones in plates, pooled gRNA libraries contain thousands of individual gRNAs in a single tube. Positive control gRNAs are included within the pool enabling options for practice screens based on 6-TG (or other) drug treatment. Non-targeting (i.e. negative) control gRNAs are included within each pool as a baseline in the statistical characterization of changes in gRNA frequency as measured by deep sequencing. Negative control gRNA share minimal homology with the target genome and should not undergo significant changes in representation throughout cell treatments. Together, these controls create many options for upfront experimental design and downstream data analysis via MAGeCK, RSA, RIGER, KS-statistics, edgeR, or other methods for qualifying “hits” relevant to drug-gene interactions.

Sigma’s pooled-gRNA design process takes into account several general considerations, including: (1) minimization of off-targeting, (2) broad distribution of unique gRNA sequences across various exons to ensure a deep survey of gene knockout possibilities, (3) careful gRNA design efforts to include small or unrepresented genes to maximize gene coverage.

Summary of Pooled LentiCRISPR Products

LentiCRISPR Pool	Species	Target Class	total # of gRNAs	gRNA design by	Vector design by	Cas9 ?	FP ?	Puro ?
HWGCRISPR-1EA	Human	Whole Genome	176,066	Sigma	GeCKOv2	No	No	Yes
HGECKO2G-1EA	Human	Whole Genome	123,411	Broad/MIT	GeCKOv2	No	No	Yes
HKCRISPR-1EA	Human	Kinases	6,012	Sigma	Sigma	Yes	GFP	Yes

Physical Material
Product Number: HWGCRISPR
The **Sigma Whole Human Genome Lentiviral CRISPR Pool** contains the following components:

- eight lentiviral pools provided as 200 uL (divided into 8 x 25 uL aliquots) of CRISPR lentivirus per pool, targeting all known human protein-coding genes
- each of the 8 pools targets approximately 2,300 genes, with an average of 9.5 gRNAs per gene contained in each pool, leading to around 22,000 gRNA designs per pool
- positive and negative (non-targeting) controls included in each pool
- employs the GeCKOv2 lentiguide-puro gRNA-only vector

Reagents not provided, but recommended:
Cas9 Delivery (Plasmid and Lentiviral Formats)
Product Number:Description
CAS9BST-1EA: A lentiviral plasmid expressing Cas9 along with a blasticidin resistance gene
LVCAS9BST-1EA: Lentiviral particles expressing Cas9 along with a blasticidin resistance gene
CAS9NEO-1EA: A lentiviral plasmid expressing Cas9 along with a neomycin resistance gene
LVCAS9NEO-1EA: Lentiviral particles expressing Cas9 along with a neomycin resistance gene

Companion product: High-titer Control Human HPRT1 CRISPR Lentivirus (**Product No: CRISPR16H-1EA)** contains the following components:

- 8 x 25 uL aliquots of high titer CRISPR lentivirus targeting the human HPRT1 locus with p24 titer ≥ 5 $X10^8$ particles/mL
- uses the same GeCKOv2 gRNA-only vector as the Sigma Human Whole Genome Lentiviral CRISPR Pooled Library

This control CRISPR lentivirus targets human HPRT1 gene at sequence: 5'-TTATATCCAACACTTCGTGGGG-3', where underlined GGG is PAM sequence. The use of the following PCR primers yields a 372 bp primary PCR product which is then cleaved into 128 bp and a 244 bp fragments in the SURVEYOR Mutation Assay (CEL-1 assay):
Forward primer: 5'-CTCAGCACGGATGAAATGAAAC-3'
Reverse primer: 5'-GTCAAGGGCATATCCTACAACA-3'

Product Number: HGECKO2G
The Gecko2 Human Whole Genome CRISPR Pool, gRNA Only Lenti Particles (Gecko2 vector) contains the following components:

- Originally designed and assembled by Sanjana et al. "Improved vectors and genome-wide libraries for CRISPR screening." Nature Methods 11.8 (2014): 783-784.
- two lentiviral CRISPR pools (A + B) provided as 200 ul (divided into 8 x 25 ul aliquots) of CRISPR lentivirus per pool, targeting a total of 19,052 human protein-coding genes in both pools and 1,864 miRNA genes in pool A only (4 gRNA designs per miRNA gene)
- each of the 2 pools contains 3 gRNAs per protein-coding gene (6 gRNA-per-gene coverage is obtained when combining pools A + B), with 65,386 gRNA designs in pool A and 58,031 gRNA designs in pool B
- positive and negative (non-targeting) controls included in each pool
- employs the GeCKOv2 lentiguide-puro gRNA-only vector

Reagents not provided, but recommended:
Cas9 Delivery (Plasmid and Lentiviral Formats)
Product Number:Description
CAS9BST-1EA: A lentiviral plasmid expressing Cas9 along with a blasticidin resistance gene
LVCAS9BST-1EA: Lentiviral particles expressing Cas9 along with a blasticidin resistance gene
CAS9NEO-1EA: A lentiviral plasmid expressing Cas9 along with a neomycin resistance gene
LVCAS9NEO-1EA: Lentiviral particles expressing Cas9 along with a neomycinresistance gene

High-titer Control Human HPRT1 CRISPR Lentivirus (**Product No: CRISPR16H-1EA)** contains the following components:

- 8 x 25 uL aliquots of high titer CRISPR lentivirus targeting the human HPRT1 locus with p24 titer ≥ 5 $X10^8$ particles/mL
- uses the same GeCKOv2 gRNA-only vector as the GeCKOv2 Human Whole Genome Lentiviral CRISPR Pooled Library

This control CRISPR lentivirus targets human HPRT1 gene at sequence: 5'-TTATATCCAACACTTCGTGGGG-3', where underlined GGG is PAM sequence. The use of the following PCR primers yields a 372 bp primary PCR product which is then cleaved into 128 bp and a 244 bp fragments in the SURVEYOR Mutation Assay (CEL-1 assay):
Forward primer: 5'-CTCAGCACGGATGAAATGAAAC-3'
Reverse primer: 5'-GTCAAGGGCATATCCTACAACA-3'

Product Number: HKCRISPR-1EA
The Human Kinase Lentiviral CRISPR Pool contains the following components:

- a single pool provided as 200 uL (divided into 8 x 25 uL aliquots) of CRISPR lentivirus targeting the entire human kinome with a minimum viral titer of ≥ $5x10^8$ particles/mL measured by p24
- employs Sigma's all-in-one lentiviral CRISPR vector expressing the gRNA, along with Cas9, puromycin resistance, and tGFP

Reagents not provided, but recommended:
Cas9 Delivery (Plasmid and Lentiviral Formats)
Product Number:Description
CAS9BST-1EA: A lentiviral plasmid expressing Cas9 along with a blasticidin resistance gene
LVCAS9BST-1EA: Lentiviral particles expressing Cas9 along with a blasticidin resistance gene
CAS9NEO-1EA: A lentiviral plasmid expressing Cas9 along with a neomycin resistance gene
LVCAS9NEO-1EA: Lentiviral particles expressing Cas9 along with a neomycinresistance gene

Companion Product: High-titer Control Human HPRT1 CRISPR Lentivirus (**Product No: CRISPR13H-1EA)** contains the following components:

- 8 x 25 uL aliquots of high titer CRISPR lentivirus targeting the human HPRT1 locus with p24 titer ≥ 5 $X10^8$ particles/mL
- uses the same all-in-one vector as the CRISPR kinase pool

This control CRISPR lentivirus targets human HPRT1 gene at sequence: 5'-TTATATCCAACACTTCGTGGGG-3', where underlined GGG is PAM sequence. The use of the following PCR primers yields a 372 bp primary PCR product which is then cleaved into 128 bp and a 244 bp fragments in the SURVEYOR Mutation Assay (CEL-1 assay):
Forward primer: 5'-CTCAGCACGGATGAAATGAAAC-3'
Reverse primer: 5'-GTCAAGGGCATATCCTACAACA-3'

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices. Though the lentiviral transduction particles produced are replication incompetent, it is recommended that they be treated as **Risk Group Level 2 (RGL-2)** organisms in laboratory handling. Follow all published RGL-2 guidelines for laboratory handling and waste decontamination.

Biosafety Features
The lack of generation of replication competent viral particles is an important safety feature of CRISPR Lentiviral Particles. Users should consult and observe their own institutional guidelines when working with viral systems.

Storage and Stability
After receiving immediately store the product at -70°C. Avoid freeze/thaw cycles, as this will severely reduce transduction efficiency.

Determination of puromycin sensitivity (kill curve)

NOTE: Prior to performing any large scale CRISPR library screen, two preliminary experiments are **strongly recommended**:

1. Determine the sensitivity of target cell type to puromycin (kill curve), and...
2. Determine CFU/ml (colony formation units per ml) for your target cell type using a control lentivirus sample similar in nature to the pooled library. This allows calculation of MOI (multiplicity of infection) to be based on the functional titer of the virus (derived from the CFU/ml assay) which is a more accurate reflection of experimental infection rates. It is highly recommended that your control lentivirus be in the same vector as the pooled library.

Overview of Puromycin-based CFU Determination
Preparatory Work (~ 2 weeks)

- Puromycin titration (kill curve).
- CFU determination for target cell type.
- Experimental planning using CFU data for target cell type (MOI calculation, media volumes, etc.)

Experimental Work (~2-4 weeks depending on cell growth rates)

- Transduction of cell populations with pooled CRISPR libraries.
- Cell treatment and growth.
- Sample prep and deep sequencing.
- Data analysis.

Detailed Protocols: puro-based CFU determination
Note: The appropriate concentration of puromycin for each cell type varies. If the killing concentration for the desired cell type is unknown, a titration experiment, or kill curve, must be performed. Typically, 2-10 µg/ml are sufficient to kill most untransduced mammalian cell types.

Puromycin Titration:
Materials required
· Target cells in culture
· Puromycin (10 mg/ml solution), (Sigma-Aldrich, Product No. P9620)
· Complete growth media specific for the target cells
· Hank's Balanced Salt Solution, (Sigma-Aldrich, Product No. H6648)
· 1x Trypsin-EDTA solution, (Sigma-Aldrich, Product No. T3924)

Puromycin titration (kill curve) should be performed when working with a new target cell type.

1. Plate $2x10^4$ cells per well in one 24-well plate with 0.5mL fresh medium.
2. The next day add 0.5–10 µg/ml of puromycin to selected wells.
3. Examine viability every 2 days.
4. Culture for 3 – 7 days depending on the growth rate of the cell type and the length of time that cells would typically be under selection during a normal experimental protocol. Replace the media containing puromycin every 2 - 3 days. The minimum concentration of puromycin that causes complete cell death after 4 days of treatment should be used for that cell type and experiment.

Note: Excess puromycin can cause many undesired phenotypic responses in many cell types.

Determination of infection rates (CFU/ml) for your target cell type

Note: Users are advised to determine the CFU-based titer for different target cell types prior to a library-scale screening. It is recommended to use cells at low passage number for transduction experiments.

Materials Required

- Target cells in culture
- Hexadimethrine bromide (Polybrene, 2 mg/mL stock); (Sigma-Aldrich, Product No. H9268)
- Puromycin (10 mg/mL stock); (Sigma-Aldrich, Product No. P9620)
- Crystal Violet Solution; (Sigma-Aldrich, Product No. HT90132)
- Dulbecco's Phosphate Buffered Saline; (Sigma-Aldrich, Product No. D8662)
- Recommended: Control High-titer HPRT1 LentiCRISPR virus (Product No: CRISPR13H-1EA or CRISPR16H-1EA). Use of this less expensive viral material will conserve pooled-CRISPR virus for subsequent experiments.

Day 1 Seeding cells

Seed 10^5 cells per well in fresh medium on three 6-well plates. Place the plates in an incubator set to 37°C, 5% CO_2 and incubate for 24 hours.

Day 2 Transduction

1. Thaw one vial of lentiviral particles (25µL) on ice. Mix by gently tapping the tube several times. Keep them stored on ice when not in use. **Note:** Lentiviruses are quite labile. Multiple freeze-thaw cycles and prolonged exposure to ambient temperatures will decrease the lentiviral titer.
2. Prepare 25 mL of media containing polybrene (final concentration 8 µg/mL).
3. Prepare 2.5 mL 10-fold serial dilutions over a range of 10^{-2} to 10^{-9} in 15 mL conical vials with virus. Mix gently by inverting the tubes 3 to 4 times.
    a. Mix 25 µL lentivirus with 2,475µL polybrene containing media to achieve 10^{-2} dilution.
    b. Mix 250 µL of the 10^{-2} dilution with 2,250 µL polybrene containing media to achieve 10^{-3} dilution.
    c. Mix 250 µL of the 10^{-3} dilution with 2,250 µL polybrene containing media to achieve 10^{-4} dilution.
    d. Mix 250 µL of the 10^{-4} dilution with 2,250 µL polybrene containing media to achieve 10^{-5} dilution.
    e. Mix 250 µL of the 10^{-5} dilution with 2,250 µL polybrene containing media to achieve 10^{-6} dilution.
    f. Mix 250 µL of the 10^{-5} dilution with 2,250 µL polybrene containing media to achieve 10^{-7} dilution.
    g. Mix 250 µL of the 10^{-7} dilution with 2,250 µL polybrene containing media to achieve 10^{-8} dilution.
    h. Mix 250 µL of the 10^{-8} dilution with 2,250 µL polybrene containing media to achieve 10^{-9} dilution.
4. Remove medium from all wells of three 6-well plates. Add 1.0 mL of polybrene containing complete growth medium to two wells on one 6-well plate as negative controls. Add 1.0 mL of each of the lentivirus dilutions to the remaining wells of three plates. Duplicate the transduction for each lentiviral dilution.
5. Place plates in an incubator set to 37°C, 5% CO_2 for 24 hours

Day 3 Medium change

Remove the medium containing the lentiviral particles from wells. Add 2.0 mL fresh media (without polybrene) to each well. Place plates in an incubator set to 37°C, 5% CO_2 for 24 hours

Day 4 Puromycin selection

Remove the medium from wells. Feed cells with fresh medium containing puromycin.

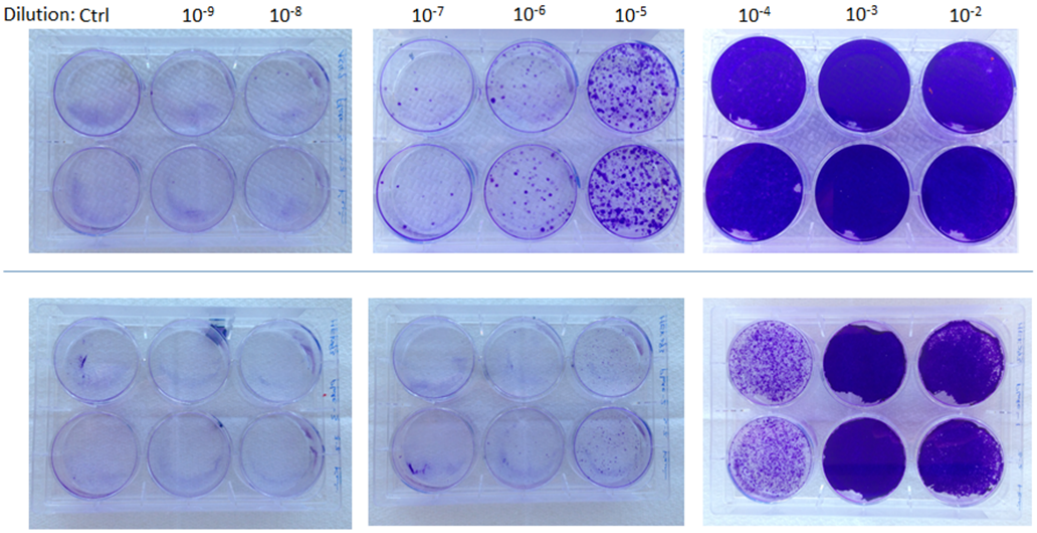
Day 5-8 Continued puromycin selection

Replace medium containing puromycin as necessary during the selection process (usually every 2 to 3 days).

Day 9 Stain and count colonies

Note: The untransduced control cells should die completely after 4-day selection with puromycin.

1. Remove medium and gently wash each well with 3.0 ml/well PBS. Add 1.0 mL of crystal violet solution per well and incubate 10 minutes at room temperature. Remove crystal violet solution. Wash twice with 3 mL Dulbecco's PBS per wash.
2. Invert plates on paper towels and let dry about 1 hour.
3. Count the blue-stained colonies (Figure 1) using a microscope at a magnification of 40X. Only count wells that contain between 10-300 colonies. Any more or less then the counts are not significant.
4. Calculate by multiplying the number of colonies per well by the dilution factor. The lentiviral titer is defined here as Colony Formation Units per milliliter (CFU/mL).



** Upper row: **A549 cells**; Lower row: **HEK293 cells**

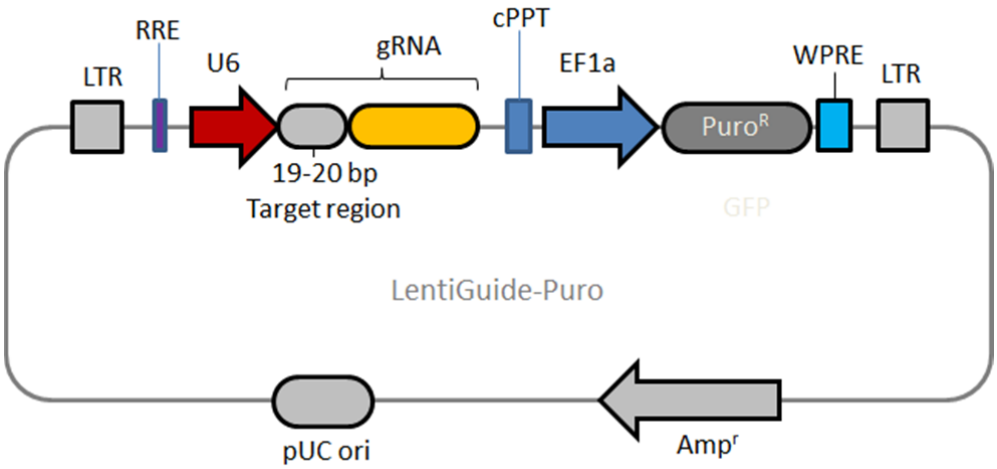
CFU count data

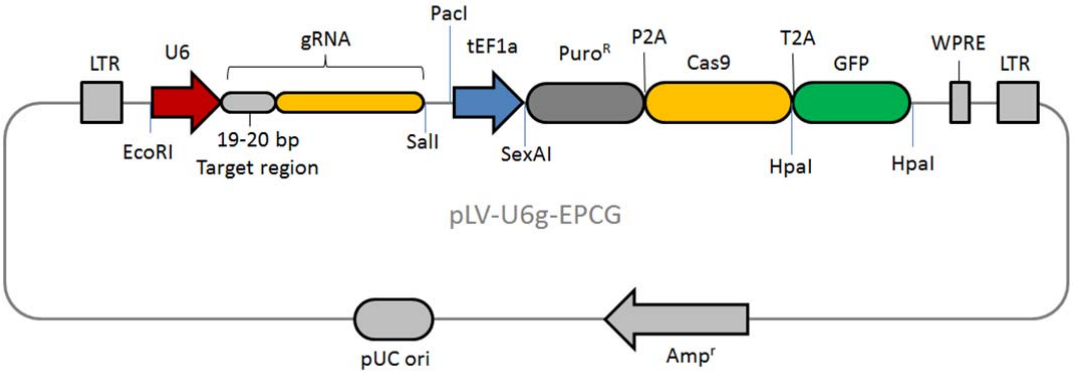
Plate Replicate	Dilution Factor	# colonies	Titer	Average	Stdev
1	1.00E+02	DNC	DNC	1.50E+08	5.59E+07
	1.00E+03	DNC	DNC		
	1.00E+04	DNC	DNC		
	1.00E+05	DNC	DNC		
	1.00E+06	189	1.89E+08		
	1.00E+07	11	1.10E+08		
	1.00E+08	DNC	DNC		
	1.00E+09	DNC	DNC		
2	1.00E+02	DNC	DNC	1.84E+08	4.95E+06
	1.00E+03	DNC	DNC		
	1.00E+04	DNC	DNC		
	1.00E+05	DNC	DNC		
	1.00E+06	187	1.87E+08		
	1.00E+07	18	1.80E+08		
	1.00E+08	DNC	DNC		
	1.00E+09	DNC	DNC		
			plates 1 and 2 combined	1.67E+08	3.786E+07

Note: If there are too many or too few colonies to count in a given well, record DNC in the # colonies field.

Figure 1. Example data for colony staining and counting to determine lentiviral CFU/ml based on puromycin resistance.

Vector Maps


RRE
cPPT
LTR
U6
gRNA
EF1a
WPRE
LTR
Puro^R
19-20 bp
Target region
GFP
LentiGuide-Puro
pUC ori
Amp^r


PacI
LTR
U6
gRNA
tEF1a
Puro^R
P2A
Cas9
T2A
GFP
WPRE
LTR
EcoRI
19-20 bp
Target region
SalI
SexAI
HpaI
HpaI
pLV-U6g-EPCG
pUC ori
Amp^r

Troubleshooting Guide

Problem	Cause	Solution
Low transduction efficiency	Cell density was not optimized: too low or too high	To determine optimal density and transduction efficiency of tested cells, first use Control High-titer HPRT1 LentiCRISPR virus, Product No: CRISPR13H-1EA or CRISPR16H-1EA. When adding cells to the wells make sure they are mixed well. Keep mixing them periodically to insure the same amount of cells for each well.
	Cell conditions were not optimal	Use low passage number cells and make sure that cells are 90% viable upon transduction.
	Cells are sensitive to polybrene	Test cells for sensitivity by including control cells incubated with media containing polybrene (8 µg/ml). If cells are sensitive to polybrene omit the addition of this reagent. Cells can still be transduced but with lower efficiency.
	Polybrene was not included during transduction	Transduce in the presence of polybrene when possible since it enhances the transduction efficiency of most cell types.
	Puromycin concentration is not optimal for cell selection	For each new cell type used, it is recommended that a puromycin kill curve be performed to determine the lowest concentration of puromycin needed to efficiently select transduced cells.

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