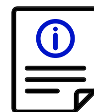
EditCo

QUICK START GUIDE 

Arrayed CRISPR gRNA Libraries

Thank you for choosing an Arrayed CRISPR gRNA Library for your experiment!

EditCo's human and mouse screening libraries utilize multi-guide sgRNA that has been strategically designed to knock out your human or mouse protein-coding gene of interest. The sgRNAs jointly cause a fragment deletion at the targeted genomic locus, consequently disrupting gene function and making it ideal for loss of function screens. The arrayed format of EditCo's libraries, in which one gene is targeted per well across multiwell plates, eliminates the need for complex data deconvolution and is compatible with a variety of functional assays. This quick start guide provides instructions on how to prepare, use, store and quantify your sgRNA library.

Materials Provided

Quantity Options for Human genes	Plate Options
150 pmol 250 pmol 300 pmol	384-well standard Nunc plates (Thermo Scientific #4309)
150 pmol 250 pmol 300 pmol	384-well Echo plates (Beckman #001-14555)
150 pmol 250 pmol 300 pmol	384-well EchoLDV plates, carboxyl coated (Beckman Coulter #001-13070)
500 pmol 1500 pmol	96-well standard Nunc plates (Thermo Scientific #260251)

Quantity Options for Mouse genes	Plate Options
150 pmol 250 pmol	384-well standard Nunc plates (Thermo Scientific #4309)
150 pmol	384-well Echo plates (Beckman #001-14555)
1500 pmol	96-well standard Nunc plates (Thermo Scientific #260251)

Note: The material listed above refers to 1-3 chemically modified sgRNA(s) per well of a plate. The molecular weight of an average 100 nucleotide sgRNA is 32µg/nmol. sgRNAs can be stored at -20°C for up to 3 years (if not repeatedly thawed).

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Additional Materials Required

Material	Ordering Information
Thermal microplate sealer	Agilent, PlateLoc
Manual plate sealer (alternative to thermal microplate sealer)	Multiple vendors (e.g., Thermo Fisher Scientific)
Plate seals	Peelable aluminum, Agilent #24210-001
Plate shaker	Multiple vendors (eg., Bioshake from QInstruments)
Nuclease-free 1X TE buffer	Multiple vendors (e.g., Thermo Fisher Scientific, Catalog #AM9849)
Nuclease-free water	Multiple vendors (e.g., Thermo Fisher Scientific)
Positive control sgRNA: Human Transfection Optimization Kit (multi-guide): includes *TRAC* multi-guide sgRNA (1.5 nmol), PCR & sequencing primers, nuclease-free water, nuclease-free Tris-EDTA buffer, & SpCas9 2NLS (S. pyogenes, 300 pmol) or Positive Control Multi-guide sgRNA (mod), Human *TRAC* (1.5 nmol) Mouse Positive Control, Mouse *Rosa26*, *mod-sgRNA* (1 nmol)	EditCo, available at checkout as an add-on
SpCas9 2NLS nuclease (*S. pyogenes*) (optional)	EditCo, available at checkout as an add-on

Rosa26* positive control is one sgRNA sequence (*not*** multi-guide)

Note: Additional materials are listed in EditCo's lipofection protocols for Cas-9-expressing cells and RNPs and for nucleofection protocol using RNPs.

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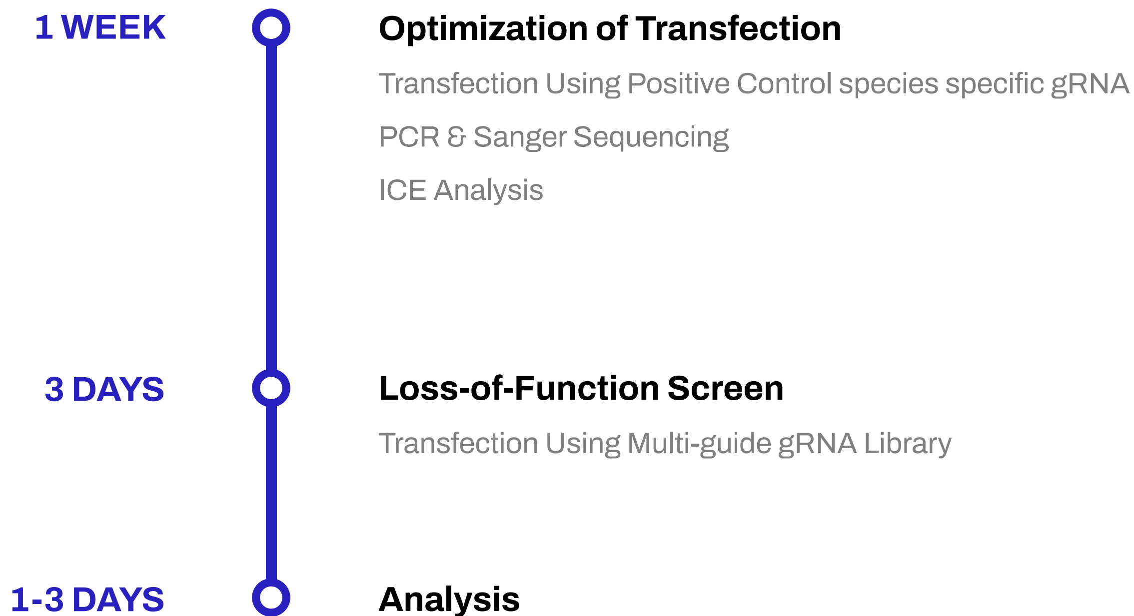
Positive Control Primer Information

Genome	Primer	Sequence
Human	*TRAC* PCR primer	Forward: 5'- TCAGGTTTCCTTGAGTGGCAGG - 3' Reverse: 5'- TAAGGCCGAGACCACCAATCAG - 3'
Human	*TRAC* Sequencing primer	5'-CTGGCCGTGAACGTTCACTGAAATCATGGC-3'
Mouse	*Rosa26* PCR primer pair	Forward: 5'-GAGGCGGATCACAAGCAATA-3' Reverse: 5'-GGGAGGGGAGTGTTGCAATA-3'

Note: Human *TRAC* primers (PCR & sequencing) are included in the Transfection Optimization Kit. Otherwise, all primers must be purchased separately. The Forward or Reverse *Rosa26* primers can be used for sequencing.

Workflow Schematic



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Step 1: Receive Plates & Remove the Plate Seal

EditCo sgRNAs ship dry at ambient temperature and remain stable for two weeks at <30°C. However, we recommend that you store the sgRNAs at -20°C immediately upon arrival. All plates are heat-sealed before shipment. Please store dried sgRNAs at -20°C for long-term storage (up to 3 years, if not repeatedly thawed). **Be sure to work in an RNase-free and in a sterile environment and use sterile, filter pipette tips throughout the entire protocol.**

1. Centrifuge the plate for a minimum of 3 minutes at 500 x g to ensure that sgRNAs collects at the bottom of each well.

2. Remove the seal by firmly holding the plate in place on a table and then gently pulling the seal off starting at one corner.

Step 2: Rehydrate & Dilute the sgRNA

Instructions on how to rehydrate sgRNAs in 384- and 96-well plates to a storage concentration are described below.

1. Add sterile nuclease-free 1X TE buffer (TE buffer can be filtered to sterilize it before use) to desired wells according to the appropriate plate format and sgRNA volume (see tables below).

384-well plates

sgRNA/ well (pmol)	Nuclease-free TE buffer* (µl)	Storage Concentration (µM)
150	15	10
250	15	17
300	15	20

96-well plates

sgRNA/ well (pmol)	Nuclease-free TE buffer* (µl)	Storage Concentration (µM)
1500	15	100
500	15	33

*TE buffer: 10 mM Tris-HCl, 1 mM EDTA, pH 8.0

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2. Re-seal plates with a thermal-based heat sealer. EditCo recommends sealing plates using the Agilent PlateLoc Thermal Microplate Sealer at 160°C for 2.8 seconds.

   Note: You might have to tweak this parameter. Please refer to the recommendations from the plate manufacturers for your plate type as different settings might seal plates differently, depending upon the plate material. Alternatively you can use a manual sealer.

3. Perform a brief spin down of the plate to ensure the TE is at the bottom of the well.

4. Mix the plate on a plate shaker for 5 minutes, slowly ramping up shaking speed from zero to 1000 x g.

5. Let the plate sit at least 72h at 4°C to fully rehydrate dried sgRNA.

   Note: rehydrated sgRNA may be stored at -20°C for up to 3 years (if not repeatedly thawed, please limit freeze-thaw cycles to five per plate).

6. When ready to use the sgRNA, let the plate warm to room temperature over 15 minutes to prevent condensation on the seal.

7. Vortex sealed plate at low speed for 30 seconds to mix.

8. Centrifuge the plate for 5-10 minutes at 500-1000 x g.

9. Remove the seal by firmly holding the plate in place on a table and then gently pulling the seal off starting at one corner to prevent cross-contamination.

10. The sgRNA can be used directly at the storage concentration or be diluted using nuclease-free water to a working stock in a sterile plate. Check your transfection protocol for the desired sgRNA concentration.

Visit editco.com/resources to explore additional protocols, guides, and resources to help your CRISPR library screen.

Step 3: Optimize Conditions & Perform Screen

We highly recommend optimizing transfection conditions in your particular cell type. For human libraries, we recommend using EditCo's chemically modified positive control multi-guide sgRNA targeting *TRAC*. This positive control is available as an add-on (1.5 nmol) or as part of EditCo's Transfection Optimization Kit (Multi-guide) (includes SpCas9 and *TRAC* primers). For mouse libraries, we recommend EditCo's chemically modified positive control sgRNA targeting *Rosa26* (1 nmol). (Note that the *Rosa26* positive control is one sgRNA sequence, ***not*** multi-guide sgRNA.)

Follow the guidelines in our genotyping protocol to prepare edited and control DNA samples for Sanger sequencing. The editing efficiency of each optimization condition can be assessed using EditCo's free Inference of CRISPR Edits (ICE) tool. Please see our ICE Knockout Analysis protocol for instructions.

Following the optimization, transfect cells with your EditCo arrayed library. EditCo has two lipofection protocols for library delivery, one for Cas9-expressing cells and one for ribonucleoproteins (RNPs) and one protocol for nucleofection in Immortalized cells lines. All protocols mentioned above include detailed optimization instructions.

Step 4: Re-Seal & Store sgRNA

1. Re-seal plates after each use with the thermal-based heat sealer at 160°C for 2.8 seconds (Note: You might need to use different heating settings depending upon your plate type. Please follow the manufacturer guidelines). Before a plate seal is removed, centrifuge the plate for 5-10 minutes at 500-1000 x g to ensure that the sgRNA collects at the bottom of each well. The plate should be gripped tightly, and the seal peeled off carefully starting from one corner. Minimize environmental exposure of sgRNA to reduce the risk of contamination.

2. Rehydrated/diluted sgRNA may be stored at -20°C or colder for 3 years (if not repeatedly thawed).

3. Limit plates to no more than 5 freeze-thaws.

Arrayed Screening Libraries Quantification for 96 well plates

1. Resuspend the dried sgRNAs in the wells, following our instructions above. Use a volume such that the theoretically expected concentration is between 10-100 µM. (Note: This will heavily depend on the plate capacity and we recommend using the working volume suggested by the microplate manufacturer to determine the concentration). A lower or higher concentration may result in increased error for the OD readings. Additionally, we cannot guarantee full resuspension of sgRNAs resuspended at concentrations higher than 100 µM. If guide concentration is higher than 100µM after quantification, please correct the resuspension volume so that final concentration is equal to or less than 100 µM and re-quantify.

2. Recommended dilution factors for 96-well plates:

Storage Concentration (µM)	Sampling Volume (µl)	Dilution Factor
50	1.2, 1	80x, 100x
100	1, 0.5	100x, 200x

We recommend using a non-absorbing liquid such as nuclease-free distilled water for UV measurements

   a. For 50 µM samples, we recommend a 100x dilution: 2 µl sample volume + 198 µl water. Please note that lower sampling volumes will increase potential error in the measurement.

   b. For 100 µM samples, we recommend a 200x dilution: 1µl sample volume + 199 µl water. Please note that lower sampling volumes will increase potential error in the measurement.

   c. Include at least one blank well with just the diluting water.

3. On a UV-reader instrument, read the absorbance at 260 nm.
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4. Using the known pathlength (specific to the instrument), the average extinction coefficient of the sgRNAs in the well (included in the QC document), and the blank adjusted absorbance, calculate the concentration of sgRNAs using the formula below.

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C= A/(ε*p) * Dilution Factor

Where:

i. C = Concentration (M)
ii. ε = Extinction coefficient (M^{-1} cm^{-1}) (found on the QC report)
iii. p = pathlength of light (cm) (specific to the instrument)
iv. A = Blank Adjusted Absorbance
v. Dilution Factor is the factor by which the sample was diluted

- You may instead use a single extinction coefficient for all values if all of your sgRNAs are the same length. We recommend using the value from the QC document for the most accurate numbers.
- Please use the setup you have available to determine your own pathlength.

5. Convert the UV obtained concentration to yield (moles) if needed, by multiplying the concentration and the resuspension volume. Convert to your unit of relevance and use this process to calculate how much material you have in each well.

Example

5 µl of resuspended sgRNA from a 100 µl volume well was taken out and mixed with 195 µl of water in a UV plate. With a 40X dilution factor, we recorded an unadjusted absorbance at 260 nm of 0.348 and a blank diluent well absorbance of 0.051. The machine's pathlength is 0.52 cm. The sgRNAs have an average extinction coefficient, ε = 1010436.667 M^{-1} cm^{-1}, per the QC document, to determine the total concentration from absorbance.

C= A/(ε*p) * Dilution Factor

C= (0.348 - 0.051) /(1010436.667 M^{-1} cm^{-1} * 0.52 cm) * 40

C=0.000022610 M

C= 22.610 µM

Converting to Yield (if needed)

Yield = C * Volume of well

Yield = (22.610 µM *100 µl)/1000 = **2.261 nmoles of total sgRNA** in the well prior to sampling

You may instead multiply by the expected volume in the well after sampling to determine the updated yield. In this case, it would be Yield = (22.610 µM *95 µl)/1000 = **2.147 nmoles**

Additional Information

For an up-to-date list of all protocols and other resources, please visit this link.

For technical assistance, contact our Scientific Support Team at technicalsupport@editco.bio.

For common FAQs, please visit this link.

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