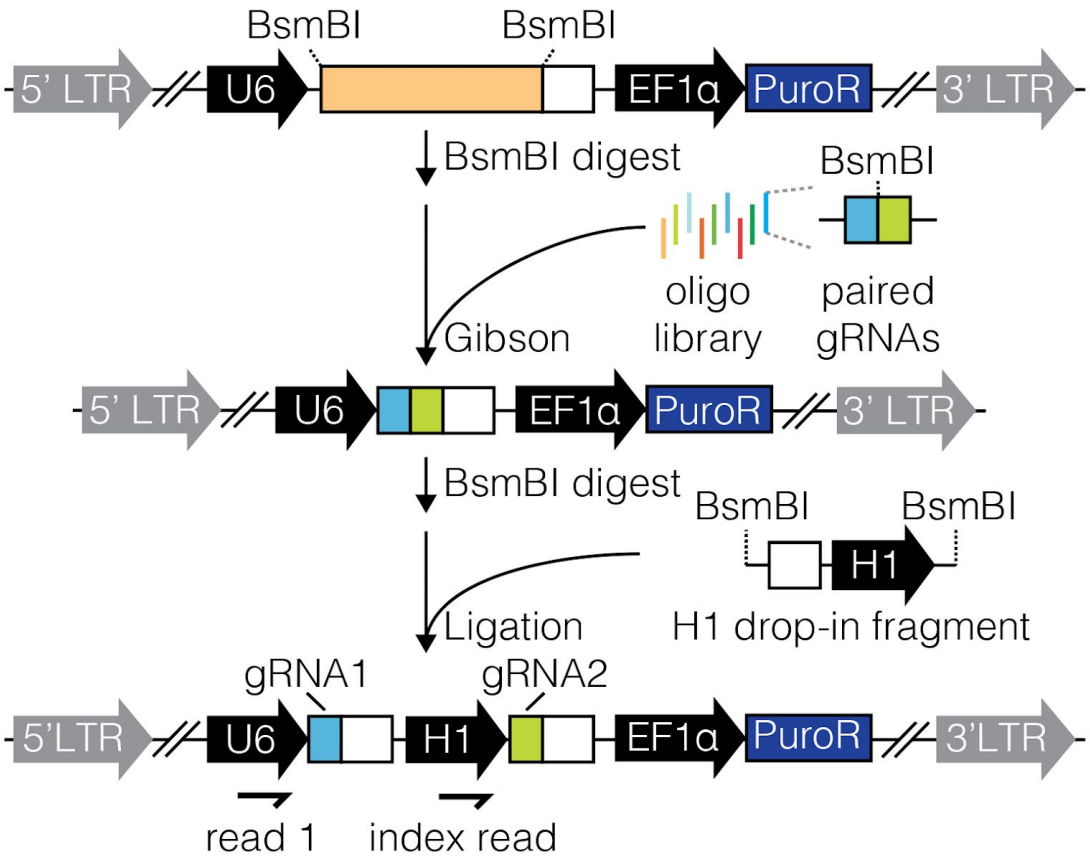
pgRNA library cloning & screening

protocol used in Thomas et al (2020), *Nature Genetics*

contact for questions/comments: jdthomas@fredhutch.org
http://bradleybiology.org/

Major steps

Procedure overview

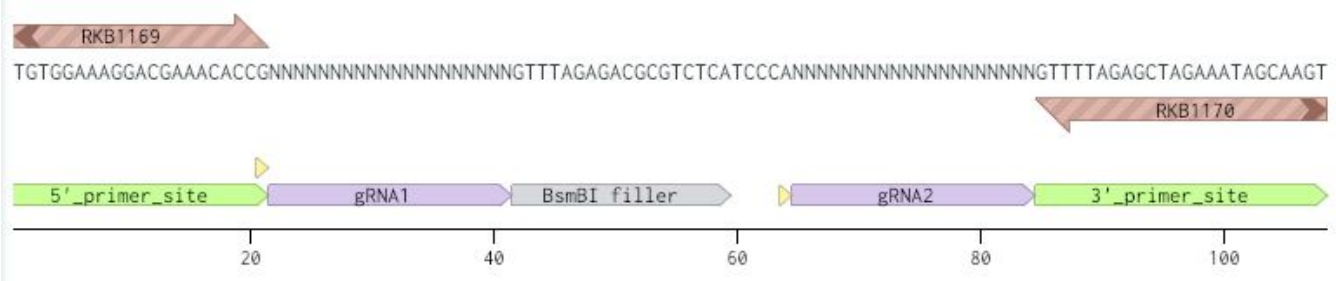


General/misc. notes:

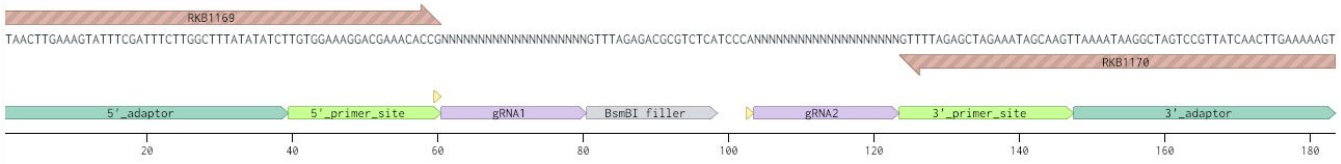
- Backbone is the lentiGuide-Puro plasmid (Addgene #52963)
- The oligo library can be ordered from Twist or a similar company
- The "H1 drop-in" fragment is ordered as a 376 bp gBlock from IDT
- These papers are useful for thinking about high-throughput CRISPR screens:
  - PMID: 28333914
  - PMID: 29651054
  - PMID: 29199283

pgRNA oligo overview

pgRNA oligo ultramer



pgRNA oligo ultramer after amplification with RBK1169 & RKB1170



RKB1169 sequence: TAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCG

RKB1170 sequence: ACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC

RKB1148 sequence: GAGGGCCTATTTCCCATGAT (used for Sanger sequencing)

Equipment Set Up

Large LB plates

Prepare 35 g/L LB agar (Invitrogen #22700-025) solution in deionized water and swirl to mix. Autoclave to dissolve and sterilize the mixture. Allow LB agar to cool to 55˚C before adding carbenicillin at 100 µg/mL and swirl to mix. On a sterile bench, pour ~300 mL of LB agar per large plate (Corning CLS431111-16EA). Place the lids on the plate (leaving a crack to allow stream to escape) and allow to cool until solidified (~30 minutes to 1 hour). Invert plates and let sit several more hours before storing at 4˚C for up to 3 months.

Important notes:

- Autoclaving should be done in a *large* vessel. For 2 L of LB agar, at least a 4 L flask is necessary. Be sure to add aluminum foil and tape the lid down to help prevent boiling over. When autoclaving, don't put the flask in a pool of water because the agar in the bottom of the flask won't completely melt.
- When you remove LB from autoclave, combine all media into a large beaker (we have 4L beaker in the lab). Add a sterile stir bar and stir on the stir plate until the media cools enough (i.e., is warm but cool enough to touch) to add carbenicillin.
- Be really careful not to introduce bubbles when pouring the plates!
- I typically make these at least 24 hours in advance of needing them.

Construct pgRNA plasmid library

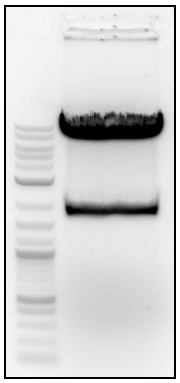
Digest and gel extract pLGP backbone

Set up restriction digest reaction as follows:

5 µg plasmid
3 µL BsmBI FastDigest (Thermo Esp3I; Cat # FD0454)
3 µL FastAP (Thermo Cat # EF0654)
6 µL 10X FastDigest Buffer
0.6 µL 0.1 M DTT
Water to 60 µL

Digest at 37˚C for at least 30 minutes.

Run product on a 0.8% agarose gel and excise backbone band (2 kb BsmBI filler will have dropped out). The product should look like this:



The upper back is the backbone (~8.3 kb) and the lower band is the insert that should be discarded (~1.88 kb).

Purify using a gel extraction kit (we prefer the one from Zymo; Cat # D4007) following the manufacturer's protocol (add the extra water step that it recommends).

Store the purified backbone at -20˚C.

Calculate number of molecules per ng DNA

Note: below we use our human poison exon knockout library (n = 9,508 pgRNAs) for calculations.

Twist ssDNA oligo = 108 bp
Avg. MW nucleotide = 330 pg/pmol

MW of oligo = 35,640 pg/pmol

(1 ng of oligo pool) x ($1x10^3$ pg/1 ng) x (1 pmol/35,640 pg) = 0.02806 pmol of oligo pool per PCR reaction

(0.02806 pmol of oligo pool) x (1 mol/ $1x10^{12}$ pmol) x (6.022×10^{23} molecules/mol) = 0.16898×10^{11} molecules per PCR reaction

1.6898×10^{10} molecules of oligo/9508 oligos in the library = 1.777×10^6 copies of each pgRNA per PCR. This is well above the desired coverage (500-1000X) to maintain library diversity.

PCR amplify and purify oligos

Note: For this step, it can be helpful to first do a single test PCR to determine the correct conditions (e.g., cycle number). It's *very* important to get a single band from this PCR reaction (see gel below). Over amplifying the library at this stage results in multiple bands and "mis-pairing" of gRNAs.

Set up eight PCR reactions on ice as follows (make this as master mix and then aliquot 50 µL reactions for PCR cycles):

25 µL NEBNext High Fidelity 2X Ready Mix (Cat # M0541S)
1.5 µL of 10 µM forward primer (RKB1169 - see above for sequence)
1.5 µL of 10 µM reverse primer (RKB1170 - see above for sequence)
1 µL of ~1 to 10 ng/µL oligo library
Water to 50 µL

Amplification conditions:

95°C	5 min	1 cycle
98°C	20 s	2 cycles
62°C	15 s	-
72°C	30 s	-
98°C	20 s	6 cycles
72°C	45 s	-
72°C	5 min	1 cycle
10°C	hold	

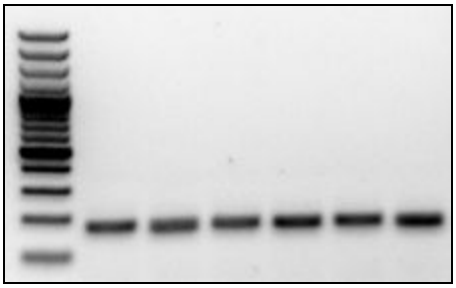
Pool the reactions and purify using a 1X SPRI bead clean-up (make 80% ethanol fresh daily for these clean-ups!):

1. Vortex SPRI beads and bring to RT for ~30 minutes.
2. Add 1X volumes of SPRI beads for every 1 volume of solution (e.g., 50 µL beads for every 50 µL reaction). Mix thoroughly by pipetting.

3. Incubate for 15 minutes at RT.
4. Place on a magnet for ~5 minutes.
5. Take off the supernatant leaving ~5 µL behind.
6. Wash with 200 µL of 80% ethanol (make this fresh daily!).
7. Incubate for 30 seconds.
8. Take off all ethanol.
9. Repeat the wash once.
10. Dry beads with caps open. This is the trickiest part because you don't want to overdry the beads. The beads should be just slightly wet when you resuspend. This typically takes < 5 minutes of drying.
11. Remove the tube from the magnet and elute in water. For pgRNA oligo amplification, I usually elute in 10 µL of water for each reaction (e.g., 80 µL for 8 total reactions)
12. Incubate for 2 minutes at RT.
13. Place on a magnet for ~5 minutes.
14. Transfer the supernatant to a new tube.

Quantify the purified pLGP backbone and PCR-amplified oligo library using a NanoDrop.

The purified PCR product should be a single band:



Having a single band is **very** important! Each lane above is a technical replicate.

Gibson assembly: combine pgRNA insert & plasmid backbone

Calculate the amount of molecules for each purified product using the following equation:

pmols = (weight in ng) x 1,000 / (base pairs x 650 daltons)

pmols insert = (3.80 ng x 1,000) / (161 bp x 650)
pmols insert = (3,800) / (104,650)
pmols insert = 0.0363

pmols backbone = (52.5 x 1,000) / (8,183 bp x 650)
pmols backbone = (52,500) / (5,318,950)

pmols backbone = 0.0099

For Gibson assembly with 2-3 fragments, the total amount of DNA should be between 0.02-0.5 pmols. Based on the calculation above, we are adding 0.0462 pmols of DNA at a 3.6:1 insert to backbone ratio.

Set up positive (4 total) and no insert (1 total) negative control reactions for Gibson assembly:

5 µL NEBuilder HiFi (Cat # E2621S)
52.5 ng of plasmid backbone (1:3.6 insert)
3.80 ng of insert
Water to 10 µL

Incubate at 50˚C for 1 hour.

Pool the positive and negative control reactions separately and purify using a 0.8X SPRI bead clean-up. Elute the DNA in 5 µL of water per Gibson reaction (e.g., 20 µL of water for 4 total Gibson reactions). Quantify the product using a NanoDrop (this isn't really necessary but can give a general sense of the yield. It's okay if the 260/230 or 260/280 values are a little wonky). This library can be stored at -20˚C for several months.

Bacterial amplification round #1

Notes:

- It is helpful to do a test transformation using 10 cm plates to make sure your Gibson assembly worked before doing the big transformation and plating on the large plates. While this adds an extra day to the protocol, it avoids accidentally using large LB plates for failed reactions.
- For a custom library such as ours, Joung et al 2017 recommends 1 bacterial transformation per 5,000 gRNAs in the library. Include 1 additional bacterial transformation for the Gibson negative control (i.e., no insert) reaction.

Electroporation

1. Bring recovery media (2 mL per transformation; Lucigen 80026-1) and LB-Carb selection plates to room temperature.
2. Pre-chill 1.0 mm cuvettes and microcentrifuge tubes on ice.
3. Thaw aliquots of Endura ElectroCompetent cells (Lucigen 60242-2)on ice (~10-20 minutes). Each aliquot is sufficient for two 25 µL transformations.
4. Mix thawed cells by tapping gently and aliquot 25 µL of cells to pre-chilled microcentrifuge tubes on ice.
5. Add 2 µL of purified Gibson product per transformation and stir briefly with pipette tip. Do not pipette up and down!

6. Carefully pipette cell/DNA mixture into pre-chilled electroporation cuvette without introducing air bubbles. Tap the cuvette on the desk to deposit cells at the bottom of the well.
7. Electroporate bacteria using standard procedures. The Bio-Rad machine we use typically returns the following values: ~1.8 kV and ~5.6 msec. Bellow are the conditions recommended by Lucigen for these cells:
    - 1.0 mm cuvette
    - 10 µF
    - 600 Ohms
    - 1800 Volts
    - Note: typical time constants are 3.5 to 4.5 msec
8. Quickly after electroporation, add 975 µL of recovery media to the cuvette, pipet up and down three times, and transfer to a culture tube containing 1 mL of media (total volume now 2 mL).
9. Place the tube in a shaking incubator at 250 rpm for 1 hour at 37˚C.

Plating

1. Combine transformants into a single tube and mix well.
2. Make two serial dilutions of pooled transformant:
    a. 10 µL of stock to 990 µL media (100-fold dilution)
    b. 100 µL of 100-fold dilution to 900 µL of media (1000-fold dilution)
3. Plate 100 µL of these two dilutions on separate LB agar selection plates. These are now 1,000-fold and 10,000-fold dilutions of the stock transformant that will be used to estimate transformation efficiency. Also, make 1,000-fold dilutions of the Gibson negative control reaction and plate that as well.
4. Add the remaining transformant to either:
    a. Liquid culture: for a total volume of 105 mL with a final concentration of 100 µg/mL Carb. I *don't* do this but I've heard it can work.
    b. Large LB plates: for this, add 1 volume of media for every volume of pooled transformant. Add 2 mL of this solution per large LB agar plate. Use a cell spreader (not glass beads) to get even cell spreading.
5. Incubate the cells for 12 to 24 hours at 30˚C. For liquid cultures, periodically measure the OD600 to capture the cells in the linear growth phase. An OD600 of 1 is equivalent to 8 x 10^8 cells/mL. An ideal OD600 is 1-3 (when measuring on the NanoDrop, it will be necessary to make serial dilutions of the stock solution to ensure linearity of the measurement). Again, I *don't* use liquid cultures. For LB plates, grow until you can see small colonies (this will be ***way*** more than enough biomass for getting high yields of bacteria).

Calculate transformation efficiency

Note: below we use our human poison exon knockout library (n = 9,508 pgRNAs) for calculations.

Count the number of colonies on the 1,000-fold or 10,000-fold dilution plate (whichever is easier). Calculate the total number of transformants using this formula (if 1,000-fold plate was counted):

cells = (mL of original transformant X 1000) X (# of colonies)

For example:

total cells = (4 X 1000) X (43)
total cells = 172,000

library coverage = 172,000/9508
library coverage = 18-fold

If the coverage is high enough (ideally greater than 200/500-fold but definitely no less than 50-fold), continue with plasmid purification.

IMPORTANT! At this stage it is a really good idea to send ~100 (this may be a little overkill, less is okay too) colonies for Sanger sequencing to test for (1) the percentage of gRNA mis-pairing and (2) a reasonable representation of library diversity (although this won't really be possible without high-throughput sequencing). At minimum, this is a good sanity check to make sure things are working.

Plasmid purification round #1

Notes:

- Getting good yield from Maxi-Preps has been a tricky part of this protocol for me (probably just my lack of experience!).
- According to Joung et al 2017, the number of maxipreps to perform can be determined using this formula: # of maxipreps = OD600 X (total volume of suspension)/1,200. I don't do this. I just use the wet weight mass metric described below.
- The Macherey-Nagel Maxiprep kit works best in my hands for this protocol.

To collect cells from LB agar plate, follow these steps:

1. Add LB to each plate and gently scrape colonies with a cell spreader. Decant this mixture into a large, sterile beaker. Be careful not to dig into the LB!
2. Repeat with another amount of LB.
3. Repeat steps 1 & 2 for each LB agar plate.
4. Aliquot the bacterial mixtures evenly amongst 50 mL conical tubes.
5. Spin at max speed at 4°C for 10 minutes.
6. Decant the supernatant. Weigh the tube and figure out the weight of each wet bacterial pellet.
7. Use 2.25-2.5 grams of "wet weight" bacterial pellet for each MaxiPrep using the Macherey-Nagel Maxiprep kit.

Digest and gel extract pLGP-2xSpacer

Notes:

- I've found that digesting the backbone overnight can reduce the number of background colonies in the subsequent ligation steps. I think poor BsmBI digestion has been one of the major culprits of failed reactions in the past. This could (probably) just be my hands though.

Set up restriction digest reactions as follows:

5 µg plasmid
3 µL BsmBI FastDigest
3 µL FastAP
6 µL 10X FastDigest Buffer
1.2 µL 0.05 M DTT (dilute stock 0.1M by half to get this)
Water to 60 µL

Digest at 37˚C for at least 1 hour (preferably overnight for me!)

Purify the product using 0.8X SPRI bead clean-up. Elute in 20-30 µL water/rxn. It can be helpful to run undigested, unpurified digested, and purified digested product on a gel to make sure the digestion efficiency was good.

Digest and purify H1 drop-in

Reaction:

25 µL of 10 ng/µL H1 drop-in (IDT GBlock, see above for link to sequence)
2.5 µL BsmBI FastDigest
5 µL 10X FastDigest Buffer
1 µL 0.05 M DTT (dilute stock 0.1M by half to get this concentration)
Water to 50 µL

Digest at 37˚C for at least 1 hour.

Purify the product using a 1.8X SPRI bead clean-up. Elute in 15 µL water/rxn. I typically make a lot of digested H1 drop in and store it for months of use.

Measure the concentration of the purified pLGP-2XSpacer backbone and purified H1 drop-in via NanoDrop.

Ligation: combine H1 drop-in insert & plasmid backbone

Notes:

- For this, be sure to include a no insert control! This is *very* important.
- It's also important to use fresh ligase buffer. I typically aliquot the buffer into one-time use tubes.

- For me (JDT), this ligation has been the trickiest part of cloning individual pgRNAs or pgRNA libraries.

Set up four ligation reactions:

5 ng insert
100 ng vector
5 µL 2X Quick Ligase buffer (comes with ligase)
Water to 10 µL
1 µL Quick Ligase (Cat # M2200S)

Incubate at room temperature for 15 minutes. The protocol says less is actually better. Don't heat inactivate the enzyme! Either proceed with SPRI bead clean-up or store at -20°C.

Combine reactions and perform 0.8X SPRI bead clean-up. Elute in 5 µL water/rxn.

Adding 2 µL of ligation reaction for each transformation seems to be very efficient.

Bacterial amplification round #2

Note:

- It is helpful to do a test transformation using 10 cm plates to make sure your ligation worked before doing the big transformation and plating on the large plates. While this adds an extra day to the protocol, it avoids accidentally using large LB plates for failed reactions.

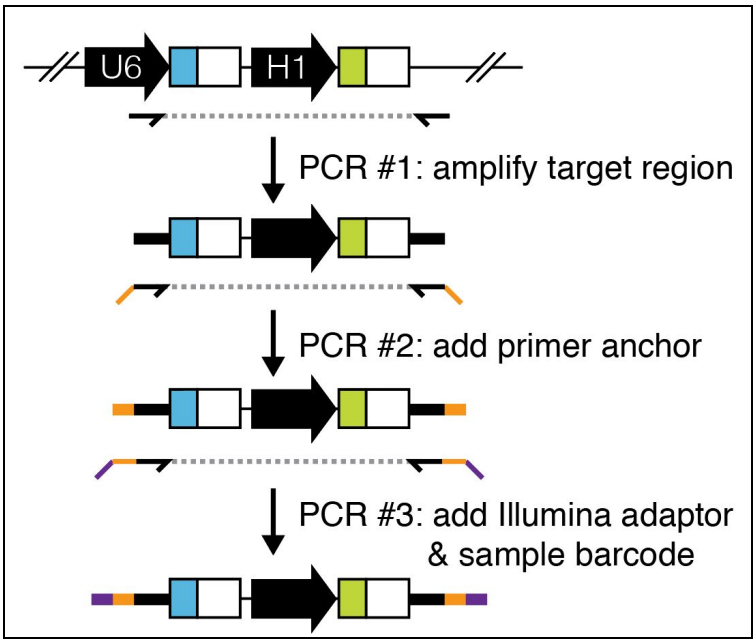
This procedure is the same as the first round of bacterial amplification. Add 2 µL of the ligation product for each transformation.

Plasmid purification round #2

This procedure is the same as the first round of plasmid purification. As before, it can be helpful to set aside some colonies (up to 100) for Sanger sequencing prior to plasmid purification.

Confirm pgRNA plasmid library diversity using NGS

Outline of the NGS library prep:



The sequence of the final amplicon can be found here.

Calculate PCR1 input molecules

Note: below we use our human poison exon knockout library (n = 9,508 pgRNAs) for calculations.

Calculate the number of molecules per ng of plasmid used in PCR

Step 1: calculate weight of plasmid

size of plasmid = 8649 bp
weight of dsDNA bp = 650 pg/pmol
weight of plasmid = (8649) * (650 pg/pmol) = 5,621,850 pg/pmol

Step 2: Determine pmol of plasmid per 1 ng

(1 ng) * (1000 pg/ng) * (1 pmol/5,621,850 pg) = 0.00017787738

Step 3: Determine the number of molecular per 1 ng

(0.00017787738 pmol) * (1 mol/ 1 x 10^{12} pmol) * (6.022 x 10^{23}) = 107,117,758

Step 4: Determine library coverage per 1 ng

(107,117,758 molecules/ng) / 9508 pgRNAs = 11,266-fold coverage

Plasmid NGS PCR #1

Note: It's a good idea to run two PCRs. One will be used for SPRI bead purification, the other reaction can be run on a gel to confirm you get a single band. It's *very* important to get a single band. If there is more than one band, run the reaction again, reducing the number of amplification cycles. Over amplifying the library results in pgRNA "mis-pairing".

Start thermocycler and pause it at the first 98°C denaturation step (see below)

Set PCR reaction on ice as follows:

25 µL NEBNext High Fidelity 2X Ready Mix
2.5 µL of 10 µM forward primer (RKB2713) (0.5 µM final concentration)
2.5 µL of 10 µM reverse primer (RKB2714) (0.5 µM final concentration)
1 ng of plasmid DNA
Water to 50 µL

RKB2713: TTGTGGAAAGGACGAAACACC
RKB2714: CAAGATCTAGTTACGCCAAGCT

Run reaction in thermocycler:

98°C	30 seconds	1 cycles
98°C	10 seconds	10 cycles (increase/decrease as needed)
65°C	10 seconds	-
72°C	1 minute	-
72°C	2 minutes	1 cycles
4°C	Hold	

Purify the product using a 0.8X SPRI bead clean-up. Elute each 50 µL PCR reaction in 50 µL of water. Quantify using NanoDrop.

Calculate PCR2 input molecules

Note: below we use our human poison exon knockout library (n = 9,508 pgRNAs) for calculations.

Step 1: calculate weight of plasmid

size of amplicon = 473 bp

weight of dsDNA bp = 650 pg/pmol
weight of amplicon = (473) * (650 pg/pmol) = 307,450 pg/pmol

Step 2: Determine pmol of plasmid per 1 ng

(1 ng) * (1000 pg/ng) * (1 pmol/307450 pg) = 0.00325256139

Step 3: Determine the number of molecular per 1 ng

(0.00325256139 pmol) * (1 mol/ 1 x 10^{12} pmol) * (6.022 x 10^{23}) = 1,958,692,469

Step 4: Determine library coverage per 1 ng

(1,958,692,469 molecules/ng) / 9,508 pgRNAs = 206,004

Plasmid NGS PCR #2

Notes:
- As before, getting a single band from this PCR is *very* important.
- Only need to set up one reaction per library for the following PCR since the library diversity should be maintained at well over 500X coverage at this point.

Set up PCR reaction on ice as follows:

25 µL NEBNext High Fidelity 2X Ready Mix
2.5 µL of 10 µM forward primer (RKB2715) (0.5 µM final concentration)
2.5 µL of 10 µM reverse primer (RKB2716) (0.5 µM final concentration)
10 ng of PCR #1 product
Water to 50 µL

RKB2715: CTAAATGGCTGTGAGAGAGCTCAGTTGTGGAAAGGACGAAACACC
RKB2716: ACTTTATCAATCTCGCTCCAAACCCAAGATCTAGTTACGCCAAGCT

Run the reaction in a PCR thermocycler using the following conditions:

98°C	30 seconds	1 cycles
98°C	10 seconds	6 cycles (increase/decrease as needed)
65°C	10 seconds	-
72°C	1 minute	-
72°C	2 minutes	1 cycles
4°C	Hold	

Purify the product using a 0.8X SPRI bead clean-up. Elute each 50 µL PCR reaction in 25 µL of water. Quantify using NanoDrop.

Plasmid NGS PCR #3

Notes:

- As before, getting a single band from this PCR is *very* important.
- Only need to set up one reaction per library for the following PCR since the library diversity should be maintained at well over 500X coverage at this point.

This is the PCR where sample specific barcodes can be added. These are the barcode primers that we use:

primer	**sequence**	**barcode**
RKB2957	CAAGCAGAAGACGGCATACGAGATTACAAGGACCGTCGGCACTTTATCAATCTCGCTCCAAACC	TACAAG
RKB2958	CAAGCAGAAGACGGCATACGAGATGTAGCCGACCGTCGGCACTTTATCAATCTCGCTCCAAACC	GTAGCC
RKB2959	CAAGCAGAAGACGGCATACGAGATAAGCTAGACCGTCGGCACTTTATCAATCTCGCTCCAAACC	AAGCTA
RKB2960	CAAGCAGAAGACGGCATACGAGATCTGATCGACCGTCGGCACTTTATCAATCTCGCTCCAAACC	CTGATC
RKB2961	CAAGCAGAAGACGGCATACGAGATTCAAGTGACCGTCGGCACTTTATCAATCTCGCTCCAAACC	TCAAGT
RKB2962	CAAGCAGAAGACGGCATACGAGATGATCTGGACCGTCGGCACTTTATCAATCTCGCTCCAAACC	GATCTG
RKB2963	CAAGCAGAAGACGGCATACGAGATATTGGCGACCGTCGGCACTTTATCAATCTCGCTCCAAACC	ATTGGC
RKB2964	CAAGCAGAAGACGGCATACGAGATCACTGTGACCGTCGGCACTTTATCAATCTCGCTCCAAACC	CACTGT
RKB2965	CAAGCAGAAGACGGCATACGAGATTGGTCAGACCGTCGGCACTTTATCAATCTCGCTCCAAACC	TGGTCA
RKB2966	CAAGCAGAAGACGGCATACGAGATGCCTAAGACCGTCGGCACTTTATCAATCTCGCTCCAAACC	GCCTAA
RKB2967	CAAGCAGAAGACGGCATACGAGATACATCGGACCGTCGGCACTTTATCAATCTCGCTCCAAACC	ACATCG
RKB2968	CAAGCAGAAGACGGCATACGAGATCGTGATGACCGTCGGCACTTTATCAATCTCGCTCCAAACC	CGTGAT

Set up PCR reaction on ice as follows:

25 µL NEBNext High Fidelity 2X Ready Mix

2.5 µL of 10 µM forward primer (RKB2717) (0.5 µM final concentration)
2.5 µL of 10 µM reverse primer (index primer) (0.5 µM final concentration)
10 ng of PCR #2 product
Water to 50 µL

RKB2717: AATGATACGGCGACCACCGAGATCTACACACGTAGGCCTAAATGGCTGTGAGAGAGCTCAG

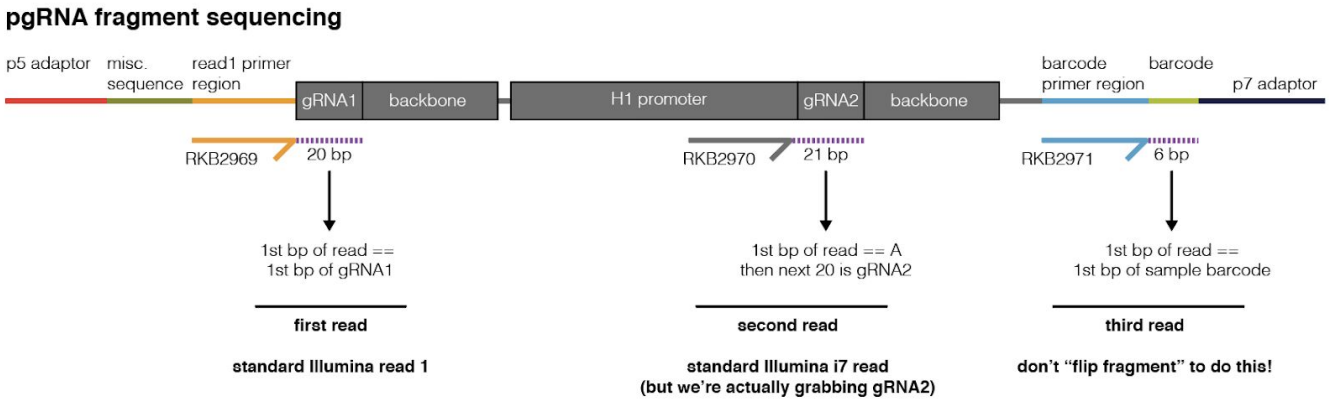
Run the reaction in a PCR thermocycler using the following conditions:

98°C	30 seconds	1 cycles
98°C	10 seconds	6 cycles (increase/decrease as needed)
65°C	10 seconds	-
72°C	1 minute	-
72°C	2 minutes	1 cycles
4°C	Hold	

Purify product using a 0.8X SPRI bead clean-up. Elute each 50 µL PCR reaction in 25 µL of water. Quantify product using Qubit to accurately determine concentration for Illumina sequencing.

Illumina sequencing

Schematic of Illumina library sequencing strategy:



Sequencing primers (order standard, desalted from IDT):
RKB2969: GAGAGCTCAGTTGTGGAAAGGACGAAACACCG
RKB2970: GGGAATCTTATAAGTTCTGTATGAGACCACTCTTTCCC
RKB2971: GGTTTGGAGCGAGATTGATAAAGTGCCGACGGTC

For sequencing with RKB2971, a custom chemistry protocol is required to allow three reads without paired-end re-synthesis. This information is contained in the file "Amplicon_mod_no_resynthesis.zip". It is important to put this in the correct directory so the MiSeq can access it. The directory should be the same as the v2 synthesis chemistry. This should be something like 'C:\Illumina\MiSeq Control Software\Recipe\v2'. The custom sequencing primers are resuspended to 100 µM and added to the Illumina Primer wells:

- combine 5 µL of 100 µM custom primer + 145 µL of HT1 buffer
- load the entire 150 µL directly into wells 12-14 of the cartridge

Remember to specify the chemistry "Amplicon_mod_no_resynthesis" on your sample sheet, as well as put the proper cycle numbers:

- Illumina-R1: as many as 25 cycles (these capture gRNA1, more cycles help to get full quality states from PhiX spike ins)
- Illumina-IX: 21 cycles (these capture gRNA2)
- Illumina-R2: 6 cycles (more if using longer sample barcodes)

Sample sheet example:

[Header]										
Investigator Name	James D Thomas									
Project Name	pgrna_sequencing									
Experiment Name	pgrna_sequencing									
Date	2/20/18									
Workflow	Generate FASTQ									
Description										
Chemistry	Amplicon_mod_no_resynthesis									
[Reads]										
20										
9										
[Settings]										
[Data]										
Sample_ID	Sample_Name	Sample_Plate	Sample_Well	Sample_Project	index	I7_Index_ID	index2	I5_index_ID	Description	GenomeFolder
samples	samples				TTGGTAGTCGTTGGTAGTCGA					C:\Illumina\MiSeq Reporter\Genomes\PhiX\Illumina\RTA\Sequence\Chromosomes

I typically submit 30 µL of a 6 nmol pooled library (can be less concentrated though; chat with people at genomics core to confirm this) for running on the MiSeq 50 bp kit (with the v2 kit, you can actually get up to a total of 66 cycles). I typically get at least 13-15 million reads per run but sometimes as many as 20 million.