Part I: Library Construction Protocol from Library Plasmid DNA

1. Library Analysis PCR 1 (LaPCR1)

Components	Amount (µL/ µg)	Final Concentration
Library Plasmid DNA	1ng	-
Lenti-Guide Forward primer (10 µM)	2.5µL	0.5 µM
Lenti-Guide Reverse primer (10 µM)	2.5µL	0.5 µM
5X Q5 Reaction Buffer	10µL	1X
Deoxynucleoside triphosphates (dNTPs; at 10 mM)	1µL	200µM
Q5 HIFI DNA Polymerase	0.5µL	1.0U/50µL PCR
Nuclease Free Water/ddH2O	To 50 µL	

2. Use the following PCR cycling conditions:

Temperature	Duration	Cycles
98 ˚C	30 seconds	1
98 ˚C	10 seconds	15/20
72 ˚C	60 seconds	
72 ˚C	5 minutes	1
4 ˚C	-	Hold

Lenti-Guide Forward primer:
TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCG

Lenti-Guide Reverse primer:
GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTTTAGTTTGTATGTCTGTTGCTATTATGTCTACTATTCTT TCCC

3. Library Analysis PCR 2 (LaPCR2): Add an index to each sample for multiplexing (Nextera primers see **Table 1**).

Components	Amount (µL)	Final Concentration
LaPCR1 Product from step 2 [Diluted 1:5]	1 µL	-
Nextera Forward Primer (Ad1_noMX) (2 µM)	1 µL	0.2 µM
Nextera Reverse Primer (Ad2.x*) (2 µM)	1 µL	0.2 µM
5X Q5 Reaction Buffer	2 µL	1X
Deoxynucleotide triphosphates (dNTPs; at 10 mM)	1 µL	200 µM
Q5 HIFI DNA Polymerase	0.1 µL	1.0 U/50 µL PCR
Nuclease Free Water/ddH2O	To 10 µL	

4. PCR cycling conditions:

Temperature	Duration	Cycles
98˚C	30 seconds	1
98˚C	10 seconds	15 (see **Note1**)
65˚C	30 seconds	
72˚C	30 seconds	
72˚C	5 minutes	1
4 ˚C	-	Hold

5. Gel purify the ~466 bp amplification product using a gel purification kit as per manufacturer's instructions.
6. Measure the DNA concentration using Qubit.
7. Mix the DNA samples and send out for NGS sequencing.

Part II: Library Construction Protocol from Genomic DNA

1. Extract genomic DNA using genomic DNA extraction kit. Measure the quality and quantity of genomic DNA using Nanodrop. Use genomic DNA immediately for library PCR or store at -20°C until further use.
2. Perform the Library PCR1 with sufficient coverage. For ACER, to achieve 1,000x coverage, at least 8,000,000 cells which is approximately 52.8 µg (the amount of genomic DNA acquired from 1 million cells is typically around 6.6 µg) must be used for library PCR. Typically, multiple reactions of PCR can be set. For example, if using 5 µg of genomic DNA per reaction, around 11 more PCR reactions is needed to reach sufficient coverage.
3. Library Analysis PCR 1 (LaPCR1): Amplify sgRNA library

Components	Amount (µL/ µg)	Final Concentration
Library Genomic DNA from STEP 1.	5 µg	-
Lenti-Guide Forward primer (10 µM)	5 µL	0.5 µM
Lenti-Guide Reverse primer (10 µM)	5 µL	0.5 µM
5X Q5 Reaction Buffer	20 µL	1X
Deoxynucleotide triphosphates (dNTPs; at 10 mM)	2 µL	200 µM
Q5 HIFI DNA Polymerase	27 µL	1.0 U/50 µL PCR
Nuclease Free Water/ddH2O	To 100 µL	

4. Use the following PCR cycling conditions:

Temperature	Duration	Cycles
98 ˚C	30 seconds	1
98 ˚C	10 seconds	25/30/35(Note2)
72 ˚C	60 seconds	
72 ˚C	5 minutes	1
4 ˚C	-	Hold

5. Pool all LaPCR1 product from the same sample together and vortex to mix well.

6. Library Analysis PCR 2 (LaPCR2): Add an index to each sample for multiplexing (Nextera primers see **Table 1**).

Components	Amount (µL)	Final Concentration
LaPCR1 Product from STEP 3 [Diluted 1:5] (Note 3)	1 µL	-
Nextera Forward Primer (Ad1_noMX) (2 µM)	1 µL	0.2 µM
Nextera Reverse Primer (Ad2.x*) (2 µM)	1 µL	0.2 µM
5X Q5 Reaction Buffer	2 µL	1X
Deoxynucleotide triphosphates (dNTPs; at 10 mM)	1 µL	200 µM
Q5 HIFI DNA Polymerase	0.1 µL	1.0 U/50 µL PCR
Nuclease Free Water/ddH2O	To 10 µL	

7. PCR cycling conditions:

Temperature	Duration	Cycles
98˚C	30 seconds	1
98˚C	10 seconds	15 (Note 4)
65˚C	30 seconds	
72˚C	30 seconds	
72˚C	5 minutes	1
4 ˚C	-	Hold

8. Gel purifies the ~466 bp amplification product using a gel purification kit as per manufacturer's instructions.
9. Measure the DNA concentration using Qubit.
10. Mix the DNA samples and send out for NGS sequencing.

Additional Notes

Note1:

Optimization of cycle number and dilution factor for PCR 1 product is required to successfully get the PCR product. Perform multiple cycles (e.g., 10, 15, and 20 cycles) and run 2% agarose gel to optimize the PCR condition. Fewer PCR cycles should be employed to decrease the potential bias during PCR amplification.

Note2:

Optimization of cycle number is required. Perform multiple cycles (e.g., 10, 15, and 20 cycles) and run 2% agarose gel to optimize the PCR condition. Fewer PCR cycles should be employed to decrease the potential bias during PCR amplification.

Note3:

For amplification from genomic DNA, instead of using the diluted LaPCR1 product, pool the LaPCR1 product and run it on a 2% agarose gel. After gel purifying the correct band, proceed with LaPCR2 using 50 ng of the purified LaPCR1 as the template. This approach is recommended to reduce non-specific amplifications.

Note4:

Optimization of cycle number and dilution factor for PCR 1 product is required to successfully get the PCR product. Perform multiple cycles (e.g., 10, 15, and 20 cycles) and run 2% agarose gel to optimize the PCR condition. Fewer PCR cycles should be employed to decrease the potential bias during PCR amplification.

Table 1. Nextera index sequences

Ad2.x*: Index primers Ad2.1 to Ad2.24 are listed below:

Index	Sequences
Ad1_noMX	AATGATACGGCGACCACCGAGATCTACACTCGTCGGCAGCGTCAGATGTG
Ad2.1_TAAGGCGA	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGGAGATGT
Ad2.2_CGTACTAG	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGGAGATGT
Ad2.3_AGGCAGAA	CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGGAGATGT
Ad2.4_TCCTGAGC	CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGGAGATGT
Ad2.5_GGACTCCT	CAAGCAGAAGACGGCATACGAGATAGGAGTCCGTCTCGTGGGCTCGGAGATGT
Ad2.6_TAGGCATG	CAAGCAGAAGACGGCATACGAGATCATGCCTAGTCTCGTGGGCTCGGAGATGT
Ad2.7_CTCTCTAC	CAAGCAGAAGACGGCATACGAGATGTAGAGAGGTCTCGTGGGCTCGGAGATGT
Ad2.8_CAGAGAGG	CAAGCAGAAGACGGCATACGAGATCCTCTCTGGTCTCGTGGGCTCGGAGATGT
Ad2.9_GCTACGCT	CAAGCAGAAGACGGCATACGAGATAGCGTAGCGTCTCGTGGGCTCGGAGATGT
Ad2.10_CGAGGCTG	CAAGCAGAAGACGGCATACGAGATCAGCCTCGGTCTCGTGGGCTCGGAGATGT
Ad2.11_AAGAGGCA	CAAGCAGAAGACGGCATACGAGATTGCCTCTTGTCTCGTGGGCTCGGAGATGT
Ad2.12_GTAGAGGA	CAAGCAGAAGACGGCATACGAGATTCCTCTACGTCTCGTGGGCTCGGAGATGT
Ad2.13_GTCGTGAT	CAAGCAGAAGACGGCATACGAGATATCACGACGTCTCGTGGGCTCGGAGATGT
Ad2.14_ACCACTGT	CAAGCAGAAGACGGCATACGAGATACAGTGGTGTCTCGTGGGCTCGGAGATGT
Ad2.15_TGGATCTG	CAAGCAGAAGACGGCATACGAGATCAGATCCAGTCTCGTGGGCTCGGAGATGT
Ad2.16_CCGTTTGT	CAAGCAGAAGACGGCATACGAGATACAAACGGGTCTCGTGGGCTCGGAGATGT
Ad2.17_TGCTGGGT	CAAGCAGAAGACGGCATACGAGATACCCAGCAGTCTCGTGGGCTCGGAGATGT
Ad2.18_GAGGGGTT	CAAGCAGAAGACGGCATACGAGATAACCCCTCGTCTCGTGGGCTCGGAGATGT
Ad2.19_AGGTTGGG	CAAGCAGAAGACGGCATACGAGATCCCAACCTGTCTCGTGGGCTCGGAGATGT
Ad2.20_GTGTGGTG	CAAGCAGAAGACGGCATACGAGATCACCACACGTCTCGTGGGCTCGGAGATGT
Ad2.21_TGGGTTTC	CAAGCAGAAGACGGCATACGAGATGAAACCCAGTCTCGTGGGCTCGGAGATGT
Ad2.22_TGGTCACA	CAAGCAGAAGACGGCATACGAGATTGTGACCAGTCTCGTGGGCTCGGAGATGT
Ad2.23_TTGACCCT	CAAGCAGAAGACGGCATACGAGATAGGGTCAAGTCTCGTGGGCTCGGAGATGT
Ad2.24_CCACTCCT	CAAGCAGAAGACGGCATACGAGATAGGAGTGGGTCTCGTGGGCTCGGAGATGT