Illumina Hiseq4000 compatible Primers (for PCR and sequencing)
Prepare roughly equal numbers of samples using Set A and Set B

Set A

Sample id	TruSeq ID	Paired End 5' Primer (orange indicates sample index for demultiplexing after sequencing)
1	12	aatgatacggcgaccaccgagatctacacgatcggaagagcacacgtctgaactccagtcacCTTGTAgcacaaaaggaaactcaccct
3	14	aatgatacggcgaccaccgagatctacacgatcggaagagcacacgtctgaactccagtcacAGTTCCgcacaaaaggaaactcaccct
5	3	aatgatacggcgaccaccgagatctacacgatcggaagagcacacgtctgaactccagtcacTTAGGCgcacaaaaggaaactcaccct

Common 3' Primer CAAGCAGAAGACGGCATACGAGATCGACTCGGTGCCACTTTTTC

Set B

Sample id	Truseq ID	Paired End 3' Primer
2	6	aatgatacggcgaccaccgagatctacacgatcggaagagcacacgtctgaactccagtcacGCCAATCGACTCGGTGCCACTTTTTC
4	10	aatgatacggcgaccaccgagatctacacgatcggaagagcacacgtctgaactccagtcacTAGCTTCGACTCGGTGCCACTTTTTC
6	1	aatgatacggcgaccaccgagatctacacgatcggaagagcacacgtctgaactccagtcacATCACGCGACTCGGTGCCACTTTTTC

Common 5' Primer CAAGCAGAAGACGGCATACGAGATGCACAAAAGGAAACTCACCCT

Submit pooled samples from both A and B for sequencing along with a mix of the following two primers:
5' Sequencing Primer GTGTGTTTTGAGACTATAAGTATCCCTTGGAGAACCACCTTGTTG
3' Sequencing Primer TGATAACGGACTAGCCTTATTTAAACTTGCTATGCTGTTTCCAGCTTA

The index that is appended to sgRNA samples allows each sample to be uniquely identified, even when pooled into a single sequencing lane. Consult with your sequencing facility to find out how many reads to expect per lane, and plan for 250-500 reads per sgRNA per sample. For example, a typical genome-scale 200k library screen should have at least 50M reads per sample (e.g. T0, treated, untreated), and each should be PCR amplified such that they have a unique index. Before proceeding plan out how you want to pool/run your samples on the flow cell. If you are sharing a lane with another lab member ask them which indices they are using. If you are running fewer than 6 samples on a lane, use indices 12,6 or 12,6,14 or 12,6,14,10, etc, to ensure the indices are read correctly.

Sample id	Truseq id	Set	Index
1	TruSeq Index 12 CTTGTA	A	CTTGTA
2	TruSeq Index 6 GCCAAT	B	GCCAAT
3	TruSeq Index 14 AGTTCC	A	AGTTCC
4	TruSeq Index 10 TAGCTT	B	TAGCTT
5	TruSeq Index 3 TTAGGC	A	TTAGGC
6	TruSeq Index 1 ATCACG	B	ATCACG
7	TruSeq Index 23 GAGTGG	A	GAGTGG
8	TruSeq Index 13 AGTCAA	B	AGTCAA
9	TruSeq Index 5 ACAGTG	A	ACAGTG
10	TruSeq Index 4 TGACCA	B	TGACCA
11	TruSeq Index 7 CAGATC	A	CAGATC
12	TruSeq Index 11 GGCTAC	B	GGCTAC
13	TruSeq Index 9 GATCAG	A	GATCAG
14	TruSeq Index 2 CGATGT	B	CGATGT
15	TruSeq Index 16 CCGTCC	A	CCGTCC
16	TruSeq Index 18 GTCCGC	B	GTCCGC
17	TruSeq Index 8 ACTTGA	A	ACTTGA
21	TruSeq Index 19 GTGAAA	A	GTGAAA
22	TrusSeq Index 20 GTGGCC	B	GTGGCC
23	TruSeq Index 21 GTTTCG	A	GTTTCG
24	TruSeq Index 25 ACTGAT	B	ACTGAT

18	TruSeq Index 22 CGTACG	B	CGTACG
19	TruSeq Index 27 ATTCCT	A	ATTCCT
20	TruSeq Index 15 ATGTCA	B	ATGTCA