Protocol for amplifying N16 barcodes for Illumina sequencing. Taken from: https://www.cell.com/cell/fulltext/S0092-8674(20)31003-5. Primer sequences: https://github.com/jbloomlab/SARS-CoV-2-RBD_DMS/blob/master/data/primers/primers.csv

Part of the Illumina seuqnecing handles are already plasmid-embedded, so it only takes a single PCR to generate Illumina sequencing amplicons:

PCR mix

component	per reaction (uL)
H_2O	12uL
fwd primer: o72 (10uM)	1.5uL
KOD 2x master mix	25uL
10uM reverse primer (Nextflex Rnd2Rev indexed primer)	(1.5uL)
template (if yeast-purified plasmid [low yield])*	(10uL)

*note, if using bacterial-purified plasmid, use much less template, replace with H2O.

Protocol: KOD_IL_58:

1. 95°C, infinity (to preheat)
2. 95°C, 2min
3. 95°C, 20s
4. 58°C, 10s
5. 70°C, 10s
6. Return to 2, 19x (20 cycles total)

Ampure purify, quantify, (pool if multiplexing), gel purify → 50bp single end Illumina sequencing

If libraries have not been bottlenecked, the vast majority of barcodes for a given library with the "SARS-CoV-2" target in this table should be sampled: https://github.com/jbloomlab/SARS-CoV-2-RBD_DMS/blob/master/results/variants/codon_variant_table.csv