PCR amplification for NGS analysis

* Plasmid DNA as templates can be PCR amplified by KAPA HiFi HotStart ReadyMixPCR Kit (KAPABIOSYSTEMS, KK2602) with 26-28 cycles of reactions using primers of Lib-NGS-F and Lib-NGS-R:

Lib-NGS-F: 5’-TACACGACGCTCTTCCGATCTTATCTTGTGGAAAGGACGAAACACC

Lib-NGS-R: 5’-AGACGTGTGCTCTTCCGATCTTCGACCTGCTGGAATCTCGTG

Up to 2 ng plasmid DNA can be used as templates in one 50-μL PCR reaction with KAPA polymerase, and 8 PCR reactions is sufficient. Then, the PCR products can be pooled together and purified with DNA Clean & Concentranter-5 (Zymo Research Corporation, D4013). Then, the purified PCR products as templates can be PCR amplified and purified using NEB Dual Index Primers Set 1 E7600, NEBNext Ultra Q5 Master Mix (M0544), and Ampure XP beads (Agencourt, A63881), which sets up DNA library for Illumina paired-end sequencing.

* Genomic DNA extracted from library cells as templates can be PCR amplified by KAPA HiFi polymerase with 26-28 cycles using the Lib-NGS-F and Lib-NGS-R primers. Up to 6 μg genomic DNA can be used in one 100-μL PCR reaction, and the number of PCR reactions should be determined according to the amount of genomic DNA extracted from cell libraries.