**Supplementary Table 1.** Primer sequences. The inner primer pairs were used to amplify the V3/V4 region of the 16S rRNA gene. The outer primers with spacer sequences and SP sequences at the 5´region of primer were used to construct SNAP-TE libraries compatible for Illumina MiSeq/HiSeq platform.

|  |  |
| --- | --- |
| **Primer** | **Sequence (5**´**→3**´**)** |
| Inner Primer Forward (314F) | ACACTGACGACATGGTTCTACAb (spacer sequenced) CCTACGGGNGGCWGCAGa |
| Inner Primer Reverse (806R) | TACGGTAGCAGAGACTTGGTCTb (spacer sequenced) GGACTACHVGGGTWTCTAATa |
| Outer Primer Forward | AATGATACGGCGACCACCGAGATCTACACc (i5 indexe) ACACTGACGACATGGTTCTACAb |
| Outer Primer Reverse | CAAGCAGAAGACGGCATACGAGATc (i7 indexe) TACGGTAGCAGAGACTTGGTCTb |

a red sequences are the amplification primers 314F and 806R of the V3-V4 region of the 16S rRNA gene.

b blue sequences are the SP sequences, which are both the amplification primer region of the outer primers and the sequencing primer of the Illumina platform.

c green sequences are the adapter sequences of P5 and P7 for Illumina.

d spacer sequences are used to improve the sequencing quality, composed of differential sequences with different lengths of bases. We designed eight spacer sequences, namely are NONE, A, TC, CTA, GATA, ACTCC, TACTAT and CTCTTCT.

e i5 and i7 are 8-base indexes (shown below). We randomly chose two indexes to identify each sample.

**Supplementary Table 2.** Index sequences used for next-generation sequencing library construction.

|  |  |
| --- | --- |
| **index ID** | **index sequence** |
| 1 | CATTGCTT |
| 2 | ACCAGACT |
| 3 | TCTTATAT |
| 4 | GTTACTTG |
| 5 | ATAACACC |
| 6 | TATCCAGA |
| 7 | CCGCACAG |
| 8 | ATCCAAGC |
| 9 | GGTATACT |
| 10 | GTCCTACG |
| 11 | TGAGGTGA |
| 12 | ATGATTCA |
| 13 | GTACATGT |
| 14 | CGGTGTTA |
| 15 | ATTGGCCG |
| 16 | GCTTACGA |
| 17 | TATTCCTA |
| 18 | TCACGTTC |
| 19 | TAACTACT |
| 20 | TATAGGCA |
| 21 | GGAGGAAG |
| 22 | ATATCGTC |
| 23 | AAGTCACC |
| 24 | CGCAGTCC |
| 25 | ATTAAGGC |
| 26 | TTGCGGTT |
| 27 | CCTCAGTC |
| 28 | TTGGAGGA |
| 29 | GCCATTGC |
| 30 | TATTATCT |

**Supplementary Table 3.** A total of 14 flexible sample/assay combination modes that can set on multi-sample nanodispenser system. Replicates can be easily done using these settings.

|  |  |
| --- | --- |
| **Sample** | **Assay** |
| 384 | 12 |
| 216 | 24 |
| 144 | 36 |
| 108 | 48 |
| 96 | 54 |
| 72 | 72 |
| 64 | 80 |
| 54 | 96 |
| 42 | 120 |
| 36 | 144 |
| 24 | 216 |
| 20 | 248 |
| 16 | 296 |
| 12 | 384 |

**Supplementary Table 4.** All parameters tested for SNAP-TE experiments. Two replicates of 24 nanowells on a chip were used for each test of PCR system conditions. Data from individual next-generation sequencing libraries from each nanowell were pooled by samples for analyses.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Tests** | **Parameters** | **Conditions** | **No. NGS Libraries generated for testing** | **No. Nanowells used** |
| PCR system condition tests | *Taq* polymerase | TE Mix | 5 | 120 |
| ImmoMix™ | 5 | 120 |
| Vazyme Mix | 5 | 120 |
| DNA input amount | 10ng | 5 | 120 |
| 25ng | 5 | 120 |
| 50ng | 5 | 120 |
| 100ng | 5 | 120 |
| Inner/Outer Primer ratioa | 1:4 | 5 | 120 |
| 1:25 | 5 | 120 |
| Reproducibility testsb | No. Replicates on the chip | 1 | 5 | 60 |
| 2 | 5 | 120 |
| 3 | 5 | 180 |
| 4 | 5 | 240 |

aThe ratio stands for the constant inner primer concentration (40 nM) over the outer primer concentration (160 or 1000 nM).

bAll tests were performed using the samples of other five individuals (F,G,H,I,J).

**Supplementary Table 5**. Cost dissection for two methods of next-generation sequencing library construction.

|  |  |  |  |
| --- | --- | --- | --- |
| **Method** | **Workflow Day** | **Steps in Fig. 1** | **Cost per sample($)** |
| **Conventional** | DAY1 | Reaction mix preparation | 0.006 |
| PCR | 0.648 |
| Purification | 0.548 |
| DAY2 | Quality control | 2.618 |
| DAY3 | Pooling | 0.054 |
| DAY4 | Purification | 0.121 |
| Quantification | 0.311 |
| Total |  | 4.307 |
| **SNAP-TE** | DAY1 | Reaction mix preparation | 0.006 |
| PCR | 0.648 |
| Product collection | 1.517 |
| DAY2 | Purification | 0.121 |
| Quantification | 0.311 |
| Total |  | 2.604 |