**Adapter**

These will always be the same.

Forward: AATGATACGGCGACCACCGAGATCTACAC

Reverse: CAAGCAGAAGACGGCATACGAGAT

**Index**

We recommend utilizing the Schloss indices. However, you could design your own. They need to be 8 bp long and have a 25% ATGC composition at each site when used together.

The number of indices you use depends on the number of samples to be sequenced. Each combination (like SA5##-SA7##) equals 96 samples. You can further mix and match 2 sets each of forward and reverse to equal 384 samples (like SA5##-SA7##, SA5##-SB7##, SB5##-SA7##, SB5##-SB7##).

Index 5 (forward)

|  |  |  |  |
| --- | --- | --- | --- |
| SA501 ATCGTACG  SA502 ACTATCTG  SA503 TAGCGAGT  SA504 CTGCGTGT  SA505 TCATCGAG  SA506 CGTGAGTG  SA507 GGATATCT  SA508 GACACCGT | SB501 CTACTATA  SB502 CGTTACTA  SB503 AGAGTCAC  SB504 TACGAGAC  SB505 ACGTCTCG  SB506 TCGACGAG  SB507 GATCGTGT  SB508 GTCAGATA | SC501 ACGACGTG  SC502 ATATACAC  SC503 CGTCGCTA  SC504 CTAGAGCT  SC505 GCTCTAGT  SC506 GACACTGA  SC507 TGCGTACG  SC508 TAGTGTAG | SD501 AAGCAGCA  SD502 ACGCGTGA  SD503 CGATCTAC  SD504 TGCGTCAC  SD505 GTCTAGTG  SD506 CTAGTATG  SD507 GATAGCGT  SD508 TCTACACT |

Index 7 (reverse)

|  |  |  |  |
| --- | --- | --- | --- |
| SA701 AACTCTCG  SA702 ACTATGTC  SA703 AGTAGCGT  SA704 CAGTGAGT  SA705 CGTACTCA  SA706 CTACGCAG  SA707 GGAGACTA  SA708 GTCGCTCG  SA709 GTCGTAGT  SA710 TAGCAGAC  SA711 TCATAGAC  SA712 TCGCTATA | SB701 AAGTCGAG  SB702 ATACTTCG  SB703 AGCTGCTA  SB704 CATAGAGA  SB705 CGTAGATC  SB706 CTCGTTAC  SB707 GCGCACGT  SB708 GGTACTAT  SB709 GTATACGC  SB710 TACGAGCA  SB711 TCAGCGTT  SB712 TCGCTACG | SC701 ACCTACTG  SC702 AGCGCTAT  SC703 AGTCTAGA  SC704 CATGAGGA  SC705 CTAGCTCG  SC706 CTCTAGAG  SC707 GAGCTCAT  SC708 GGTATGCT  SC709 GTATGACG  SC710 TAGACTGA  SC711 TCACGATG  SC712 TCGAGCTC | SD701 ACCTAGTA  SD702 ACGTACGT  SD703 ATATCGCG  SD704 CACGATAG  SD705 CGTATCGC  SD706 CTGCGACT  SD707 GCTGTAAC  SD708 GGACGTTA  SD709 GGTCGTAG  SD710 TAAGTCTC  SD711 TACACAGT  SD712 TTGACGCA |

**Amplicon primer**

Now, consider your chosen amplicon primers. They should be 15-20 bp long to give good specificity to your region of interest. The amplicon needs to be within range of whatever kit you plan on using on the MiSeq. We recommend maximizing Read 1 and 2 overlap as well as only going up to the 2x250 bp kit to reduce sequencing error (the additional 50 bp of the 2x300 bp kit are very poor quality). This means you want an amplicon of ~250 bp for the 2x250 kit. You could