Amplicon-seq design for Illumina library architecture

*IDT for Illumina UD Indexes:

(https://support-docs.illumina.com/SHARE/AdapterSeq/Content/SHARE/AdapterSeq/Nextera/UDIndexesSequencesNXT.htm)

These unique dual (UD) index adapters are arranged in the plate to enforce the recommended pairing strategy. The index adapters are 10 bases long, instead of the typical eight bases. The IDT for Illumina UD Indexes include IDT for Illumina–DNA/RNA UD Indexes, IDT for Illumina-PCR UD Indexes, and IDT for Illumina–Nextera DNA UD Indexes.

Index 1 (i7) Adapters:
CAAGCAGAAGACGGCATACGAGAT[i7]GTCTCGTGGGCTCGG

Index 2 (i5) Adapters:
AATGATACGGCGACCACCGAGATCTACAC[i5]TCGTCGGCAGCGTC

*Two strategies for Illumina indexed amplicon-seq:

有關 MiSeq 進行 amplicon sequencing 可採二種方式，
1) two-step PCR，第一次 PCR 之 primer 如下:
Forward primer:
5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-[locus specific sequence]
Reverse primer:
5’ GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-[locus specific sequence]
PCR 產物給 NGS Core 做第二次 PCR；PCR 時合成 sample index and Illumina flow cell oligo seq

2) one-step PCR，PCR primer 含 Illumina flow cell oligo seq and sequencing primer，如下:

PE PCR Primer 1.0:
5' AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-[locus specific sequence]
PE PCR Primer 2.0:
5' CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT-[locus specific sequence]

Note: oligo 較長，合成純化方式用 PAGE or HPLC。