1st Step PCR

**OneTaq DNA polymerase (M0480, NEB)**

**50 ul/reaction:**

5 X OneTaq standard reaction buffer: 10 ul

dNTPs (10 mM, # N0447, NEB): 1 ul

P\_forward: 1 ul

P\_reverse: 1 ul

OneTaq DNA polymerase: 0.25 ul

DNA template (Up to 200 ng genomic DNA/reaction has been working well): X ul

water: 36.75 ul - X ul

94 C 10min

**94 C 3 min**

**55 C 1 min**

**68 C 1 min (3 cycles total)**

68 C 5 min

4 C

Purify the PCR products with **NucleoSpin Gel and PCR clean up kit (#740609**), elute with 50 ul EB buffer. **Important**: Purify the PCR products again with **NucleoSpin Gel and PCR clean up kit (#740609**), elute with 33 ul EB buffer. It is important to remove all remaining primers, and a single column does not do this.

When doing large scale of PCR reactions, we usually pool 10 50ul reactions into one column and eluted with

33 ul EB buffer (30 ul will be used in one 2nd step PCR)

2nd step PCR:

**PrimerSTAR HS DNA polymerase (Takara, R010A or R010B)**

Note: this is not a high fidelity enzyme, but that’s OK

50 ul/reaction:

5 X PrimeSTAR buffer: 10 ul

dNTPs (2.5 mM each): 4 ul

PE1: 1.25 ul

PE2: 1.25 ul

PrimeSTAR HS DNA polymerase: 0.5 ul

Each sample: 30 ul

water: 3 ul

98 3 min

**98 C 10s**

**69 C 5s**

**72 C 15 s (23 cycles total)**

72 C 1min

4 C

Purify the PCR products with NucleoSpin Gel and PCR clean up kit (#740609), elute with > 30 ul EB buffer.

Run a gel or Egel to check for a primer dimer. If necessary, gel extract to remove primer dimer. Heteroduplex DNA is common and will run higher than the expected band. It is very important that you include heteroduplex DNA in your gel band cut-out or biases in barcode frequencies will result.