Method for generating blunt ends from a 5’ or 3’ overhang using Klenow DNA polymerase

Digest plasmid DNA (1-2 µg) with restriction enzyme in 20 µl volume; restriction sites that generate 5’-overhangs (e.g. BamHI G/GATCC) will be "filled-in" and 3’-overhangs (e.g. KpnI GGTAC/C) will be "chewed back" by Klenow DNA polymerase

Run 4 µl of digest on gel to check completeness of digest. Do not proceed if uncut vector present.

For fill-in/ chew back reaction mix the following:

15 µl digested DNA
2 µl 10X Klenow DNA polymerase buffer
0.64 µl 1:1 dilution of 2.5 mM each dNTP mix – final conc. 40 µM
0.4 µl 1:10 dilution of acetylated BSA (20 µg/ml) – final 0.4 µg in 20 µl
1.46 µl H20
1.0 µl Klenow enzyme (1U/µl) or adjust to ~1 U/µg DNA

Mix reaction components and incubate at room temperature for 10 min.

Stop reaction by heating for 10 min at 75° C in PCR machine.

PCR clean-up (up to 10Kb) or gel-extract fragment (resuspend in final volume of 25-30 ml H2O).

If necessary, proceed to dephosphorylation using Shrimp Alkaline Phosphatase

20 µl digested DNA
3 µl 10X SAP reaction buffer
___ µl H2O to final volume of 30 µl
___ µl SAP enzyme (1U/µl) – adjust to ~1U/µg DNA (usually 3 µl)

Incubate reaction at 37C for 30 min.

Inactivate SAP by heating to 65C for 15 min in PCR machine.

Run 2-4 µl of DNA on gel to quantify and use for ligation reaction.