# Insertion of oligonucleotide adapters into plasmids.

- Digest plasmid with appropriate restriction enzymes (usually 10 μl of Wizard miniprep with 10 units of enzyme in a final volume of 20 μl). Digest for at least 90 minutes.

- Make a 1:500 dilution of the oligo stocks in water (stock is ~ 1 μg/μl). ***Make sure the oligos are phosphorylated at their 5' end if you treat the vector with calf intestine phosphatase (CIP) ***

- Mix diluted oligos together, heat at 94$^{o}$C for 5 minutes in a PCR machine and slowly cool to room temperature (usually done by turning off the machine and leaving the samples to reach room temperature).

- Meanwhile, treat the digested plasmid with CIP[1] (NEB, 2 μl for 15 minutes at 37$^{o}$C) and run on an agarose gel. Cut the band corresponding to the linearized plasmids, geneclean according to procedures (use 5 μl of glassmilk) and elute in 20 μl of elution buffer.

- Mix plasmid and oligos: 10 μl of genecleaned plasmid
  8 μl of oligo mixture
  2 μl of 10X T4 DNA ligase buffer
  1 μl of T4 DNA ligase (NEB)

- Incubate overnight at 15$^{o}$C and transform in a suitable E.coli strain (I normally use TOP10F').

**NOTE:**

[1]If the digested plasmid can be separated from the undigested one, it is not necessary to treat with CIP and use phosphorylated oligonucleotides. However, it might be harder to insert the oligo linker.

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