General plasmid subcloning.

- Inoculate a colony or glycerol stock into 4 ml of LB broth with the appropriate antibiotic selection and incubate with shaking overnight at 37^{o}C.

- Make a glycerol stock by adding 500 ul of LB:glycerol (1:1) to 500 μl of the overnight culture. Isolate the plasmid DNA using either the Wizard (Promega), Qiagen or Zippy miniprep kit.

- Take 10 μl of the miniprep, 7 μl of H2O, 2 μl of 10X restriction buffer and 1 μl (5-20 units) of the restriction endonuclease.

- If the plasmid has to be digested with two enzymes, add 1 μl of each restriction endonuclease. Try to choose a buffer that will be compatible with both enzymes. You can try buffers from different companies (even though the enzyme is not from that company, it generally works). If it is not possible, than try to choose a buffer that can be easily modified to the buffer of the second enzyme. For example, NEB #2 can easily be changed to NEB #3 by adding 1 μl of 1M NaCl. If not, use a Qiaquick column to exchange buffers.

- Incubate for ~ 1 hour to overnight.

- Treat the plasmid into which the insert will be cloned with calf intestine alkaline phosphatase (CIAP). I usually add 2 μl of CIAP (either NEB's or Pharmacia's) and incubate it at 37^{o}C for 15 min. This will work in NEB #2, 3, 4 EcoRI and BamHI buffers but not NEB#1.

- Add 2 μl of 6X agarose loading dye to the digested plasmid and load onto a 0.8-2% TAE (Tris-acetate, not TBE) agarose gel (depending on the size of the band to be cut) and migrate at 80-90 volts until the bands can be easily cut.

- Cut the band with a razor blade and determine its weight.

- Add 3X volumes of NaI (i.e. 0.05 g band will need 150 μlof NaI).

- Heat the tube at 45-55^{o}C to melt the agarose. Add 5-8 μl of glassmilk and incubate at room temperature for at least 5 minutes (60 minutes maximum). Spin at maximum speed for 15 seconds.

- Remove supernatant and resuspend the pellet with 700 μl of cold New Wash. Spin at maximum speed for 15 seconds and remove supernatant. Repeat twice.

- After the last wash, remove as much of the New Wash solution as possible using a yellow tip. Let the pellet dry at room temperature (10 min to overnight).

- Resuspend the pellet in 7-20 μl of ddH_2O. Incubate at 50-55^{o}C for 2-3 minutes.

- Spin at maximum speed for 1 min. Keep supernatant and put it in the ligation tube. Ligate the insert and the receiving plasmid as follows:

    10 µl insert
    8 µl plasmid
    2 µl 10X T4 DNA ligase buffer
    1 µl T4 DNA ligase

- Incubate the ligations at 15^{o}C overnight. Transform into a suitable *E.coli* strain. I normally use TOP10F' (Invitrogen).

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