Partial digestion protocol*

*This protocol was adapted from Currents Protocols in Molecular Biology.

Notes for efficient cloning using partial digests:

1) The selected restriction enzyme should not cut the plasmid more than twice.
2) The partially digested band should be of a size that can be separated from other partial or complete digestion products.
3) Carry out the complete digestion first (use 10 ul of miniprep (Zymo, Promega or Qiagen), 7 ul of H_2O, 2 ul of 10X buffer[1] and 1 ul (5-20 units) of restriction enzyme and incubate at least one hour at the recommended temperature).

- Once the first digestion is complete, add 72 ul of H_2O, 8 ul of 10X restriction enzyme buffer for the second enzyme, for a final volume of 100 ul. Label the tube "A" and place on ice.

- Aliquot 20 ul from tube "A" to 3 tubes labeled "B", "C", "D" and 10 ul in a tube labeled "E". There should be 30 ul left in tube "A". Keep all tubes on ice.

- Add 1 ul of restriction enzyme ONLY to the tube labeled "A" . Mix well. The restriction enzyme will now be serially diluted from tube "A" through tube "E".

- Transfer 10 ul from tube "A" to tube "B". Mix well.

- Changing pipette tip every time, transfer 10 ul of tube "B" to tube "C", then 10 ul of tube "C" to tube "D", and finally 10 ul of tube "D" to tube "E". Mix well every time. Each tube now contains 20 ul. It is important that all the tubes be kept on ice.

- Incubate all tubes at the recommended temperature for the restriction enzyme between 30 seconds to 4 minutes, depending on the enzyme[2]. Put the tubes back on ice.

- Add 100 ul of buffer PB (Qiaquick PCR purification kit) to each tube, and pool them in one tube.

- Add the pooled samples to one Qiaquick column and centrifuge at maximum speed for one minute. Discard the flowthrough.

- Add 750 ul of buffer PE (wash buffer), and centrifuge 1 minute at maximum speed. Discard the flowthrough.

- Dry the column by centrifuging 2 minutes at maximum speed.

- Insert the Qiaquick column in a fresh eppendorf tube and add 30 of buffer EB. Incubate one minute at room temperature and centrifuge for one minute at maximum speed. Discard the column.

- Add at least 5 ul of 6X agarose loading dye before loading on the agarose gel[3].

- Load on the agarose gel in either one or two lanes and migrate until the desired band can be separated from the other bands.

- Cut the band from each lane(s), pool them if necessary, and purify the DNA by geneclean[4].

- Ligate the partially digested bands to the plasmid using the general cloning protocol[4].

[1]If the restriction buffer for the first enzyme is not compatible with the second enzyme, I do a buffer exchange using a Qiaquick PCR purification column.

[2]For most enzymes, an incubation of 2-3 minutes is sufficient. Shorter times are recommended for enzymes of higher concentration.

[3]There will be residual ethanol from the PE buffer in the sample, even after the two minute centrifugation. If insufficient loading dye is used, the sample will float to the surface.

[4]The latest version of the protocols can be downloaded at: http://ericcampeau.com/manuals.html.

Eric Campeau version 1.0, November 2009.