General Geneclean Protocol

- Take 10 μl of miniprep DNA and digest with selected restriction enzyme(s) in a final volume of 20 μl for 1-18h.

- If necessary, add 2 μl of CIP to the reaction and incubate 15min at 37^{o}C.

- Add 2 μl of 6X agarose loading dye and load on a 0.8-1.0% agarose gel.

- Migrate at 80-90 volts[1] until the band can be clearly seen and easily cut. Cut the band with a razor blade and determine its weight.

- Add 3X volumes of NaI and 1/10 volume of TBE modifyer (geneclean kit, Bio 101) if the agarose gel was run in TBE buffer (ex. 0.05 g band will need 150 ul of NaI and 15 ul of modifyer).

- 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.

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