Steps

Plasmid DNA Prep

1) Bind

1) Resuspend bacteria in 250 uL P1
2) Lyse with 250 uL P2, wait 1-5 min
3) Neutralize with 350 uL chilled N3
4) Spin 5 min, load 600-650 uL SN on column
5) Incubate at RT for 2 min
5) Spin, discard FT

Gel Extraction

1) Bind

1) Dissolve every 100mg of gel slice with 300 uL QG
(No heat needed w/ low melt agarose, invert or vortex)
(Some protocols suggest 400 uL/100mg, also works)
2) Load column with 600 uL, incubate at RT for 2 min
3) Spin, discard FT
4) Repeat until all dissolved gel passed through column
Only first incubation at RT is necessary to activate silica

PCR/Enzymatic Reaction Purification

1) Bind

1) Add 5 volumes of PB to 1 volume PCR/Enz reaction
2) Load 600 uL onto column, incubate at RT for 2 min
3) Spin, discard FT
4) Repeat until all product passed through column
Only first incubation at RT is necessary to activate silica

2) Wash

1) Add 600-700 uL 1X PE **(diluted with EtOH!!!)**, spin, discard FT
2) Repeat for second wash
3) Discard final FT, spin additional 2 minutes
4) Place column into fresh 1.5 mL tube

3) Elute

1) Add 15-100uL of elution buffer, pre-warm to 50C for DNA >5-10 kb
2) Spin, check concentration, transfer to fresh tube if feeling fancy.
***** Elution with volumes >50 uL will likely require concentration, Centrivap is king here**

Recipes

1) Bind

Buffer N3
4M Guanidine-HCl
0.5M Potassium Acetate pH 4.2

Buffer QG
5.5 M Guanidine Thiocyanate
20 mM Tris HCl pH 6.6

Buffer PB
5 M Guanidine-HCl
20 mM Tris-HCl pH 6.6
30% ethanol

***** Refer to Plasmid Prep Cheat sheet for all recipes *****

2) Wash

5X Buffer PE
80 mM NaCl
8mM Tris-HCl pH 7.5
DILUTE WITH ETHANOL

→

1X Buffer PE
1 vol. 5X Buffer PE
4 vol. 100% Ethanol

3) Elute

Elution Buffer: 5 mM Tris-HCl pH 8

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SN = Supernatent **W/** = with
FT = Flow through **Vol.** = Volume
Spin = Centrifuge at 16kxg for 30s