

Steps

1) Bind

- ### Plasmid DNA Prep
- 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) 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) 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) Incubate at RT for a minimum of 2 minutes, longer can slightly increase yield
- 3) 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

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

- ### 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

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 = Supernatant W/ = with
FT = Flow through Vol. = Volume
Spin = Centrifuge at 16kxg for 30s
RT = Room temperature (~22C)