<table>
<tr><th>Buffer Name</th><th>Silica Column Prep (Biobasic, Qiagen or generic column)</th><th>DEAE Column Prep (Qiagen or generic column)</th><th>Phenol/Chloroform Prep</th></tr>
<tr><td>Solution P1/1/A
(Resuspension)
(*** 4uL of 25mg/mL RNase PER mL, transfer some buffer to new tube and add RNase A, use once and throw out. Do not make large volumes.)</td><td>Solution P1<table><tr><th></th><th>F.C.</th><th>Stock</th><th>50mL</th><th>100mL</th></tr><tr><td>Tris pH 8.0</td><td>50 mM</td><td>1M</td><td>2.5 mL</td><td>5 mL</td></tr><tr><td>EDTA pH 8.0</td><td>10 mM</td><td>0.5M</td><td>1 mL</td><td>2 mL</td></tr><tr><td>RNase A</td><td>100 ug/mL</td><td>25 mg/mL</td><td>***</td><td>***</td></tr></table></td><td>Solution P1

<---------- Same</td><td>Solution 1/A etc

<---------- Same</td></tr>
<tr><td>Solution P2/2/B
(Lysis, Make fresh every 2-4 weeks, old stocks will ruin your day.)</td><td>Solution P2<table><tr><th></th><th>F.C.</th><th>Stock</th><th>50mL</th><th>100mL</th></tr><tr><td>NaOH</td><td>200 mM</td><td>5M</td><td>2 mL</td><td>4 mL</td></tr><tr><td>SDS</td><td>1%</td><td>10%</td><td>5 mL</td><td>10 mL</td></tr></table></td><td>Solution P2

<---------- Same</td><td>Solution 2/B etc

<---------- Same</td></tr>
<tr><td>Solution N3/3/C
(Neutralization, keep chilled at 4C for best results)</td><td>Solution N3<table><tr><th></th><th>F.C.</th><th>Stock</th><th>50mL</th><th>100mL</th></tr><tr><td>Guanidine Hydrochloride</td><td>4M</td><td>Powder</td><td>19.11g</td><td>38.20</td></tr><tr><td>Potassium Acetate</td><td>0.5M</td><td>Powder</td><td>2.45g</td><td>4.91g</td></tr><tr><td>Acetic Acid</td><td>pH 4.2</td><td>Liquid</td><td>pH to 4.2</td><td>pH to 4.2</td></tr></table></td><td>Solution P3<table><tr><th></th><th>F.C.</th><th>Stock</th><th>50mL</th><th>100mL</th></tr><tr><td>Potassium Acetate</td><td>3M</td><td>Powder</td><td>14.72g</td><td>29.44g</td></tr><tr><td>Acetic Acid</td><td>pH 5</td><td>Liquid</td><td>pH to 5</td><td>pH to 5</td></tr></table></td><td>Solution 3/C etc

<---------- Same as DEAE Column
Different from Silica column</td></tr>
<tr><td>5X PE/ Buffer 1X QC
(Wash buffer, dilute 5X PE with ethanol*)

DILUTE WITH ETHANOL
DILUTE WITH ETHANOL
DILUTE WITH ETHANOL</td><td>5X PE Wash Buffer<table><tr><th></th><th>F.C.</th><th>Stock</th><th>50mL</th><th>100mL</th></tr><tr><td>NaCl</td><td>80 mM</td><td>5M</td><td>0.8 mL</td><td>1.6 mL</td></tr><tr><td>Tris pH 7.5</td><td>8 mM</td><td>1M</td><td>0.4 mL</td><td>0.8 mL</td></tr></table>* Dilute to 1X with 100% Ethanol, i.e. 10 mL 5X PE + 40 mL 100 % EtOH</td><td>1X Buffer QC<table><tr><th></th><th>F.C.</th><th>Stock</th><th>100mL</th><th>500mL</th></tr><tr><td>NaCl</td><td>1M</td><td>5M</td><td>20 mL</td><td>100 mL</td></tr><tr><td>MOPS pH 7.0</td><td>50 mM</td><td>1M</td><td>5 mL</td><td>25 mL</td></tr><tr><td>Isopropanol</td><td>15%</td><td>100%</td><td>15 mL</td><td>75 mL</td></tr></table></td><td>N/A</td></tr>
<tr><td>EB / Buffer QF / TLE
(Elution or resuspension, warm to 50C for larger plasmids)</td><td>EB<table><tr><th></th><th>F.C.</th><th>Stock</th><th>50mL</th><th>100mL</th></tr><tr><td>Tris pH 8.0</td><td>10 mM</td><td>1M</td><td>0.5 mL</td><td>1 mL</td></tr></table></td><td>Buffer QF<table><tr><th></th><th>F.C.</th><th>Stock</th><th>50mL</th><th>100mL</th></tr><tr><td>NaCl</td><td>1.25M</td><td>5M</td><td>12.5 mL</td><td>25 mL</td></tr><tr><td>Tris pH 8.5</td><td>50 mM</td><td>1M</td><td>2.5 mL</td><td>5 mL</td></tr><tr><td>Isopropanol</td><td>15%</td><td>100%</td><td>7.5 mL</td><td>15 mL</td></tr></table></td><td>TLE (Tris Low EDTA)<table><tr><th></th><th>F.C.</th><th>Stock</th><th>50mL</th></tr><tr><td>Tris pH 8.0</td><td>10 mM</td><td>1M</td><td>0.5 mL</td></tr><tr><td>EDTA pH 8.0</td><td>0.1 mM</td><td>0.5M</td><td>10 uL</td></tr></table></td></tr>
<tr><td>Buffer QBT
(Equilibration Buffer for DEAE columns ONLY)</td><td>N/A</td><td>Buffer QBT<table><tr><th></th><th>F.C.</th><th>Stock</th><th>100mL</th><th>500 mL</th></tr><tr><td>NaCl</td><td>750 mM</td><td>5M</td><td>15 mL</td><td>75 mL</td></tr><tr><td>MOPS pH 7.0</td><td>50 mM</td><td>1M</td><td>5 mL</td><td>25 mL</td></tr><tr><td>Isopropanol</td><td>15%</td><td>100%</td><td>15 mL</td><td>75 mL</td></tr><tr><td>Triton X-100</td><td>0.15%</td><td>10%</td><td>1.5 mL</td><td>7.5 mL</td></tr></table></td><td>N/A</td></tr>
</table>

F.C. = Final Concentration SN = Supernatant

Silica Column Prep (1:1:1.4 Sol'n 1/Sol'n 2/Buffer N3)

***** Centrifugation done at 16,000xg in benchtop centrifuge at room temperature (RT) unless otherwise indicated *****

1) Grow up 3-5mL of *E. coli* for 20 hours overnight at 37C and 275 RPM. Spin down culture in 1.5 mL tube, pour off SN, quick spin, pipette off remainder of media.

2) Re-suspend pellet with **250 uL** of Solution 1 (w/ RNase). Add **250 uL** of Solution 2, invert GENTLY until thoroughly mixed, incubate at RT for 1-5 minutes.

3) Quick spin and add **350 uL** pre-chilled Buffer N3. Invert until thoroughly mixed and precipitate resembles white fluffy coconut without any yellowish goo.

4) Centrifuge at 16,000xg for 5 minutes at 4C. Take 600-650 uL of SN and avoid white goop as much as possible (Genomic DNA/Proteins). **Optional: Centrifuge SN again in fresh tube.**

5) Transfer SN to fresh silica column, let sit for 2 minutes. Centrifuge for 30s, discard flow through. **Optional: Pass SN through column a second time for slight increase in yield.**

6) Add 700 uL 1X PE **(Diluted w/ EtOH)** to column, centrifuge for 30s, discard flow through. Repeat for a total of two washes. Centrifuge a final time for 2 minutes to dry silica column.

7A) Transfer column to clean 1.5 mL tube and add 15-30 uL of Elution Buffer. Incubate at RT for 5 minutes and centrifuge for 30s to elute plasmid DNA.

Optional: Pre-warm elution buffer to 50C for larger plasmids.

OR

7B) For maximum yield, elute twice for 5 minutes with 50 uL Elution buffer. Concentrate 100 uL down to 20 uL with CentriVap concentrator.

Maxi DEAE Column Prep (1:1:1 P1/P2/P3)

1) Grow up 100-200 mL of *E. coli* for 20 hours overnight at 37C and 275 RPM. Spin down culture at 3200xg in 50 mL centrifuge tube and pour off SN.

2) Re-suspend pellet with **12 mL** P1 (w/ RNase). Add **12 mL** P2 and invert GENTLY to mix, incubate at RT for 1 minute, quick spin at 3200xg.

3) Add **12 mL** pre-chilled P3 and invert gently to mix until precipitate resembles desiccated coconut. Centrifuge at 3200xg at 4C for 20 minutes.

4) Prepare a 125 mL glass flask with a funnel. Fold up two layers of thin filter paper and wet it with dH2O to keep it in place. Add a single layer of mira cloth on top.

5) Pour centrifuged lysate onto mira cloth. Lift mira cloth so majority of liquid goes onto filter paper.

6) Equilibrate DEAE maxi column with 30 mL Buffer QBT. Add filtered lysate to column and let flow through by gravity (Or gentle pressure with pump). Wash twice with 30 mL of Buffer QC.

7) Elute with 15 mL of Buffer QF into a 50 mL tube. Add 10.5 mL cold isopropanol and invert to mix. **Optional: Pre-warm Buffer QF to 50C for larger plasmids.**

8) Centrifuge at 16,000 xg for 20 minutes at 4C (Either many 1.5 mL tubes or in one large tube, larger pellet is easy to dislodge).

9) Discard SN, add volume of 70% ethanol equal to originally precipitated volume. Invert a few times and centrifuge at 16,000xg for 5 minutes.

10) Pour off SN, quick spin, pipette off remainder of ethanol. Re-suspend pellet in 200 uL pre-warmed (50C) TLE (10 mM Tris pH 8.0, 0.1 mM EDTA) and transfer to a clean 1.5 mL tube.

Phenol/Chloroform Prep (1:1:1 Sol'1/Sol'n 2/Sol'n 3)

***** Centrifugation done at 16,000xg in benchtop centrifuge at room temperature (RT) unless otherwise indicated *****

Grow up 3-5mL of *E. coli* for 20 hours overnight at 37C and 275 RPM. Spin down culture in 1.5 mL tube, pour off SN, quick spin, pipette off remainder of media.

2) Re-suspend pellet with **250 uL** of Solution 1 (w/ RNase). Add **250 uL** of Solution 2, invert GENTLY until thoroughly mixed, incubate at RT for 1 minute.

3) Quick spin and add **250 uL** pre-chilled Solution 3. Invert until thoroughly mixed and precipitate resembled desiccated coconut.

4) Centrifuge at 16,000xg for 5 minutes at 4C. Take 600-650 uL of SN and avoid white goop as much as possible (Genomic DNA/Proteins). **Optional: Centrifuge SN again in fresh tube.**

5) Transfer SN to fresh tube, add equal volume isopropanol. Centrifuge at 16,000xg for 20 minutes at 4C.

6) Pour away supernatant and let air dry. Re-suspend pellet in 200 uL TLE. Add equal volume basic PCI and vortex for 30s. Centrifuge for 5 minutes. Transfer SN to new tube and repeat PCI extraction.

7) Transfer SN to new tube and add 2.6 volumes of Precipitation Mix (50 mL 100% EtOH + 2 mL 3M NaOAC pH 5.2 in 50mL tube). Centrifuge for 20 minutes.

8) Pour off SN, add 1 mL 75% EtOH, invert several times, centrifuge for 1 minute and pour off SN. Quick spin, pipette off remainder of liquid. Air dry pellet and re-suspend in 15-30 uL TLE.