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| **Tris-Cl (1 M)**Dissolve 121.1 g of Tris base in 800 ml of H2O. Adjust the pH to the desired value by adding concentrated HCl <!>.pH HCl7.4 70 ml7.6 60 ml8.0 42 ml ßour most commonAllow the solution to cool to room temperature before making final adjustments to the pH. Adjust the volume of the solution to 1 liter with H2O. Dispense into aliquots and sterilize by autoclaving.If the 1 M solution has a yellow color, discard it and obtain Tris of better quality. The pH of Tris solutions is temperature dependant, and decreases ~0.03 pH units for each 1 C increase in temperature. For example, a 0.05 M solution has pH values of 9.5, 8.9, and 8.6 at 5 C, 25 C, and 37 C, respectively. **20X SSC**Dissolve 525.9 g of NaCl and 264.6 g of sodium citrate in 2.4 liters of H2O. Adjust the pH to 7 with a few drops of a 14 N solution of HCl <!>. Adjust the volume to 3 liters with H2O. Dispense into aliquots. Sterilize by autoclaving. The final concentrations of the ingredients are 3.0 M NaCl and 0.3 sodium citrate.**Extraction/Lysis Buffers and Solutions**Alkaline Lysis Solution I (Plasmid Preparation)50 mM glucose25 mM Tris-Cl (pH 8.0)10mM EDTA (pH 8.0)Prepare solution I from standard stocks in batches of ~500 ml, autoclave for 15 minutes at 15 psi (1.05 kg/cm2) on liquid cycle, and store at 4 C.**Alkaline Lysis Solution II (Plasmid Preparation)**5 M potassium acetate 180.0 mlglacial acetic acid <!> 34.5 mlH2O 85.5 mlThe resulting solution is 3 M with respect to potassium and 5 M with respect to acetate. Store the solution at 4 C and transfer it to an ice bucket just before use.**Electrophoresis and Gel-loading Buffers****TAE 50 X** (stock solution/Liter)242 g of Tris base57.1 ml of glacial acetic acid <!>100 ml of 0.5 M EDTA (pH 8.0)**TBE 5X (3.5 Liters)**189 g of Tris base96.25 g of boric acid70 ml of 0.5 M EDTA (pH 8.0)**LB Freezing Buffer**36 mM K2HPO4 (anhydrous)13.2 mM KH2PO41.7 mM sodium citrate0.4 mM MgSO4\*7H2O6.8 mM ammonium sulfate4.4% (v/v) glycerol100 ml LB brothDissolve the salts into 100ml of LB to the specified concentrations. Measure 95.6 ml of the resulting solution into a fresh container and then add the 4.4 ml of glycerol. Mix the solution well and then sterilize by passing it through a 0.45-ul disposable Nalgene filter. Store the sterile freezing medium at a controlled room temperature (15-25 C).**EDTA (0.5 M, pH 8.0)**Add 186.1 g of disodium EDTA\*2H2O to 800 ml of H2O. Stir vigorously on a magnetic stirrer. Adjust the pH to 8.0 with NaOH (~20 g of NaOH pellets). Dispense into aliquots and sterilize by autoclaving. The disodium salt of EDTA will not go into solution until the pH of the solution is adjusted to ~8.0 by the addition of NaOH.**NaCl (Sodium Chloride, 5 M)**Dissolve 292 g of NaCl in 800 ml of H2O. Adjust the volume to 1 liter with H2O. Dispense into aliquots and sterilize by autoclaving. Store the NaCl solution at room temperature.**SDS (20% w/v)**Also called sodium lauryl sulfate. Dissolve 200 g of electrophoresis –grade <!> in 900 ml of H2O. Heat to 68 C, and stir with a magnetic stirrer to assist dissolution. If necessary, adjust the pH to 7.2 by adding a few drops of concentrated HCl <!>. Adjust the volume to 1 liter with H2O. Store at room temperature. Sterilization is not necessary. Do not autoclave. **Sodium Acetate (3 M, pH 5.2 and pH 7.0)**Dissolve 102.07 g of sodium acetate\*3H2O in 200 ml of H2O. Adjust the pH with glacial acetic acid <!> or adjust the pH to 7.0 with dilute acetic acid. Adjust the volume to 250 ml with H2O. Dispense into aliquots and sterilize by autoclaving.**5X Denaturing Solution** 140g NaOH613.6g NaClbring to 3.5L H2Oautoclave**2X Neutralizing Solution** ~2L H2O613.63g NaCl363.42g TrispH to 7.5 with concentrated HClbring volume to 3.5L with H2Oautoclave**CTAB Extraction Buffer**

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| **Final Con.** | **Stock Conc** | **Amt of stock to add** |
| 0.1 M Tris pH 7.5 | 1 M Tris pH 7.5 | 100ml |
| 0.75M NaCl | 5 M NaCl | 140ml |
| 0.01M EDTA | 0.5M EDTA | 20ml |
| 1% CTAB | CTAB | 10g |
| ddH2O |   | 730ml |
|   |   | **1000ml** |
| 1% B-mercaptoethanol | 14 M BME | 10 ml/1000 ml CTAB Buffer (add just before use) |

CTAB =1% mixed alkyl trimethyl-ammonium bromideDNA Wash Solution76% ethanol \_\_\_\_\_\_\_\_\_\_\_\_ 304 ml of 100% ethanol10 mM NH4Ac \_\_\_\_\_\_\_\_\_ 0.4 ml of 10 M NH4AcBring volume to 400 ml with ddH2O.**Antibiotics**

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|  | Stock Solution Concentration | Storage | Working Concentration Stringent Plasmids | Working Concentrations Relaxed Plasmids |
| Ampicillin | 50 mg/ml in H2O | -20 C | 20 ug/ml | 20 ug/ml |
| Carbenicillin | 50 mg/ml in H2O | -20 C | 20 ug/ml | 60 ug/ml |
| Chloramphenicol | 34 mg/ml in ethanol | -20 C | 25 ug/ml | 170 ug/ml |
| Kanamycin | 10 mg/ml in H2O | -20 C | 10 ug/ml | 50 ug/ml |
| Streptomycin | 10 mg/ml in H2O | -20 C | 10 ug/ml | 50 ug/ml |
| Tetracycline | 5 mg/ml in ethanol | -20 C | 10 ug/ml | 50 ug/ml |
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| 0.25% (W/V) Bromophenol blue |
| 0.25% (W/V) Xylene cyanol FF |
| 30% (V/V) Glycerol in H2O store at 4C |