SOB Medium

1. Put 950 ml of d.d. H_2O into a 1 L sterile bottle.
2. Add 20 g of Tryptone
3. Add 5 g of Yeast Extract
4. Add 0.5 g of NaCl
5. Shake unitl the solutes have dissolved
6. Add 10 ml of a 250 mM KCl solution to the medium (see prep below)
7. Adjust the pH to 7.0 using 5 M NaOH.
8. Adjust the total volume to 1 L
9. Autoclave
10. Right before use, add 5 ml of 2 M $MgCl_2$ (sterile by autoclave, see prep below) to the 1 L bottle (or 0.05% of a smaller volume).

Note 1: 250 mM KCl solution: 1.86 g of KCl in total volume of 100 ml d.d. H_2O. Dissolve and use.

Note 2: 2 M $MgCl_2$ solution: 19 g $MgCl_2$ in total volume of 100 ml d.d. H_2O. Dissolve, autoclave, and use.

SOC Medium

1. Follow the same procedure above, including the addition of 2 M $MgCl_2$.
2. Make a 1 M glucose solution (See below) and sterilize through filtration.
3. Add 20 ul of this sterile solution to 1 ml of SOB medium (or 2%).

Note 3: Dissolve 6 g of glucose in 40 ml of d.d. H_2O. Adjust volume to 50 ml once glucose is dissolved. Sterilize through syringe powered filtration through a 0.22 μm filter.

Inoue Transformation Buffer

1. Prepare a 0.5 M PIPES solution. Dissolve 15.1 g PIPES in 80 ml d.d. H_2O. Adjust pH to 6.7 with 5 M KOH. Adjust volume to 100 ml. Sterilize through filtration through a 0.45 μm filter. Divide into 20 ml aliquots and store at -20C.
2. In a sterile bottle, collect 800 ml of d.d. H_2O.
3. Add 10.88 g of $MnCl_2*4H_2O$ (55mM concentration)
4. Add 2.2 g of $CaCl_2*2H_2O$ (15 mM concentration)
5. Add 18.65 g of KCl (250 mM concentration)
6. Add 20 ml of the PIPES solution from above.
7. Dissolve these solutes and adjust the total volume to 1 L with d.d. H_2O.

8. Sterilize by filtration through 0.45 μm filter. Divide into 50 ml and 20 ml aliquots and store at -20C.