YPD

1% yeast extract, 2% peptone, 2% glucose; pH 6.8

1. Add 10 g of yeast extract.
2. Add 20 g of peptone.
3. Add 20 g of dextrose.
4. Add 20 g of agar (for plates only).
5. Dissolve reagents in 1 L of distilled and deionized water. Autoclave for 20 min at 121 °C.

PLATE mix

1 mL of freshly prepared PLATE mix (make enough mix for N+1 transformations)

1. Add 875 µL of 50% PEG 3350 (NOTE: make sure PEG solution is ≤2 months old).
2. Add 100 µL of 10x TE (100 mM Tris, pH 7.4; 10 mM EDTA, pH 8).
3. Add 25 µL of 1 M LiOAc, pH 7.0; adjust pH with acetic acid (NOTE: ensure LiOAc solution is ≤2 months old).
4. Mix by inversion and gentle flicking to ensure complete mixing.

Roswell Park Memorial Institute (RPMI)-1640 medium

1. Add 700 mL of distilled and deionized water.
2. Add 10.4 g of RPMI.
3. Add 34.5 g of MOPS.
4. Use 5 N NaOH to adjust the pH of the solution to 7.2 and bring the solution to a final volume of 1000 mL with distilled and deionized water.
5. Filter-sterilize by passing thesolution through a 0.22 µm filter.
6. Place a small aliquot of media in a 37 °C incubator overnight to check for contamination.

Ficoll Buffer

Make this buffer fresh on the day of the experiment.

1. Add 500 µL of 1 M PIPES, pH 6.3, stock solution.
2. Add 12.5 µL of 1 M $CaCl_2$.
3. Add 15 mL of sterile distilled and deionized water.
4. Add 2.25 g of Ficoll 400.
5. Incubate at room temperature on a shaking platform until dissolved.
6. Bring up to 25 mL volume with sterile distilled and deionized water.
7. Cool to 4 °C before use.

1 M $CaCl_2$

1. Dissolve 55.49 g of $CaCl_2$ in 40 mL of distilled and deionized water.
2. Bring to final volume of 50 mL.
3. Filter-sterilize by passing the solution through a 0.22 µm filter.

Solution can be stored at room temperature up to 1 year.

5 M NaCl

1. Add 292.2 g of NaCl to 1 L of distilled and deionized water and stir at room temperature until dissolved.
2. Filter-sterilize by passing the solution through a 0.22 µm filter.

Solution can be stored at room temperature up to 1 year.

1 M PIPES (pH 6.3)

1. Add 151.2 g of PIPES (free acid) to 400 mL of distilled and deionized water and stir at room temperature.
2. While monitoring pH, add NaOH pellets during mixing until pH reaches 6.0.
3. Add 1 N NaOH until pH reaches 6.3, and bring the volume up to 500 mL with distilled, deionized water.
4. Filter-sterilize by passing the solution through a 0.22 µm filter.

Solution can be stored at 4 °C for up to 6 months.

2 M Sorbitol

1. Dissolve 182.17 g of sorbitol in 400 mL of distilled and deionized water.
2. Bring to final volume of 500 mL.
3. Filter-sterilize by passing the solution through a 0.22 µm filter.

Solution can be stored at room temperature for up to 6 months.

1 M Tris-Cl (pH 8.0)

1. Dissolve 60.57 g of Tris (hydroxymethyl) aminomethane in 400 mL of distilled and deionized water by stirring at room temperature.
2. Adjust the pH to 8.0 with concentrated HCl and bring the volume up to 500 mL with distilled and deionized water.
3. Filter-sterilize by passing the solution through a 0.22 µm filter.

Solution can be stored at room temperature for up to 6 months.

Sorbitol Buffer

1. Add 35 mL of 2 M sorbitol.
2. Add 2 mL of 1M HEPES-KOH, pH 7.5, stock.
3. Add 250 µL of 100 mM $MgCl_2$ stock.
4. Add 12.75 mL of distilled and deionized water; mix until the solution is clear.
5. Filter-sterilize by passing the solution through a 0.22 µm filter.

Buffer can be stored at 4 °C for up to 6 months.

Resuspension Buffer

Combine the following volumes of stock solutions to make 600 mL of resuspension buffer:

1. Add 360 mL of 2 M sorbitol.
2. Add 60 mL of 1 M potassium phosphate buffer, pH 7.5.
3. Add 300 µL of 1 M $CaCl_2$.
4. Add 179.7 mL of distilled, deionized water.
5. **On the day of experiment:** Prepare 30 mL aliquot(s) of Resuspension Buffer and add 15 µL of 1 M 2-mercaptoethanol and one protease inhibitor cocktail tablet per aliquot.

Stock solution can be stored at 4 °C for up to 6 months.

Aliquots with 2-mercaptoethanol and protease inhibitor added can be stored at 4 °C for 1–2 days.

SPC Buffer

Combine the following volumes of stock solutions to make 50 mL:

1. Add 25 mL of 2 M sorbitol.
2. Add 1 mL of 1 M PIPES, pH 6.3.
3. Add 5 µL of 1 M $CaCl_2$.
4. Add 24 mL of distilled and deionized water; mix until the solution is clear.
5. **On the day of experiment:** Add a tablet of protease inhibitor cocktail.

Buffer without protease inhibitor can be stored for up to 6 months at 4 °C. Once protease inhibitor is added, buffer should be used within 1–2 days.

Bead Activation Buffer (for ConA beads)

1. Add 400 µL of 1 M HEPES-KOH at pH 7.9.
2. Add 200 µL of 1 M KCl.
3. Add 20 µL of 1 M $CaCl_2$.
4. Add 20 µL of 1 M $MnCl_2$.
5. Bring the final volume to 20 mL with distilled and deionized water.

Buffer can be stored at 4 °C for 6 months.

Wash Buffer

1. Add 1 mL of 1 M HEPES at pH 7.5.
2. Add 1.5 mL of 5 M NaCl.
3. Add 12.5 µL of 2 M spermidine.
4. Bring the final volume up to 50 mL with distilled and deionized water.
5. Add one Roche Complete Protease Inhibitor (EDTA-free) tablet.

Buffer can be stored at 4 °C for up to 1 week.

Cell Permeabilization Buffer
Mix 2 mL of 2% (wt/vol) Digitonin with 40 mL of Wash Buffer.

Buffer can be stored at 4 °C for up to 1 day.

Antibody Buffer
Mix 1.6 mL of Cell Permeabilization Buffer with 6.4 µL of 0.5 M EDTA.

Made fresh on the day of experiment and can be stored at 4 °C for up to 1 day.

Stop Buffer

1. Add 4.1 mL of distilled and deionized water.
2. Add 340 µL of 5 M NaCl.
3. Add 200 µL of 0.5 M EDTA.
4. Add 100 µL of 0.2 M EGTA.
5. Add 250 µL of 2% (wt/vol) Digitonin.
6. Add 25 µL of 20 mg/mL RNase A.
7. Add 1.5 pg per sample of heterologous spike-in DNA.

Buffer can be stored at 4 °C for up to 1 week.

TBE Buffer
Prepare a 5x stock solution in 1 L of distilled and deionized water:

1. Add 54 g of Tris base.
2. Add 27.5 g of boric acid.
3. Add 20 mL of 0.5 M EDTA (pH 8.0)
4. Mix until completely dissolved.

NOTE: TBE is usually made and stored as a 5x or 10x stock solution. The pH of the concentrated stock buffer should be 8.3. Dilute the concentrated stock buffer just before use and make the gel solution and the electrophoresis buffer from the same concentrated stock solution. Some investigators prefer to use more concentrated stock solutions of TBE (10x as opposed to 5x). However, a 5x stock solution is more stable because the solutes do not precipitate during storage. Filter-sterilize the 5x or 10x stock solutions using a 0.22 µm filter.

DNA Buffer (Ingredients for 50 mL**)**

1. Add 48.54 mL of nuclease-free water.
2. Add 500 µL of 1 M Tris base.
3. Add 500 µL of disodium 0.1 M EDTA.
4. Add 250 µL of Tween 20, 10% (v/v).
5. Add 186 µL of 1 N HCl.

DNA Purification Bead solution

1. Add 25 mL of 5 M NaCl
2. Add 3.58 mL of nuclease-free water.
3. Add 500 µL of 1 M Tris base.
4. Add 500 µL of 0.1 M disodium EDTA.
5. Add 168 µL of 1 N HCl.
6. Add 20 mL of PEG 8000, 50% (w/v).
7. Add 250 µL of Tween 20, 10% (v/v).
8. Add 1 mL of Sera-Mag bead suspension.

DNA Purification Bead solution instructions

1. Mix the Ampure XP beads to resuspend well.
2. Quickly transfer 1.25 mL to a 1.5 mL microcentrifuge tube. **NOTE**: Beads settle quickly.
3. Place the tube on a magnet stand until the supernatant is clear, about 30 s.
4. Remove and discard the supernatant.
5. Add 250 µL of previously prepared DNA Buffer.
6. Remove the tube from the magnet and resuspend the beads by mixing for at least 15 s.
7. Pool 4 tubes into one (total volume = 1 mL).
8. Place the tube back on the magnet until the beads clear.
9. Remove and discard the supernatant.
10. Add 1 mL of previously prepared DNA Buffer.
11. Remove the tube from the magnet and resuspend the beads by vortexing for at least 15 s.
12. Put the tube back on the magnet until the beads clear.
13. Repeat steps 10–12 for a total of 2 washes, leaving the supernatant in the tube after the last wash.

    In a new 50 mL conical tube, combine the following:

    1. Add 3.58 mL of nuclease-free water.
    2. Add 25 mL of 5 M NaCl.
    3. Add 168 µL of 1 N HCl.
    4. Add 500 µL of 1 M Tris base.
    5. Add 500 µL of 0.1 M EDTA.
14. Cap and mix well.
15. Remove the buffer supernatant from the bead tube still on the magnet.
16. Add 1 mL of incomplete binding buffer (prepared in step 10) to the bead tube on the magnet.
17. Remove the bead tube from the magnet and resuspend by vortexing for 15 s.
18. Add the washed beads to the incomplete binding buffer. Cap and vortex for 30 s.
19. With a 25 mL serological pipette, add 20 mL of 50% PEG 8000 stock.
20. Add 125 µL of 20% Tween 20.

21. Cap the tube and mix by inversion gently but thoroughly until the color appears homogeneous.

The solution can be stored at 4 °C for up to 1 week.