

Making solutions:

Make Transformation Buffer (TB):

50 mM Tris
0.35 M sucrose
0.38 M NaCl
1 mM MgCl₂
0.00001 % rezasurin
Adjust pH to 7.5

Make PEG solution:

TB with 40% (wt/vol) PEG8000
Start with volume of TB that is no more than 75% of the final volume. Use heat to dissolve the PEG. Then add more TB to final volume.

Make 1 M Tris base

Make TB, PEG, and Tris base anaerobic by leaving open in anaerobic chamber overnight with occasional agitation. Also make some anaerobic water.

Make reducing agent:

Dissolve in anaerobic water in anaerobic chamber:
cysteine-HCl·H₂O to 1 %
dithiothreitol to 50 mM
Adjust to pH 7.5 anaerobically by adding Tris base using pH papers

Add 1/50 volume of reducing agent to TB and PEG. Slight pink should turn to colorless in a few hours.

Store all solutions at 4°C under 100% N₂.