SOB frozen competent cells (Yoram Riter lab) – yields ~50 220ul aliquots

Preparation:

1) 500 ml erlenmeyer for cell growth
2) 89 ml DI H_2O autoclaved
3) 200 ml SOB, autoclaved (need 120ml + a few ml for blanks):
   - 4gr bacto-tryptone
   - 1gr bacto yeast exatract
   - 0.1gr NaCl
   - 0.038gr KCl
4) 100 ml CCMB MADE SAME DAY, sterile filtered with 0.22um filter (need 50ml):
   - 1.18gr $CaCl_2$ $2H_2O$
   - 0.4gr $MnCl_2$ $4H_2O$
   - 0.2gr $MgCl_2$ $6H_2O$
   - 1ml KOAc 1M (pH 7.5) (on Orna's bench)
   - 10ml glycerol 100% autoclaved

   CHILL ON ICE.
5) CHILL: glass pippettes, tips, 3 50ml falcons, eppendorfs or deep 96-well plate + cover,
metal holder for eppendorfs or tray for 96-well plate, sterile trough for dispensing
cells (OPTIONAL)
6) fill liquid N_2

Protocol:

0) grow cells overnight in LB at 37C, 250rpm
1) set centriguge temperature to 4C, get 3-4 buckets of ice
2) dilute 1:100 into 120ml SOB (+antibiotics) in 500ml flask, grow to OD600=0.3 at 37C, 250rpm (this is a good time to prepare CCMB and chill it)
3) chill cells on ice for 10min (in falcons – after this NO FIRE)
4) pellet: spin 5000rpm, 10min, 4C, in 3 falcons, 40ml each
5) 1st resuspension:
   on ice, 10ml ice-cold CCMB per falcon (~1/3 original volume)
   using chilled glass pippettes. combine into 1 falcon, add another 10ml CCMB.
6) chill on ice for 20min
7) pellet: spin 5000rpm, 10min, 4C
8) 2nd resuspension:

on ice, total of 10ml ice-cold CCMB
using chilled glass pippettes
9) chill 10min on ice
10) aliquot 220ul into precooled eppendorfs or precooled sterile 96-well plate with
cover using multipipettor, chilled tips and sterile trough (place tips in freezer)
11) throw into liquid N_2 to freeze quickly
12) remove from liquid N_2 with latex-gloved hands quickly (can use lid of -80 box),
move to -80C box and store at -80C
use heat shock protocol to transform.