Day before: make 50ml "superbroth" in 250ml flask and autoclave it:

For 50ml broth mix 1.25g LB broth powder, 1.25g peptone, 0.75 g yeast extract.

Autoclave two Oak Ridge tubes.

1) Inoculate 5 ml overnight culture in "superbroth special" with some leftovers of Top10 cells. Grow the culture at 37ºC overnight.
2) Next morning transfer 2 ml of o/n culture into an autoclaved 250ml Erlenmeyer flask with 50 ml "superbroth" and grow at 37ºC to an $O.D_{600}$ 0.4-0.5 (4 to 5hr) with 350rpm shaking (it never actually get to 0.4, just grow for 4 to 5hr). Place sterile ddH_2O and 10% sterile glycerol in the ice/water slurry. Turn centrifuge upstairs on, put small rotor SS34 in and set centrifuge for 1^0C.
3) After 4-5hr place flask containing the bacteria in an ice/waterbath slurry for five minutes, transfer 25 ml of culture into each pre-cooled Oak Ridge tube and spin down at 8000 RPM in prechilled rotor for 10 min at 1ºC.
4) Discard the supernatant and invert the tube on a paper towel for just a couple of seconds. Add 1 ml cold ddH_2O while keeping the tube in the ice/water. Resuspend the pellet in the ddH_2O by gently swirling the tube in the ice/water (this can take a while the first time, around 30 minutes). When resuspended, fill up to 10 ml with ice cold ddH_2O, invert gently a couple of times and spin again for 5 minutes at 8000 RPM.
5) Pour off supernatant and resuspend the pellet in 1 ml cold 10% glycerol by gently swirling the tube in the ice/water (resuspension will be easier this time). Transfer cells to a chilled 2 ml round bottom tube using 1ml blue tip with cutoff end. Spin at 12,000 g for 30s at 1ºC (Begun lab has cold centrifuge).
6) Pipet supernatant off (pellet is super loose, it is not even a pellet, just settled cells). Resuspend the pellet in 1 ml cold ice-cold 10% glycerol by rocking tube

gently on ice/water. Spin at 12,000 g for 30s at 1ºC (Begun lab has cold centrifuge).

7) Gently remove all supernatant. Resuspend the cell pellet in 1ml ice-cold 10% glycerol by rocking tube gently on ice/water and keep on ice.
8) Prechill a lot of microfuge tubes on ice.
9) Pipet 55ul into each tube using 200ul tip with cutoff end, close and freeze by lowering and leaving in LiqN2. Leave tubes in LiqN2 until ready to transfer to -70^{0}C.
10) Go to the basement and transfer tubes to -70^{0}C using big spoon to fish them out.