Heatshock Transformation after Ligation

Information:
- Get a vector into a bacteria (vector can be plasmid or ligated vector+insert -> this protocol is for after the ligation, for a ready-to-use plasmid)
- Before: Ligation
- After: Plating, Miniprep/Midiprep
- Time: 2h

What we need:
- vector (Ligation)
- competent bacteria (thaw 30 mins, DH5alpha, right freezer, shelf 5, rack III, A4 -> 200µl aliquots)
- LB (37°C)
- Resistance plates (37°C)

Proposed Pipline:
Get bacteria on ice in ice bucket -- Get LB or SOC from kitchen and resistance plate into 37 deg incubator -- Warm up heating block to 37 deg --

Procedure - Ligation:
- Use the ligates from Ligation, either directly from RT or from ice if shortly stored there.

During the 30mins incubation of the ligation:
- Label tubes, (One with the control and the other with insert), keep on ice.
- Get competent bacteria from -80°C (DH5alpha in right fridge, shelf 5, rack III, A4 -> 200µl aliquots) -> let them thaw on ice.
- Put 55ul bacteria in to both of the labeled eppis on ice.
- Get LB (growth medium), warm up at 37°C.

After incubation time:
- Add 5µl Ligation to 55µl competent bacteria in eppis still on ice!

If you want to transform with plasmid that was not ligated: We want to add 10ng Plasmid to each plate (if we do more, then we'll get too many colonies).
- *Notes: we do not use all ligation volume because the enzyme actually inhibits transformation. Keep the ligation tubes until the next day though so you could do it again if it didn't work. (Where did you keep them ?)*
- *Note: When working with bacteria, keep close to ice! Open tubes before pipetting, but beware about ice contamination!*
- Put tubes from ice on 42°C tube shaker for 1 min
- Back on ice for 2 mins
- add 110µl warm LB or SOC (do double volume LB than bacteria)
- Incubate on tube shaker at 37°C for 1h (shaking slowly, ~350) -> do this before plating so that resistance genes can become active!
- Proceed with plating on resistance plates (50-150µl per plate), don't forget positive and negative controls! Incubate at 37°C ON
- Next day: pick colonies, set up liquid culture with antibiotics, proceed with Miniprep / Midiprep