One Shot ccdB Survival Competent Cell Transformation

- Turn on hot block (42°C) and fill a few wells with water
- Turn on 37°C shaker to let it warm up
- Rapidly thaw 50 uL of DB3.1 cells by holding tube in your hand. Once thawed, immediately put tube on ice
- Add 1 ng of ccdB-containing plasmid DNA (unreacted pENTR or pDEST) to cells, mix by gently tapping the tube several times. **DO NOT MIX BY PI PETTING UP AND DOWN**
- Incubate the cells on ice for 30 minutes
- Heat shock the cells for 45 seconds at 42°C in hot block
- Incubate the cells on ice for another 2 minutes
- Add 450 uL of SOC media (500 uL total volume)
- Shake the cells horizontally for 1 hour at 37°C
- Plate 200 uL of the cells
- Incubate the plate overnight (agar side up) at 37°C