* 1. **Production of *E. coli* DH5α competent cells**.

Note: All steps handling living cells should be performed in a clean laminar flow hood and with the Bunsen burner on. Alternative: use commercially available competent cells.

* 1. Inoculate 5 mL of sterilized Luria-Bertani (LB) – 0.02 mg/mL nalidixic acid broth with freshly grown *E. coli* DH5α culture from a petri dish. Grow culture overnight at 37 ºC and 220–250 rpm.
	2. Dilute culture to get 100 mL with an optical density 600 nm (OD600nm) of 0.05. Grow the culture at 37 ºC and 220–250 rpmuntil it reaches OD600nm = 0.45-0.55
	3. Transfer the culture to ice-cold sterilized bottles. Keep the culture on ice from now on. Centrifuge 10 min at 5,836 *x g* and 4 ºC. Discard supernatant.
	4. Resuspend the cellular pellet with 20 mL of ice-cold sterilized 100 mM calcium chloride (CaCl2) solution by vortexing. Leave culture on ice for 2 h.
	5. Centrifuge 10 min at 5,836 *x g* and 4 ºC. Discard supernatant.
	6. Resuspend the cellular pellet with 2 mL of ice-cold sterilized 100 mM CaCl2 solution by vortexing. Add 2 mL of 40% glycerol solution and mix well by vortexing.
	7. Aliquot 100 μL of the sample in sterilized tubes of 1.5 mL volume. Keep at -80 ºC until use.
	8. **Transformation of *E. coli* DH5α competent cells.**

Note: All steps handling living cells should be performed in a clean laminar flow hood and with the Bunsen burner on. Alternatively, use electroporation for bacterial transformation for CAPRRESI protocol. If commercially available competent cells are used then follow manufacturer's guidelines.

* 1. Thaw *E. coli* DH5α competent cells on ice.
	2. Add 3-5 μL of purified plasmid DNA (150–300 ng/μl) and mix well. Keep on ice for 20 min.
	3. Place the samples at 42 ºC for 1 min.
	4. After heat shock, place the samples on ice for 5 min.
	5. Add 1 mL of sterilized LB broth to the samples. Put samples at 37 ºC and 220-250 rpmfor 30-60 min.
	6. Plate 100 μL of the transformed culture into solid LB broth supplemented with appropriate antibiotics.
	7. **Plasmid DNA purification from *E. coli* DH5α**.

Note: Nucleic acids extraction could also be done using commercially available kits.

* 1. Inoculate 5 mL of sterilized liquid LB broth with the desired strain. Add appropriate antibiotics. Use freshly grown culture plates.
	2. Incubate sample at 37 ºC and 220-250 rpm until culture reaches OD600nm = 0.6-0.8 (takes approximately 6–8 h).
	3. Transfer 1 mL of the culture into 1.5 mL tubes, centrifuge 1 min at room temperature and 18,407 *x g*. Discard supernatant. Repeat until the entire culture has been centrifuged.
	4. Add 1 mL of Tris-EDTA (TE) 10:1 pH 8.0 solution and resuspend cell pellet by vortexing. Centrifuge 1 min at room temperature and 18,407 *x g*. Discard supernatant.
	5. Resuspend cell pellet in 100 μL of Solution I by vortexing.
	6. Add 200 μL of Solution II. Mix gently by inversion.
	7. Add 200 μL of Solution III. Mix gently by inversion. Put samples on ice for 5 min.
	8. Centrifuge for 10 min at 4 ºC and 18,407 *x g*. Transfer supernatant to a new tube.
	9. Add 1 mL of absolute ethanol. Mix by vortexing. Freeze sample at -20 ºC for 10 min.
	10. Centrifuge samples for 10 min at 4 ºC and 18,407 *x* *g*. Discard supernatant. Dry samples in a vacuum concentrator for 10 min at medium temperature. Make sure to eliminate all traces of ethanol.
	11. Add 120 μL of sterilized water. Resuspend pellet by vortexing.
	12. Add 15 μL of 1 M magnesium chloride (MgCl2) solution. Mix by vortexing. Put samples on ice for 10 min.
	13. Centrifuge 2 min at room temperature and 18,407 *x g*. Transfer supernatant to a new tube.
	14. Add 300 μL of absolute ethanol and 15 μL of 3 M sodium acetate (CH3CO2Na) solution. Mix by vortexing. Freeze samples at -20 ºC for 10 min.
	15. Centrifuge samples for 10 min at 4 ºC and 18,407 *x g*. Discard supernatant.
	16. Resuspend pellet in 1 mL of 70% ethanol solution. Centrifuge 2 min at room temperature and 18,407 *x* *g*. Discard supernatant. Dry samples in a vacuum concentrator for 10 min at medium temperature. Make sure to eliminate all traces of ethanol.
	17. Resuspend pellet in 50-100 μL of sterilized water.
	18. **Genomic DNA purification**.

Note: Nucleic acids extraction could also be done using commercially available kits.

* 1. Inoculate 5 mL of sterilized liquid LB broth with the desired strain. Add appropriate antibiotics. Take the inoculum from freshly grown culture plates.
	2. Incubate samples at the right temperature and agitation until cultures reach early stationary phase. For example, grow *E. coli* DH5α at 37 ºC and 220–250 rpm until OD600nm = 0.6-0.8.
	3. Transfer 1 mL of culture to 1.5 mL tubes. Centrifuge samples for 1 min at 9,391 *x g*. Discard supernatant. Repeat until the entire culture has been centrifuged.
	4. Resuspend cell pellet in 1 mL of Tris-EDTA (TE) 50:20 pH 8.0 solution by vortexing.
	5. Centrifuge samples for 1 min at 9,391 *x* *g*. Discard supernatant.
	6. Resuspend cell pellet in 400 μL of TE 50:20 pH 8.0 solution by vortexing.
	7. Add 50 μL of 10% sodium dodecyl sulfate (SDS) solution and 50 μL of proteinase K solution [2.5 mg/mL]. Mix gently by inversion. Incubate samples at 56 ºC for 1 h.
	8. Transfer cell lysate to a new tube using a sterilized No. 20 syringe. Pass samples through the syringe 3 times.
	9. Pass samples through a sterilized No. 25 syringe two more times.
	10. Add 500 μL of phenol:chloroform:isoamyl alcohol 24:24:1 solution. Mix well by vortexing.
	11. Centrifuge samples for 10 min at 9,391 *x g*. Recover aqueous phase using a micropipette. Repeat previous step.
	12. Recover the aqueous phase and add 2 volumes of chloroform:isoamyl alcohol 24:1 solution.
	13. Recover the aqueous phase and add 1/10 volume of 3 M potassium acetate (CH3CO2K) solution and 2 volumes of absolute ethanol. Mix by vortexing. Centrifuge samples for 10 min at 4 ºC and 9,391 *x g*. Discard supernatant.
	14. Resuspend pellet with 1 mL of 70% ethanol using vortex. Centrifuge for 2 min at room temperature and 9,391 *x g*. Discard supernatant. Repeat one more time.
	15. Dry samples in a vacuum concentrator for 10 min at medium temperature. Make sure to eliminate all traces of ethanol. Resuspend pellet in 100 μL of sterilized water.
	16. **Colony PCR**.

Note: This technique is useful to identify candidate transformant colonies for subsequent sequencing reaction. This method does not represent a final verification of the integrity of the sequences.

* 1. Number colony stripes on the petri dish. Number microcentrifuge tubes and fill them with 100 μL of TE 10:1 10 mM sodium chloride (NaCl) solution.
	2. Take a part of the culture stripe with a sterilized toothpick. Suspend it in its corresponding tube. Vortex samples.
	3. Heat samples at 95 ºC for 10 min in a thermoblock.
	4. Centrifuge samples at room temperature for 2 min and 18,407 *x g*.
	5. Number new tubes and transfer corresponding supernatants.
	6. Use 5 μL of these supernatants as DNA template for a 50 μL PCR. Include DNA template controls: Negative control - empty plasmid where constructions will be inserted; Positive control - DNA sequence containing the desired amplicon (*e.g*. total genomic DNA).