Protocol for Transforming e. coli

This is the protocol used at BOSSLAB to transform plasmids into e. coli.

Equipment and Reagents:

Name	Amount per Transformation	Notes
Plasmid DNA (pDNA)	.1 ug (micrograms)	Carolina sells various plasmids at .005ug/ul concentrations. This means you need 20 ul per transformation
Plate of e. coli	.5 cm^2 patch	Use a plate inoculated in the last week.
50mM Calcium Chloride	250 ul	Make a large stock solution, and be sure that you use the right molecular weight for the calcium chloride you have- it comes in many hydrates, but the molecular weight should be on the bottle.
Ice bath	1	These can be made by putting ice and water in a shallow tray or cooler. Something deep enough to hold eppendorf tubes.
42 C water bath	1	This can be made by microwaving water until it is 42 C. Test with a thermometer. Make sure 42 C (107.6 F) is withing the range of your thermometer
LB+Ampicillin (or other antibiotic) plates	1	Luria Broth agar plates. 100 ug/ml ampicillin. Amount added to LB agar will depend on concentration.
LB broth	250 ul	For the recovery step.
Microcentrifuge “Eppendorf” tubes	1	Little plastic tubies. You coud probably use a tic-tac container or something if you added more liquid.
P 1000 pippettor and tips	1	Expensive, consider using fixed volume pippets that can accurately pipette near the volumes listed below.
P 2-20 pippettor and tips	1	For pipetting up tiny amounts of pDNA
Innoculating loop and flame or	For transporting bacteria to the	Can be made by bending wire

sterilized spreaders/loops	tube from the plate, and for spreading bacteria across the plate	into a small loop with a long wire handle

Preparation:

You will need to prepare some things ahead of time. The first thing will be growing a plate of e. coli on LB agar media, the day of you will need to make the ice and hot water baths. The ampicillin plate should be made the day before or the day of and stored at 4 C until use. Other antibiotics are hardier, so you may be able to make them earlier and store them under different conditions. You will also want to pre-chill your calcium chloride solution. Pippette 250 ul into your microcentrifuge tube and put it in the water bath 5 or so minutes before you begin.

Procedure:

Action	Notes	Rationale
Transfer .5 cm^2 of e. coli to the microcentrifuge tube of ice cold calcium chloride solution using a sterilized loop.	The bacteria can be sticky. Try twirling or whisking it around in the calcium chloride. An alternative method is to put log-phase liquid cells into a microcentrifuge tube and spin them down for 30s-1 min, pour off the supernatant, and then resuspend them in ice cold calcium chloride.	The calcium ions will coat the negatively charged outside of the cell, neutralizing the charge
Flick/whisk and tap the tube to distribute the bacteria and to break up clumps. It should come out as an opaque yellowish mixture.	Do not vortex or violently pippette up and down- the cells are fragile at this point. Keep as cold as possible.	By breaking up the clumps, you are increasing the surface area of the cells and therefore increasing the likelihood of causing a transformation.
Place on ice for 5 minutes.	Put in ice bath. BE SURE THE LID IS CLOSED. You may have to wedge the tube(s) in between ice cubes to make sure it doesen't float away	Allow Ca2+ ions to coat cells
Add .1ug of DNA	How much volume of suspended DNA you add will depend on the concentration of your DNA in your buffer. Check the label. Keep DNA on ice!	Well, you have to add DNA so the bacteria can take it up
Place bacteria+pDNA on ice for 5 minutes	Again, make sure the lid is closed	Allow Ca2+ions to coat the negatively charged DNA. Without the salt, the DNA backbone and the cellular membrane would repel each

		other.
Put the tube in the 42C water for 90 seconds, aka “heat shock”	Make sure the tube is closed and that it does not float. 42 C is not that hot; you can use your hand to hold it in the bath.	Increase temperature and chance that DNA can slip into the bacteria.
Add 250 ul of LB broth	Add broth to tube.	This is to help the bacteria survive. You have just put them in a very cold and very hot, and unusually salty place. They tend to die when you do that, but enough will survive so that you get transformants.
Plate 100-200 ul onto antibiotic plates immediately IF you are using ampicillin, OR incubate at room temperature for an hour for other antibiotics.	To plate, pippete 100-200 ul (older, dryer plates can absorb more liquid) of liquid from the microcentrifuge tube onto the antibiotic plate. Then spread it around with a sterile spreder, or sterile loop. Try to get it spread evenly over the plate.	Two points to make here: 1. ampicillin is pretty easy to destroy. So if you let the bacteria recover for very long, and you have an ampicillin resistance plasmid, they will make a lot of enzyme to break it down. This means that you will destroy some of the ampicillin on the plate, allowing non-transformed “satellite” colonies to survive/overgrow your transformants. 2. Spread them out over the plate as much as possible so you get individual colonies(not big groupings which are prone to kicking out plasmid/satellite colonies) when the transformants grow up.

Afterwards:

Depending on your plasmid, you may have to add another chemical to see expression, or have to incubate the bacteria at a certain temperature. Incubating at room (20 C) works well for GFP and bioluminescence plasmids.