Growing bacterial cultures (*E.coli*)

NOTES: It is good practice in most kinds of bacteriology experiments to be sure that the culture you are working with is PURE and FRESH.

To check purity, make a streak plate on non-selective medium (e.g. plain LB or TSA or R2A), and incubate at 30°C for at least a week to check for contaminants. (don't use this plate for any further work, as it will have lost any plasmids).

To ensure freshness, use a -80°C stock of the culture, or use a streak plate that is <1 week old. Ideally, when starting up an experiment like a plasmid prep or a competent cell prep, you should use a culture that is only 1-3 days old.

1. Inoculation: cultures can be inoculated from broths or plates or glycerol stocks. Agar plates are best because you can see by eye that the culture is pure (anything could be hiding in a broth!). When inoculating from a plate, use a loopful of growth from the streakline (if you are already sure the culture is pure!). If you pick a single colony, there is a small risk that you will pick some kind of mutant in the culture, ie. that the single cell that founded that colony was not representative of the whole culture. You should use enough inoculum to give you 'just visible' turbidity; this corresponds to an OD600 of ~0.05. In practice this is a small loopful into ~10 ml broth, or a fat loopful into ~100 ml broth. At this inoculation level, the culture will grow up well overnight (16 hr), and be in early stationary phase the next morning.

2. What kind of culture vessel? One of the nice things about E.coli is that you can grow it in any kind of culture vessel, this can be a 'proper' vessel ie. an Erlenmeyer flask, or a 'makeshift' vessel, ie. a media bottle. The bacteria will grow fastest and to highest densities if you grow them aerobically, with shaking, in a system where there is a very large headspace to liquid ratio – the Erlenmeyer flask is always better if you can be bothered. 'Baffled' Erlenmeyer is even better still, since this gives excellent aeration. If you are trying to overexpress proteins, this stuff matters (since it impacts the cellular physiology), but if you are doing a plasmid prep, it is less important.

   What you DON'T want to do is fill the growth vessel all the way to the top with liquid, they will grow very poorly in that case. A nice compromise between convenience and good growth is to use e.g. 50 ml of culture in a 250 ml media bottle (ie. the vessel is only 1/5-full of liquid). You can tightly cap the bottle, since there is plenty of air in the headspace (200 ml) for them to grow aerobically to stationary phase, and this minimises the risk of spills if the bottle falls over in the shaker. Also keep in mind that E.coli is a facultative anaerobe, and can grow by fermentation even when the oxygen runs out. (though it grows much better aerobically).

3. Storage. *E.coli* cultures will remain viable for at least 2 months when kept either at 4°C or at room temp (if kept wrapped in parafilm or plastic bag so they don't dry out), but plates as old as this are not ideal, and may lose plasmids, or their plasmids may undergo rearrangements etc. Fresh is best ! So either restreak them at approx. monthly intervals, or make glycerol stocks (below).

   The best practice for long-term storage is to make a glycerol stock of the culture – do this by growing up a streak plate of the culture on whatever is the appropriate antibiotic agar, then scrape off all the growth from the plate into 500 µl of sterile 20% glycerol (in ROW), vortex well, and then put at -80°C. Label the tube on the top and on the side! Use high-quality labels. Cheap labels or 'permanent' marker do not survive freezing very well.