

# Hands-on DNA: *Bacterial Evolution* – Top Tips, and Health and Safety

---

## Top tips

### Use of equipment

- It is **essential** that centrifuges are balanced correctly. Always ensure centrifuges are properly balanced before starting them.
- When loading microfuge tubes into a centrifuge, always have the hinges facing outwards (that is, towards the rim of the rotor). Pellets will then always form on the edge of the tube directly underneath the hinge, which makes them easier to locate.
- The contents of a microfuge tube should always sit at the base, with no air-bubbles. Droplets of liquid on the side of a microfuge tube can be 'encouraged' to move to the bottom of the tube by tapping the tube gently on a desk, or by holding the tube and sharply flicking your wrist.
- Large air-bubbles, sitting at the bottom of a microfuge tube, underneath the liquid can be removed by sharply flicking the tube with a finger; the tube will probably need to be briefly centrifuged after this, since droplets will almost certainly stick to the side in the process. Small air bubbles on the surface can be removed by centrifuging for a short period (around 10s).
- When using a vortex mixer to resuspend pellets or enzyme, hold two tubes (one in each hand) such that their bases, near the pellet, are touching. Their hitting each other will speed up resuspension.
- Thinner agarose gels are easier to visualise, while thicker agarose gels are more robust. However, since students will not be handling gels directly, thin gels should be sufficient. When pouring the gels, you can stop pouring almost as soon as the surface of the casting tray is covered.
- After pouring gels or melting the agarose, rinse out the conical flask immediately using hot water to prevent residual agarose building up inside the flask.
- When students are loading gels, encourage them to hold their micropipettes at an angle so they can more clearly see where the end of the tip is. Their free hand should stabilise the barrel of the micropipette.
- When visualising gels, the darker the room, the better. If total darkness is difficult to achieve, a simple viewer can be made by cutting a slit out of a cardboard box that sits over the transilluminator.
- For practice gels, the loading dye in the wells will diffuse out if the gels are left submerged in water overnight. It is best to store practice gels in the fridge, submerged in water, so they can be used repeatedly.

## Consumables/ chemicals

- It's often a good idea to centrifuge tubes (for example, containing SYBR Safe and EcoRI) very briefly (around 5-10s) before opening them. This will ensure all liquid/enzyme is in the base of the tube, not on the sides, or, in the case of SYBR Safe, caught in the threading of the lid, which is difficult to see and can easily result in leaks onto gloves. Also, tubes which might appear empty often have liquid in, which will be spun to the bottom of the tube by centrifugation.

## Health and safety

### Basic issues

Very few of the chemicals used in *Bacterial Evolution* pose any real health risk. All chemicals that are used have a 'Material Safety Data Sheet' (MSDS) that will be available either from the website of the supplier, or it may even be delivered with the chemical itself. Centres should be familiar with the MSDSs of the different chemicals.

### Storage

No chemicals have unusual storage requirements; nothing needs to be kept in fire-proof or locked cabinets. All chemicals should all be stored either at room temperature, 4°C or -20°C; they will say on them at what temperature they should be stored.

Because SYBR Safe is light sensitive, it should not be left out for long periods of time. SYBR Safe should be stored at room temperature in a dark cupboard/ drawer.

### Disposal

- Pipette tips
  - Pipette tips are sharp and should be treated with care. Tips should be disposed into a suitably robust container (Nowgen uses plastic tubs), which can then be disposed directly into normal waste.
- Microfuge tubes
  - These *can* go directly into normal waste, but for convenience it's probably easier to dispose of them with pipette tips (also, the hinges are sharp enough to tear waste bags).
- Agarose gels
  - At time of writing, SYBR Safe is considered non-toxic and non-hazardous; gels containing SYBR Safe can therefore be disposed of in normal waste. (See note below on SYBR Safe).
- TBE/ TAE Buffer can be disposed of directly down the sink.
- Gloves can be thrown away as normal waste

## Important additional considerations

### **Gloves**

Centres should be aware that powdered latex gloves **must not** be used under any circumstances. Powdered gloves, in general, should be avoided since they can induce allergies. Nitrile rubber gloves are best since they have least potential to cause any allergic reactions.

### **SYBR Safe**

SYBR Safe is one of a number of fluorescent chemicals that allow DNA to be visualised. Such chemicals work by 'intercalating' within the major or minor groove of DNA. When placed on an appropriate light source (such as a blue-light transilluminator) these chemicals absorb light at one wavelength (colour) and emit it at another.

Most commonly used in laboratories is the chemical ethidium bromide (abbreviated to EtBr or just ethidium). Ethidium is a known mutagen. By binding within the DNA it can cleave it in two, resulting in gross changes to DNA. Ethidium also has low levels of toxicity. It is worth noting, however, that ethidium has **not** been conclusively shown to be a carcinogen.

SYBR Safe is a proprietary formulation, created by Invitrogen (part of Life Technologies) to address some of the safety concerns surrounding the use of ethidium in laboratories. To-date, no toxic or mutagenic properties have been described with SYBR Safe. However, we would still recommend caution when using concentrated, liquid SYBR Safe.

SYBR Safe is dissolved in a solvent called dimethyl sulfoxide (DMSO); a non-polar solvent that is capable of easily permeating nitrile gloves. As such, demonstrators and technicians should be aware when handling liquid SYBR Safe, and should any spill onto their gloves, the gloves should be changed as soon as is practical (within minutes). If there is a spillage of liquid SYBR Safe, demonstrators should wear two pairs of gloves (one over the other) while cleaning it up. Spills should be cleaned up using water and 70% ethanol solution.

Once SYBR Safe is diluted into agarose and the agarose has solidified the likelihood of it being a health risk is negligible. However, demonstrators and technicians should avoid inhaling agarose vapours once SYBR Safe has been added.

More information on SYBR Safe is available on Invitrogen's website: <http://bit.ly/SYBRSafe>.