Making and Mixing Solutions: Dilutions, Vortexing, and Centrifugation

Introduction

- When you make a solution from several components, each is diluted and they may not mix very well
  - Some are denser than others
  - Some are ‘stickier’ than others
- If you pull some solution from unmixed contents
  - Your reactions will each be different
  - Some reactions may not work at all
- Mixing can be done in a number of ways
  - Cap and invert
  - Pipette up and down a number of times, using a setting that is close to the total volume
  - Vortex
- Once you are done mixing some of the solution may be on the inside of the lid or sides of the tube
  - Centrifuge at low speed to collect the solution
  - Don’t forget to balance tubes at 180° from each other

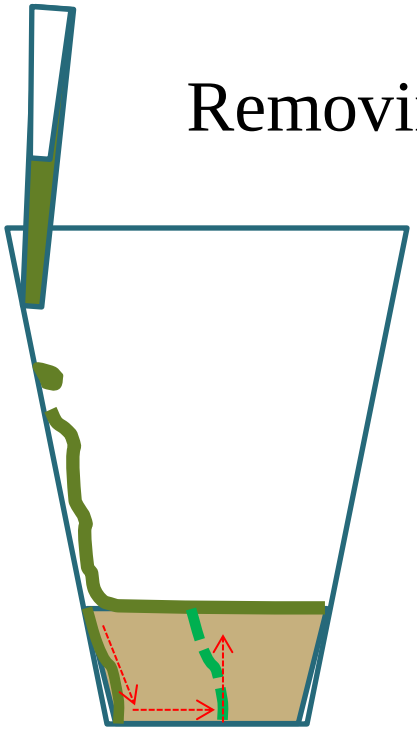
Dense and Sticky components - problems

If I run the solution down the side from above it will slide across the layer below it – little mixing

If I put the solution in from below, it will make a column and rise up to the top – little mixing.

It the solution is very sticky and I run it down the side, droplets may stick to the side instead of flowing down with the rest – little mixing.

Removing solution from a poorly mixed stock.



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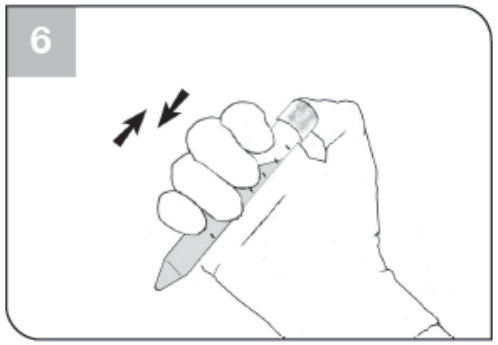
Over time diffusion will occur- → very slow mixing.

When you freeze a solution sometimes one part freezes first – crystals may float or sink.

Mixing Modes

A.

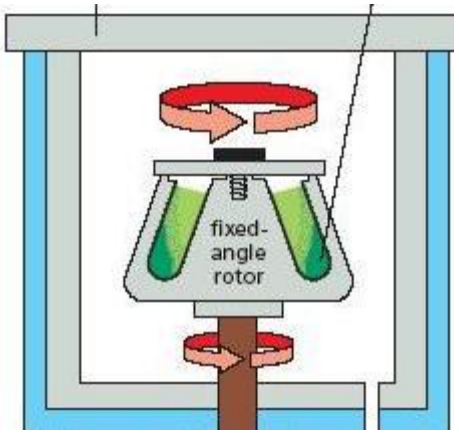


B.

C.

Protein will ‘whip’

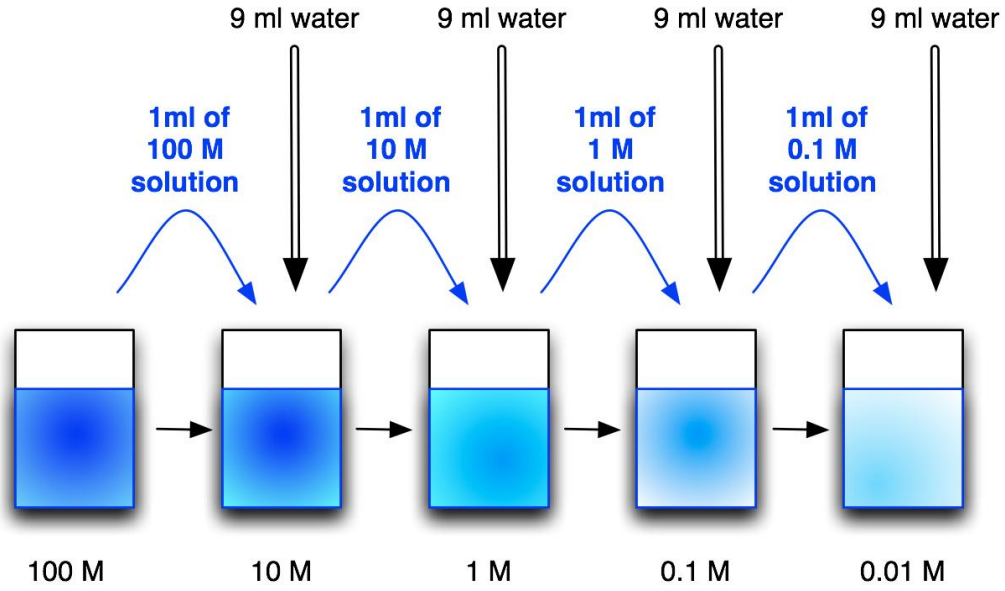
Centrifugation to collect solution



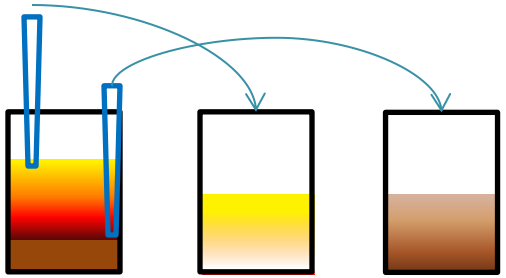
If the tubes across from each other not balanced (by mass) the rotor will start to rock, and can eventually break.

Denser material will collect at the bottom of the tube.

Diluting/dispensing from mixed solutions



If each solution is completely mixed before taking out 1ml to make the next solution in the series then your set will differ by a factor of 10 each time.



If the solution is not mixed then you get different amounts of the components depending on where the tip goes into the starting solution – all of the resulting samples will be different, and in a series you won't know what factor they differ by.