Buffer Preparation

General Guidelines:

- Calculate your buffers carefully before you start.
- When working with acids and bases, remember to add concentrated to dilute (acid or base to water), rather than dilute to concentrated (water to acid or base). If you are mixing together acids and bases, dilute both in water first, and add slowly (usually in an ice bath) to minimize the effects of the exothermic interaction.
- When adjusting the pH, use the appropriate acid (or base). For instance, sodium phosphate buffer is adjusted up and down with monobasic or dibasic sodium phosphate, not HCl or NaOH.

Buffer Selection:

For all of the experiments you will be performing (thermal analysis of UV melting curves, fluorescent titrations), a buffer is necessary to maintain your DNA samples in the appropriate protonation state and environment. Different buffers have different properties and tolerances. Generally, sodium ions (from 50-200 mM) are used to stabilize the double helix, so sodium-salts of buffering agents are used. Cacodylate buffers are very useful, but carry very high environmental toxicity- Agent Blue, made from cacodylic acid and sodium cacodylate was used as one of the selection of herbicides in the Vietnam war (along with Agent Orange)- and so their use is avoided unless absolutely necessary for an experiment. For the experiments we'll be doing in lab, three properties of these buffers are considered important:

1. **pK_a**. A buffer's capacity for pH changes is highest close to its pK_a, so choosing a buffer with a pK_a close to your working pH is important.
2. **Temperature stability of pK_a**. Tris (tris(hydroxmethyl)aminomethane) is a widely used buffer with a good pH range, but has very low temperature stability. Over a 60 °C temperature range, it will change almost 2 pH units!
3. **Compatibility with divalent metals**. While sodium ions are mainly used to stabilize duplex structures, Mg^{2+} can be used to show interesting structural transitions, and is sometimes added in small amounts to buffers.

The table below gives some commonly used buffers and their properties, and is taken from John Santalucia's chapter "The use of Spectroscopic Techniques in the Study of DNA Stability" that's on e-reserve for the class:

Table 1 Commonly used buffers for UV thermal denaturation studies[a]

Buffer	pK_a (@ 25°C, I = 0.1 M)	$\Delta pK_a/\Delta T$ (°C^{-1})	Compatible with divalent metals
Sodium cacodylate	6.27	–0.0015	Y
Sodium phosphate	7.20	–0.0028	N
MES	6.26	–0.011	Y
Tris[d]	8.06	–0.028	N
PIPES	6.80	–0.0085	Y
HEPES	7.55	–0.014	Y
Acetic acid	4.76	+0.0002	?