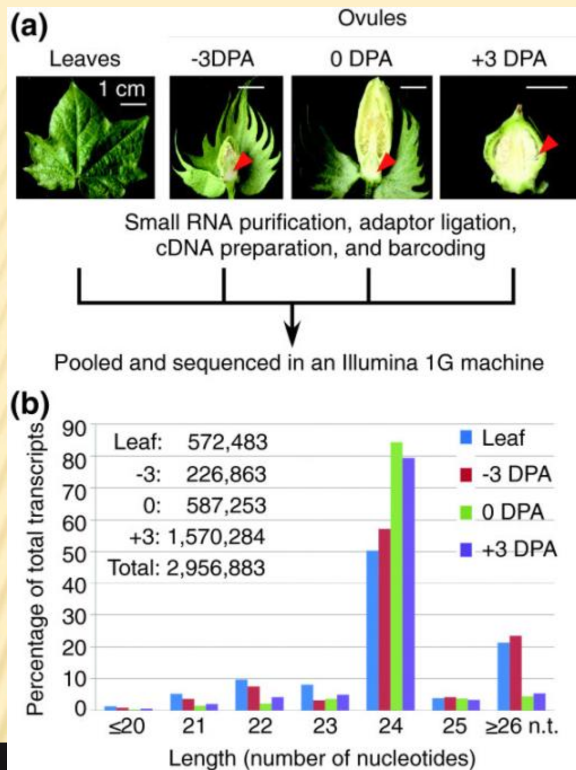


WORKFLOW DURING THE B3 CAMP

MAKING SOLUTIONS FROM STOCKS

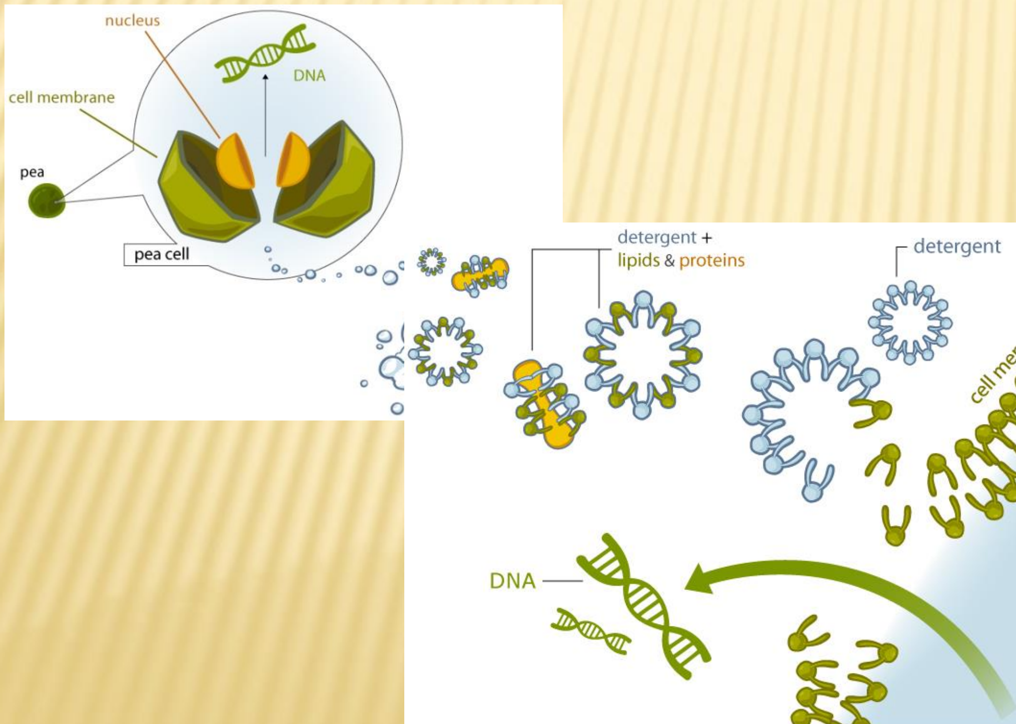
B3 Summer Science Camp
at Olympic High School

LAB WORKFLOW OVERVIEW

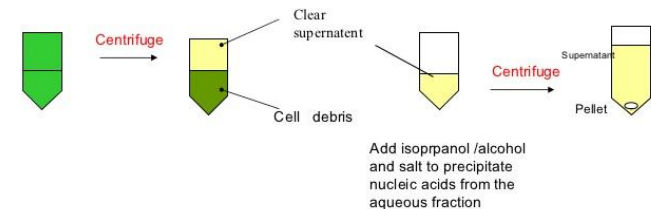
- Collect Samples (June 12th)
- Extract DNA from the samples (June 17th)
  - Break open the cells (contents will mix)
  - Use chemistry to extract nucleic acid from other cell contents and prevent degradation
  - Use biochemistry and chemistry to degrade the RNA and then concentrate the DNA
- Test the DNA for quality (June 18th and June 22nd)
  - How long are the pieces?
  - How much do we have?
  - How pure is it?
- Carry out two types of enzymatic reactions (June 23rd)
  - PCR for chloroplast segment, and agarose gel
  - Restriction Endonuclease (RE)digestion and size selection
- Construct a sequencing library with the RE material (June 24-25th)
  - Size select
  - Add small DNA ‘adaptors’ (with barcodes to tell apart) using DNA ligase
  - Carry out more PCR
  - Clean up the product to remove unwanted or unused components
- Test the library and quantify it (June 26th)
  - Verify the average length and concentration
  - Make the proper dilutions and pool the samples
- Sequence the pooled libraries (June 26th)

EXTRACT DNA

- Extract DNA from the samples (June 17th)
  - Break open the cells (contents will mix)
  - Use chemistry to extract nucleic acid from other cell contents and prevent degradation
  - Use biochemistry and chemistry to degrade the RNA and then concentrate the DNA



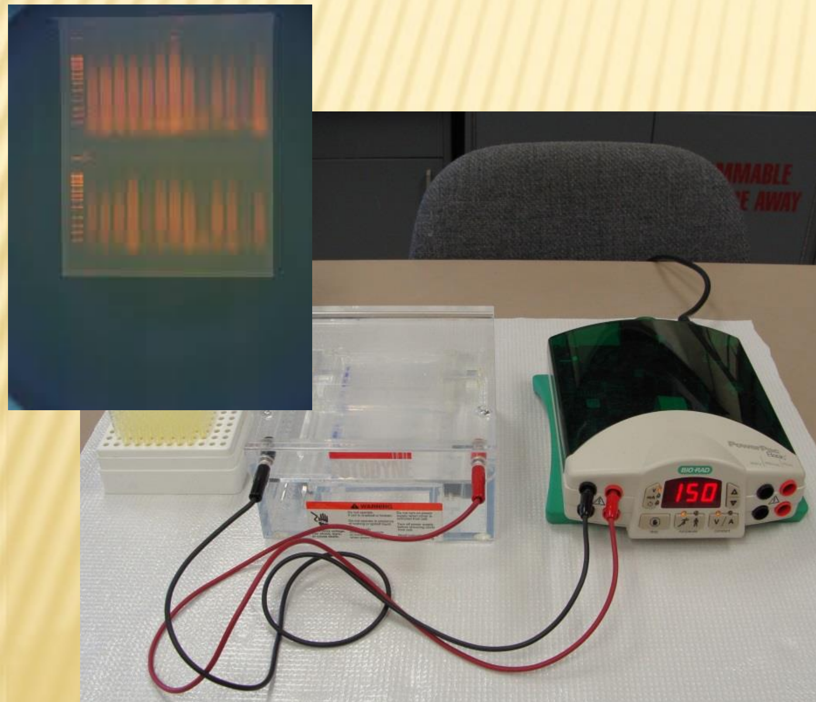
Non-Phenol Chloroform based extraction of DNA for PCR detection of Citrus yellow mosaic virus and Citrus greening bacterium

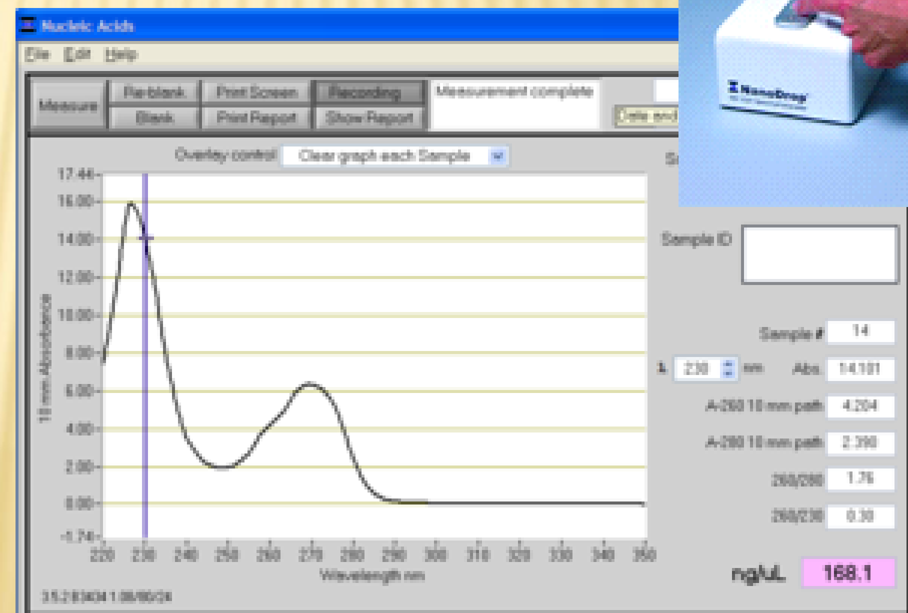


Learn.genetics.utah.edu

QUALITY TESTING DNA

- Test the DNA for quality (June 18th and June 22nd)
  - How long are the pieces?
  - How much do we have? How pure is it?

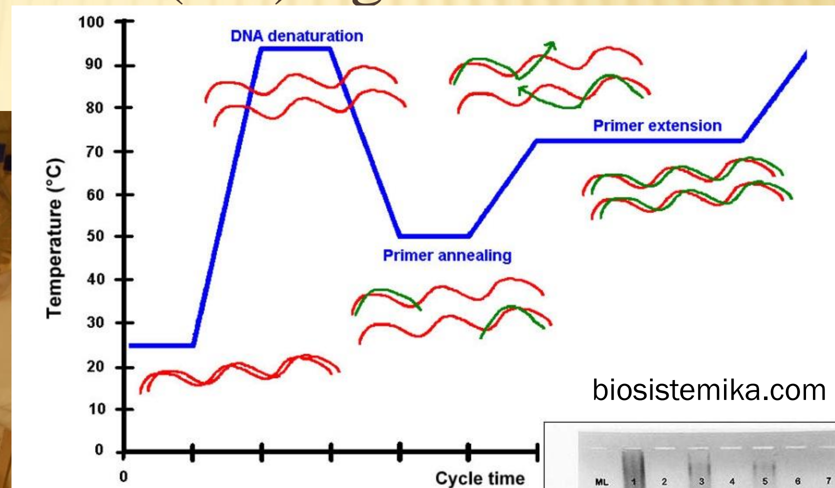




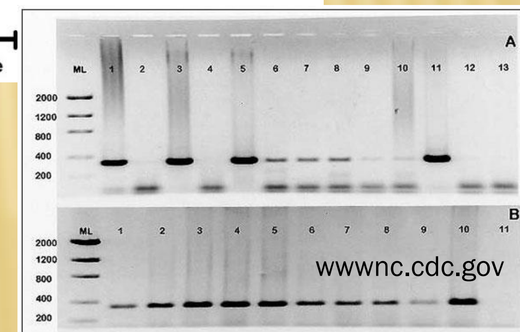
PCR, RESTRICTION DIGESTION, LIGATION

- Carry out two types of enzymatic reactions (June 23rd)
  - PCR for chloroplast segment, and agarose gel
  - Restriction Endonuclease (RE)digestion and size selection

en.wikipedia.org



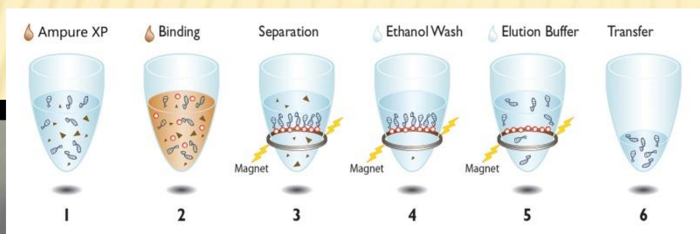
biosistemika.com

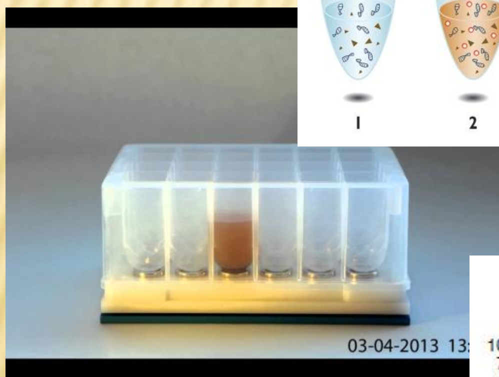


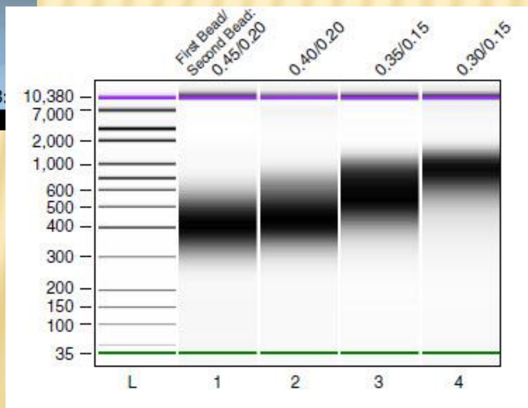
wwwnc.cdc.gov

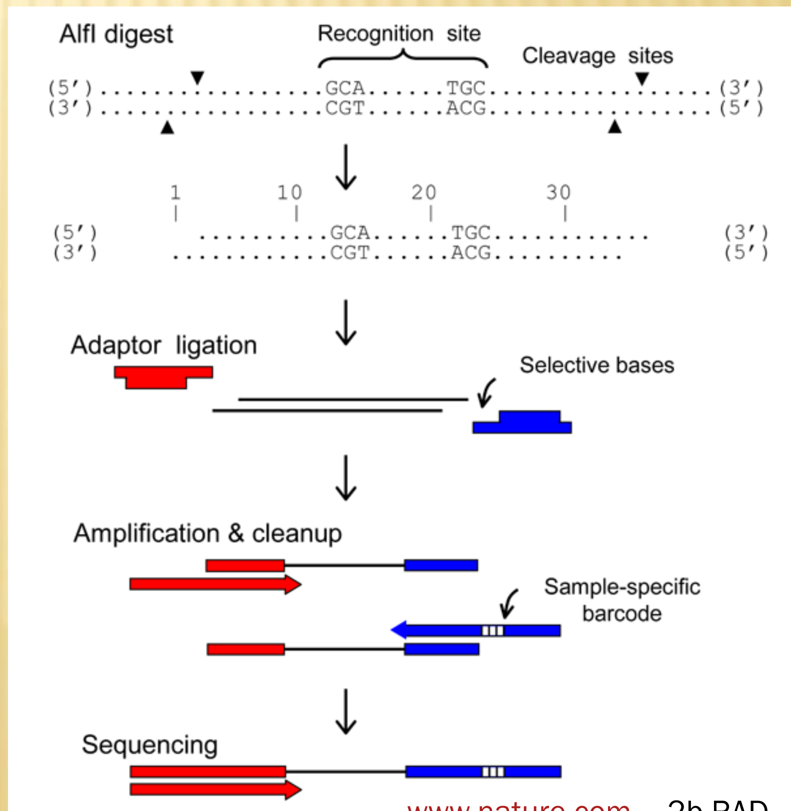
SEQUENCING LIBRARY CONSTRUCTION

- Construct a sequencing library with the RE material (June 24-25th)
  - Size select with magnetic beads
  - Add small DNA 'adaptors' (with barcodes to tell apart) using DNA ligase
  - Use beads to clean up the product







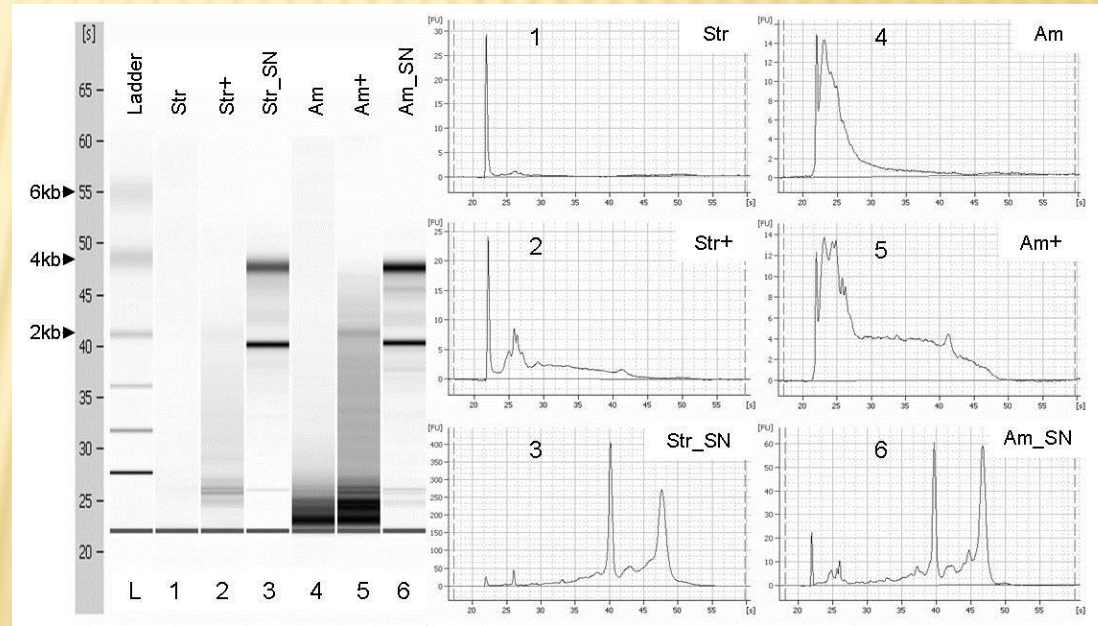


www.nature.com – 2b-RAD

SEQUENCING LIBRARY QUALITY TESTS

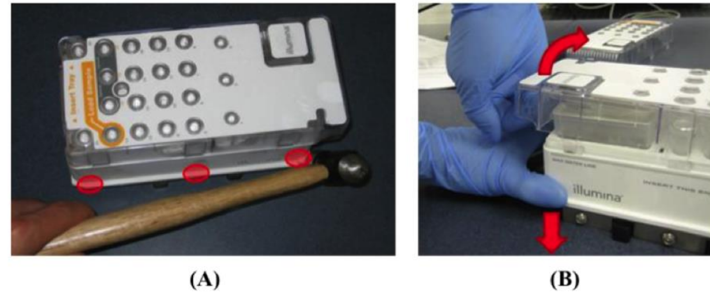
- Test the library and quantify it (June 26th)
  - Verify the average length and concentration
  - Make the proper dilutions and pool the samples

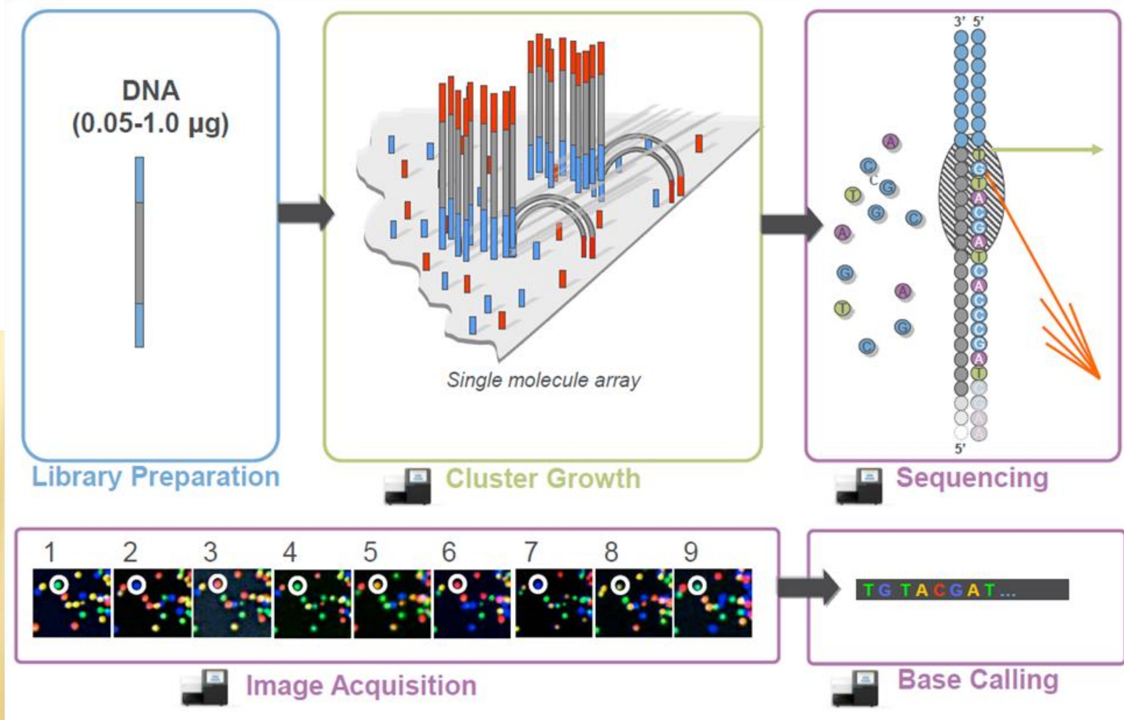
Agilent Bioanalyzer 2100



SEQUENCING THE LIBRARY

- Sequence the pooled libraries (June 26th)





LAB WORKFLOW - TODAY

- Can you make solutions from stocks?
- Can you use microbalances properly?
- Do you know how to use micropipetters?

LAB WORKFLOW - TODAY

- Can you make solutions from stocks?
  - Do you understand what different types of stocks are?

LAB STOCKS – 'FOLD' STOCKS

- What is a 10X stock? How do you use it?

10-times more concentrated than standard use.

Take 1 part of the stock and add 9 parts of the other components.

If you are only adding water and you want 100ml (0.1L) of 1X TAE buffer

C1V1 = C2V2

(1X) 100ml= (10X)?ml

[(1X) 100ml/10X] = 10ml

For the rest of the solution: subtract 10 from 100 → 90ml of H_2O if that is the only other component.

Result will be 100 ml of 1X TAE buffer.

LAB STOCKS – 'MOLAR' STOCKS

- What is a 1.0 Molar stock?

Contains 1 mole of Tris molecules for every liter for solvent (Water in this case).

1 mole is Avogadro's number: $6.023 * 10^{23}$.
Tris has a mass of 157.56 gm/mol so weigh out 157.56 grams, bring up to 1L with H2O (adjust the pH also).

If I want 100ml (0.1L) of a 50mM solution (0.05M), use the formula

C1V1 = C2V2
(0.05M) 0.1L= (1M)XL
[(0.05M) 0.1L/1M] = 0.005L or 5ml

So I will use 5ml of the stock and bring it to 100ml with the other components, which might be just water (so add 95ml) or several other things.

LAB STOCKS – 'MASS PER VOLUME' STOCKS

- What is a Mass/Volume stock?

Contains 50milligrams of the protein lysozyme (0.05gm) per 1ml (0.001L) of solution.

This does not tell you what the solution contains – for proteins it is most likely some buffer like Tris at pH that stabilizes the enzyme, in water, and it might have some salt like Ca^{++} in it, and a small amount of glycerol (5% is common).

If I am making a 100microliter (0.1ml or $100*10^{-6}L = 10^{-4}L$) solution and I want it to have 1mg/ml of lysozyme.

C1V1 = C2V2

1mg/ml(0.1ml) = 50mg/ml(Xml)

[1mg/ml(0.1ml) /50mg/ml] = 0.002ml or 2ul

So I will use 2ul of the stock and bring it to 100ul with the other components to end up with 1mg/ml of the lysozyme.

LAB STOCKS – ‘PERCENT’ STOCKS

- What is a Percent stock?

Contains 10grams of the chemical SDS (sodium dodecyl sulfate, a detergent) per 100ml of solution (in this case the solvent is water – you have to read the chemical information sheet to know that).

Note: In molecular biology, when the main solvent (that dissolves or otherwise carries the other compounds) is not named it is assumed to be water.

If I am making a 100microliter (0.1ml or 100×10^{-6}L = 10^{-4}L) solution and I want it to have a final concentration of 0.1% SDS:

C1V1 = C2V2

1%(0.1ml) = 10%(Xml)

[1%(0.1ml) /10%] = 0.010ml or 10ul

So I will use 10ul of the stock and bring it to 100ul with the other components to end up with 1% SDS.

LAB STOCKS – 'UNIT' STOCKS

- What is a Unit/Volume stock?

Contains 10grams of the chemical SDS (sodium dodecyl sulfate, a detergent) per 100ml of solution (in this case the solvent is water – you have to read the chemical information sheet to know that).

Note: In molecular biology, when the main solvent (that dissolves or otherwise carries the other compounds) is not named it is assumed to be water.

If I am making a 100microliter (0.1ml or $100*10^{-6}L = 10^{-4}L$) solution and I want it to have a final concentration of 0.1% SDS:

C1V1 = C2V2

1%(0.1ml) = 10%(Xml)

[1%(0.1ml) /10%] = 0.010ml or 10ul

So I will use 10ul of the stock and bring it to 100ul with the other components to end up with 1% SDS.

LAB STOCKS – USING CORRECTLY

- Many protocols ask you to combine concentrated stocks with some of each kind listed above.
- The Using Lab Stocks Handout has examples of doing the calculations for a couple of the types of molecular biology protocols we will be using.