Basic Laboratory Principles
Topics

- UV spectroscopy of nucleic acids and proteins
- What is happening in the Mini-Prep?
- Plasmid migration in agarose gel electrophoresis
- SDS PAGE
- Bacterial strains and electroporation
- Levels of confidence in your genetic construct
- Protein induction systems
- IMAC System
UV Absorption of Nucleic Acids and DNA

- Nucleotides have similar absorbance
- Sugar / phosphate backbone absorbs at higher energies
- Absorption feature near ~ 260 nm due to $\pi - \pi^*$ transition of bases
- Nearly the same for nucleotides and nucleosides in this region
- In general, polynucleotides absorb less per nucleotide than their constituent molecules
- Native, double-stranded DNA absorbs less than denatured (“melted”) DNA
  - Increase in A is called hyperchromicity
DNA UV Spectra
UV Spectroscopy of Nucleic Acids and Proteins

• 260/280
  – DNA ~1.8
  – RNA ~2.0
  – Lower by 0.2-0.3 acidic solution, protein, phenol, other contaminants
  – Higher by 0.2-0.3 basic solution

• 260/280 nucleotides
  – Guanine 1.15, Adenine 4.5, Cytosine 1.51, Uracil 4.0, Thymidine 1.47
UV Spectroscopy of Nucleic Acids and Proteins

• 230/280
  – Higher than 260/280 often in range of 2.0-2.2
  – Lower indicates presence of contaminants like phenol that absorb at 280 nm.
UV-visible Absorption of Amino Acids & Proteins

UV spectrum of Bovine Serum Albumin (BSA)

- Contains information on conformation and concentration

- Most proteins are colorless in the visible region.
- Absorption maxima at 190-200 nm (large)
  - π to π* transition amide backbone
- Absorption maxima at 280 nm (smaller)
  - π to π* transition in F, W, Y aromatics

π to π* transition

Absorbance

Wavelength (nm)
Secondary Structure Affects Absorption Properties

- Changes in abs properties reflect changes in “electronic environment by:
  - local charge re-distribution of chromophore
  - conformational changes altering the $\pi$ to $\pi^*$ transition amide backbone
  - bonding interactions
  - PROVIDES INFORMATION!

- Circular dichromism is more sensitive then UV for conformational changes

[Graph showing molar absorptivity vs. wavelength for different secondary structures (beta, random coil, helix)].
What is happening in the Mini Prep?

• Miniprep is a convenient version of a classic method identified as alkaline lysis of bacteria to recover a plasmid.

• Cells include lipids, proteins, salts, chromosomal DNA, plasmid DNA and RNA.

• Plasmid must be separated from other bacterial components including chromosomal DNA.
What is happening in the Mini Prep?

• Step 1- Lysis by NaOH/SDS
  – SDS is a detergent, dissolves lipids cell wall.
  – NaOH *denatures* DNA, including plasmid DNA.

• Step 2- Neutralization by KOAc
  – RNAse is added at this step
  – KOAc *precipitates* SDS and its associated lipids & proteins; chromosomal DNA is caught in this precipitate. *Plasmid DNA & RNA remain in solution.*
  – RNAse is degrading RNA to nucleoside monophosphate

• Step 3 is centrifugation
  – Pellets KOAc/SDS/lipids/proteins/Chromosomal DNA precipitate
  – Plasmid DNA remains in solution
  – RNA degradation by added RNAse is complete.
What is happening in the Mini Prep?

• Step 4- Binding of DS plasmid DNA to silica gel column.

• Step 5- Wash #1 of silica gel column to remove RNA, RNAse, detergent, and other residual contaminants.

• Step 6- Wash #2 of silica gel column to remove residual salts.
  – High alcohol content retains plasmid on column

• Step 7- Elution of DS plasmid DNA from column with water or TE buffer.
Plasmid Migration In
Agarose Gel electrophoresis

• Movement of a long polymer through a matrix is complex.
• Many theories for movement of DNA through agarose-
• One theory explaining many of the observed properties of DNA moving through agarose including very long DNA in pulsed fields is the reptation theory (with many variants).
Reptation with hernia

Fig. 1. (A) Schematic representation of the chain (full line) and its "tube" (dotted lines) in the gel (bold circles). (B) The same with a loop or "hernia."
Size Importance On Movement Through the Agarose or PAGE Gel

Fig. 2. Different regimes of migration in constant-field electrophoresis (A) Ogston sieving; (B) reptation without orientation; (C) reptation with orientation.
Fig. 5. Simulation of the migration of a 100-kbp molecule in a constant electric field (after ref. 90). The gel fibers, represented by the dots, constrain the chain’s motion. (A) The molecule is initially oriented along the field direction (arrowed); (B) it starts to bunch at the head, and a hernia begins to grow; (C) the hernia unravels to leave the molecule stretched in a U-shaped conformation; (D) the chain slides like a taut rope around a pulley; (E) the molecule is once again oriented.
Uncut Plasmids on a Gel

• When you run an uncut plasmid prep on a gel, you will get a pattern of bands (at least 3-4)
  
• Which band of DNA is
  – the supercoiled plasmids?
  – Nicked circles?
  – Linear DNA?
  – Multimers?
  – Single strand closed circle?
Multimers/Concatenates
Multimers/Concatenates

• Multimer Reference-

• RecA gene involved in some cases

• Can occur as-
  – Covalently closed circle tandem repeats (or more) of plasmid
  – Concatenates

• Often supercoiled
• Protein is first denatured
  – Heat unfolds tertiary and secondary structure
  – Beta-Mercaptoethanol or Dithiotheritol disrupt disulfide cross linkages
• SDS and Molecular Radius
  – SDS-coated proteins are linear molecules, 18 Angstroms wide w/length proportional to their molecular weight.
  – Molecular radius and mobility in the gel is determined by side chains in AA sequence, and the length (i.e. molecular weight of the protein).
  – SDS-coated proteins have the same approximate charge to mass ratio so no differential migration based on charge.
SDS Ensures Protein Remains Denatured During Electrophoresis and Provides Negative Charge
Tris-HCl Gel Protein Migration Chart

Precision Plus Protein™ Standards Run on Criterion™ Tris-HCl Gels

Tris-HCl gels with Tris/glycine/SDS running buffer
Bacterial Strains and Electroporation

• Strains used for Plasmid propagation are RecA-
• Strain used for Protein Production are RecA+
• Electroporation
  – High Field induces local breakdown of plasma membrane to form electropore.
  – Pores reseal over time
  – Mechanism of DNA entry is unknown but has been suggested to be electro-osmosis by Dimitrov and Sowers (1990)
Fig. 1. Pore resealing kinetics indicated by dye uptake. The fraction \( f_c \) of colored cells as a function of the time \( t = t_{\text{add}} \) of dye addition after the pulse. B-lymphoma cells (line IIA1.6) were exposed to one rectangular electric field pulse (\( E = 1.49 \text{ kV cm}^{-1} \); pulse duration \( t_E = 110 \mu\text{s} \)) in the presence of the dye SERVA blue G (\( M_r = 854 \)). (From ref. 9, with permission.)

From Chapter 1- Principles of membrane electroporation and transport of macromolecules. Electrochemotherapy, Electrogenetherapy, and Transdermal Drug DeliveryMethods in Molecular Medicine Volume 37, 2000, pp 1-35
Levels of confidence in your genetic construct

- Low confidence- DNA construct is the right size in agarose gel.
- Higher confidence- Cut DNA with restriction enzymes and get the right size pieces.
- Higher confidence- PCR of DNA yields correct size PCR products.
- Others?
- Gold Standard- Sequence it! It is $4 per 800 bases, CHEAP and best information.
Protein Induction Systems

Protein Induction Systems

Chapter 9 Production of Proteins from Cloned Genes In
Gene Cloning and DNA Analysis an Introduction 2010 by
T. Brown.
IMAC and 6x his Tag

Structure pictures taken from Wikipedia
IMAC / SUMO System Expression Vector
IMAC / SUMO Proteins

His 6X- SUMO Solubility TAG Target Gene (p53)

His 6X- SUMO Protease
Steps In IMAC SUMO Production and Purification

1. Clone Gene into SUMO Vector.
2. Express SUMO/Target Gene Fusion Protein in E. coli or other host.
3. Purify SUMO Target Fusion Protein on Ni or Co column (IMAC).
4. Digest SUMO Target Fusion Protein with SUMO protease.
5. Re-purify Target Protein from SUMO protease and SUMO domain w/6x his tag using Ni or Co column (IMAC).