Overview of Current Molecular Biology Techniques
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Outline

- DNA – preparation and analysis
- RNA – preparation and analysis
- RNA-Protein Complexes
- Proteins
- Cell Culture
- Organ-on-a-chip
- These all require sterile technique.
- Contamination is always a major concern.
DNA Preparation and Analysis

• DNA Extraction Protocols consist of two parts
  • Lyse cells and solubilize DNA
  • Removal of RNA, Protein, and other macromolecules

• Many labs now use purpose specific kits for extraction
  • Kits include:
    • Lysis Buffer
    • Pre-made columns
    • Other Buffers (Wash buffers and Elution buffers)

• Advantages of these kits
  • Saves time
  • Easy and straight forward
  • Enabling extraction of DNA from different sample types
DNA Preparation and Analysis (cont.)

• Techniques following extraction
  • PCR
  • Restriction digestion

• Agarose gels are used to confirm successful reaction
  • PCR product
  • Complete digestion

• Retrieval of products from gel for downstream applications
  • Kits are available for:
    • Extracting product from gel
    • Cleaning-up PCR reaction after gel confirmation

• Some examples of downstream applications
  • Sequencing: Next Generation Sequencing (NGS), Sanger sequencing
  • Cloning applications
NGS Workflow

http://www.biotechniques.com/multimedia/archive/00231/BTN_A_000114133_O_F_231434a.jpg
Gel Electrophoresis

- Nucleic acids are negatively charged.
- Separate DNA based on size.
- Three major factors to consider when running a gel.
  - Pore size of gel
  - Voltage gradient
  - Salt concentration of running buffer
- There are many different types of gels that can be used for differing applications.
  - Agarose gels
  - Acrylamide gels
  - Polyacrylamide gels with urea for denaturation
Gel Electrophoresis (cont.)

• **Voltage Gradients**
  - Ohm’s law: \( V=IR \)
  - \( V \) = voltage
  - \( I \) = current
  - \( R \) = resistance

• **Factors that effect gradient**
  - Thickness of gel
  - Ionic strength of buffer

• **Increasing gradient increases mobility of sample**

• **Another factor to consider is the heat generated**
  - \( P= I^2R \)
  - \( P= \) Power (watts)
  - Others same as above
  - Gel apparatus can displace a certain amount of heat
  - Exceeding the heat capacity can lead to deleterious effects
RNA Preparation and Analysis

• Extraction of RNA is very similar to DNA
• However, samples are generally treated with proteases to remove ribonucleases that can degrade RNA
• RNA is not as stable as DNA and rapidly degrades
  • Must be kept on ice
• Why extract RNA?
  • Transcriptomics – mRNA
  • Viral RNA
  • 16S rRNA sequencing
  • microRNA
RNA to dscDNA

1. vRNA
2. GSP
3. First Strand cDNA Synthesis
4. RNA-cDNA Hybrid
5. Second Strand Synthesis
6. Double Stranded cDNA
7. Purify and measure concentration
8. Nextera XT Sample Prep
9. Dilute to 0.2ng/μL
10. Purified PCR Product
RNA-Protein Complexes

- Several techniques for detecting and separating RNA-Protein complexes
  - Density-gradient sedimentation
  - Vertical native polyacrylamide gels
  - Gel filtration

- Assembly of complexes
  - RNA is transcribed that contains $^{32}$P.
  - Incubate under conditions that promote the formation of complexes

- Complexes are run on an agarose gel.
- Gels are visualized on a Phosphorimager.

Why study RNA-Protein complexes?
- Protein misfolding
- DNA/RNA does not show any mutations but there is no protein expression or protein is non-functional
- Secondary structure in RNA

What are some advantages of using agarose gels?
- Simple
- Inexpensive
- Rapid
- Multiplex
Protein: Molecular Modelling

- Isolate and purify experimentally expressed proteins
- Obtain structures using NMR or X-Ray Crystallography
- Structures are energy minimized to obtain the lowest energy structure
- Simulations require a structure for initial system setup
- There are many different simulations available for protein complexes. Selection depends on the goal.
  - Molecular Dynamic (MD) simulations: Atomistic
  - Coarse-grain simulations
  - QM/MM simulations
  - Ab initio MD

PDB code: 1DT7
Molecular Modelling (cont.)

• Simulations are commonly used for drug screening
  • Protein-drug interactions
  • Drug docking
• In some cases protein structures can not be solved with NMR or X-Ray crystallography and require some computational studies.
  • Homology Modelling
  • De-novo Modelling
  • Foldit – video game where players try to fold a protein sequence into a 3D structure
Why do we use molecular modelling?

• Proteins have dynamic structures.
• Reduce the cost for experiments.
• However, there are limitations:
  • Size of the system that can be simulated
  • Computational cost
  • Accuracy of the force field
  • Experience of the user
Cell Cultures

• Some common uses in labs include
  • Titrations
  • Immunocytochemistry
  • Virus amplification
  • Virus Neutralization
  • Plaque Assays

• There are many cell lines available that are immortalized
  • MDCKs
  • BHK
  • Hela cells
  • Vero cells
  • etc

• Some experiments many require cell lines that are not available, so primary cell lines maybe required
  • These are cells isolated from a tissue sample and propagated in tissue culture flasks or petri dishes
  • They have a reduced passage capacity
Cell Culture (cont.)

- Cells require nutrients which are supplied by the cell media which will vary depending on the type of cells used.
  - In general, the media contains:
    - Amino acids, glucose, salts, vitamins, antibiotics and antifungal agents.
    - FBS and L-glutamine may be added.
- Depending on the cell line replication time will vary.
- Once cells have reached 85-95% confluency, they can be split.
  - Things to avoid:
    - Splitting cells too soon.
    - Splitting cells when they are overgrown.
- Since the cells are grown on the surface of a flask, they require the use of trypsin for detachment.
- Several new flasks can be made from one confluent flask.
- Labs generally keep frozen stocks of cell lines commonly used by the group.
  - Reasons:
    - Contamination.
    - Cells get old.
    - Certain cell lines may not be used all of the time.
    - Reduce cost of cell maintenance.
- Some experiments may require a certain cell density or cell viability:
  - Hemacytometer.
  - Trypan Blue Staining.
Organ-on-a-chip

• Integration of 3D organ specific tissue and microfluidic network.

• This technology enables the study of drug and toxin metabolism by different organs.

• Reduce the use of animals for toxicity and early drug testing.

• Disadvantages
  • Experimental/novel technology – requires validation
  • Low throughput
  • Costly
  • Use of immortalized cells can lead to misrepresentation of organ function
Human-on-a-chip Example