Overview of Current Molecular Biology Techniques

VRSP Journal Club June 5, 2017

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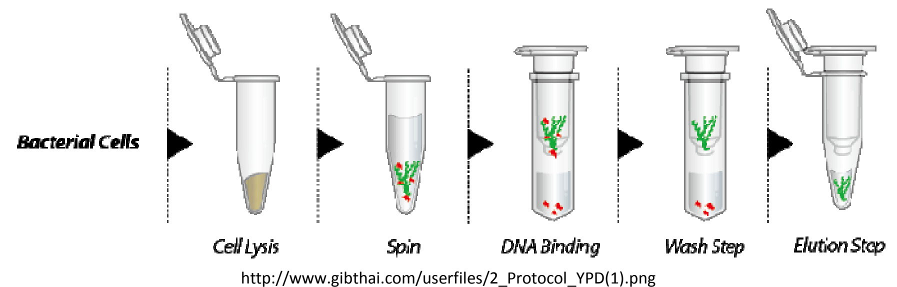
Outline

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

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



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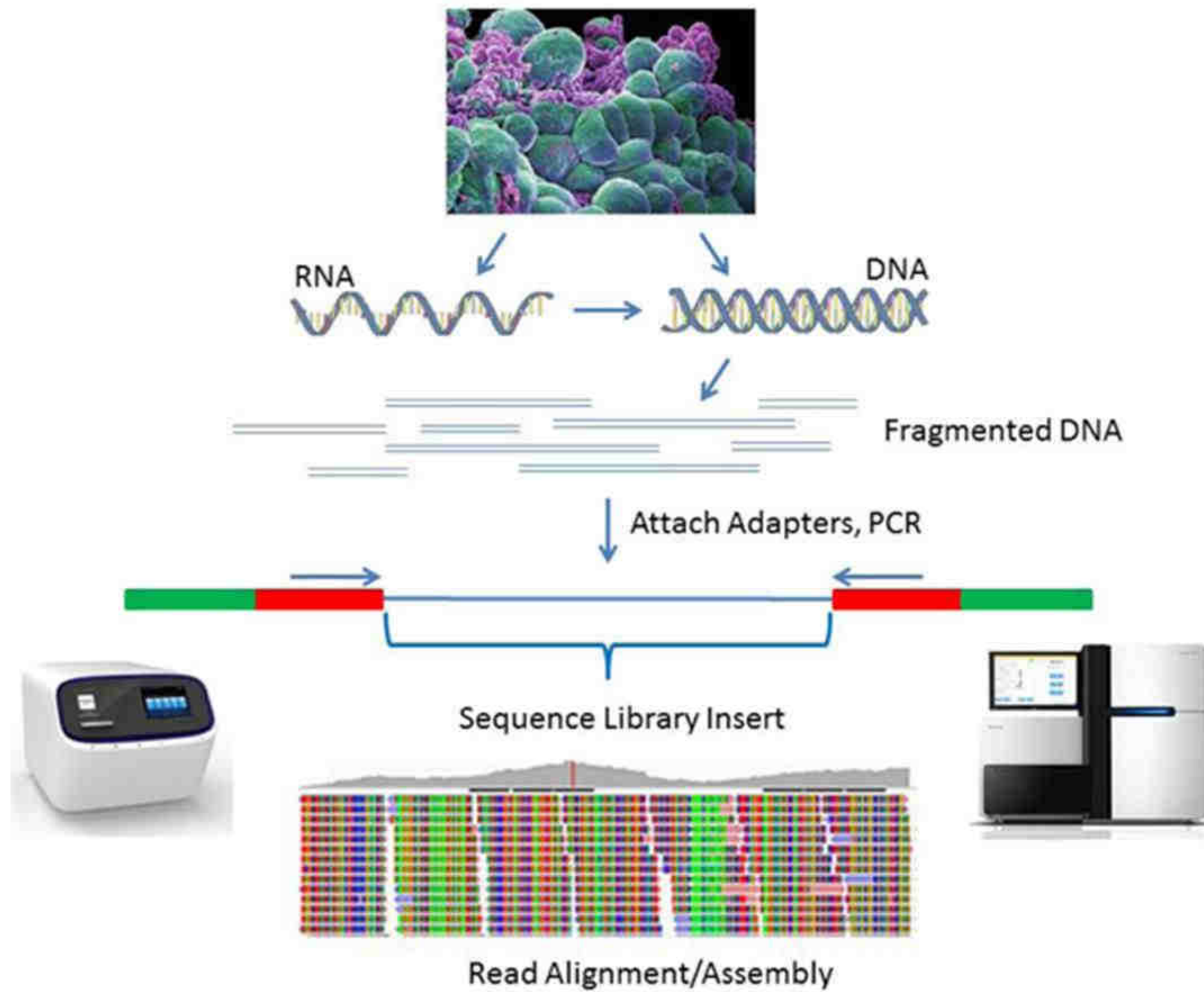
DNA Preparation and Analysis (cont.)

- 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



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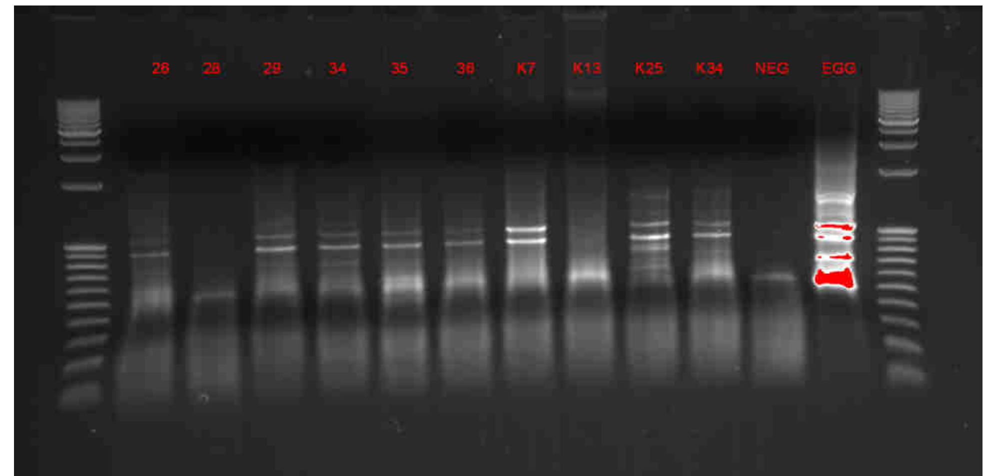
Gel Electrophoresis

- 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



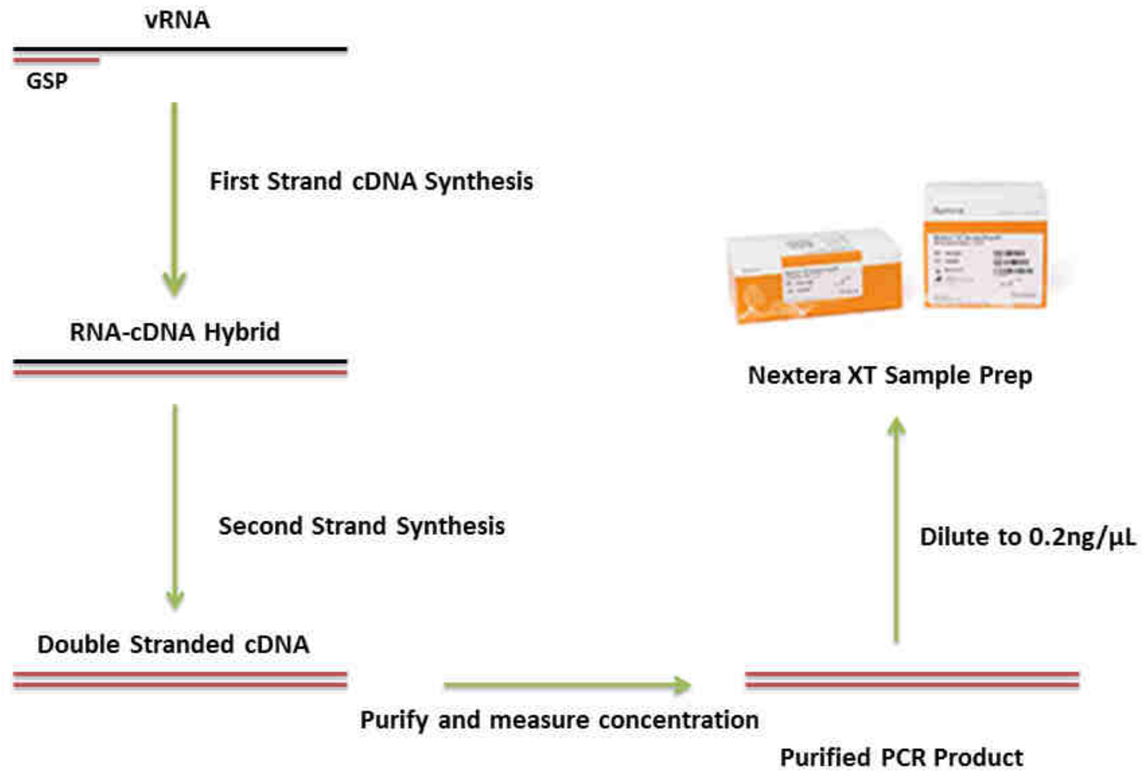
Gel Electrophoresis (cont.)

- 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

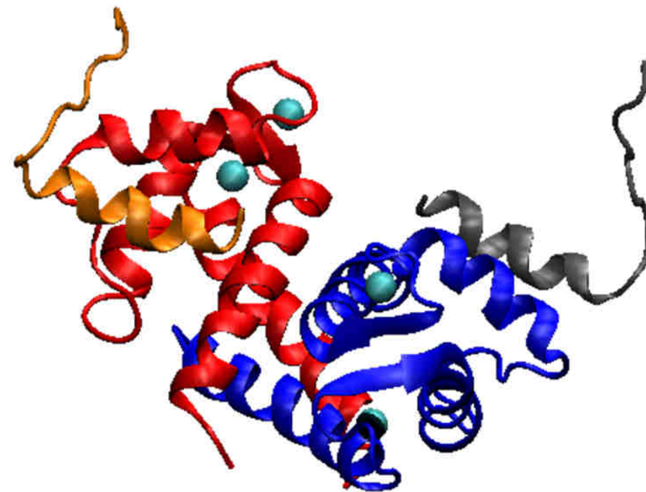


RNA-Protein Complexes

- 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



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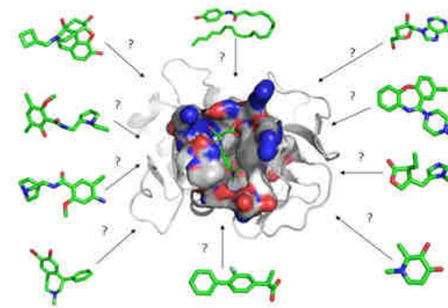
Molecular Modelling (cont.)

- 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



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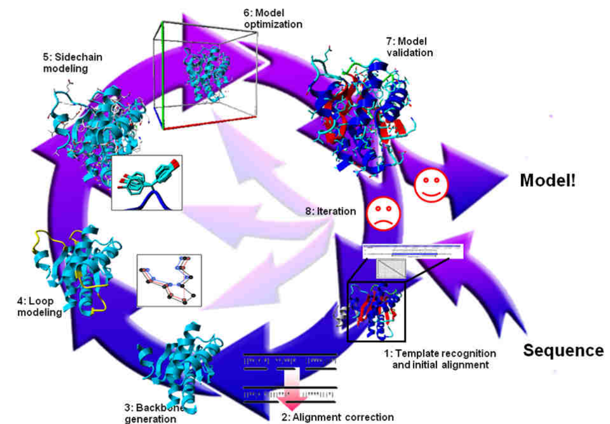
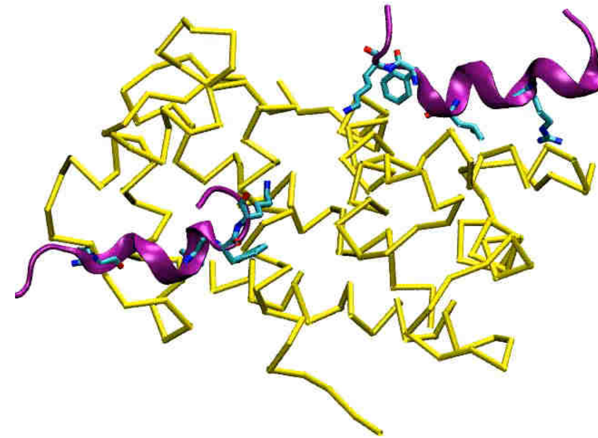


http://swift.cmbi.ru.nl/teach/EMHOMX/IMAGE/Fig2.png

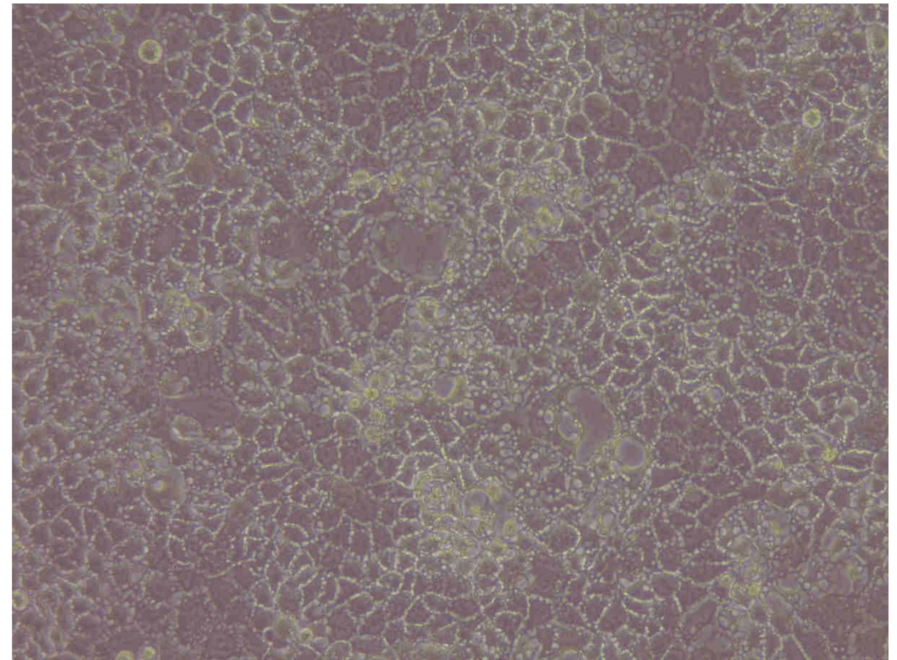
Why do we use molecular modelling?

- 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



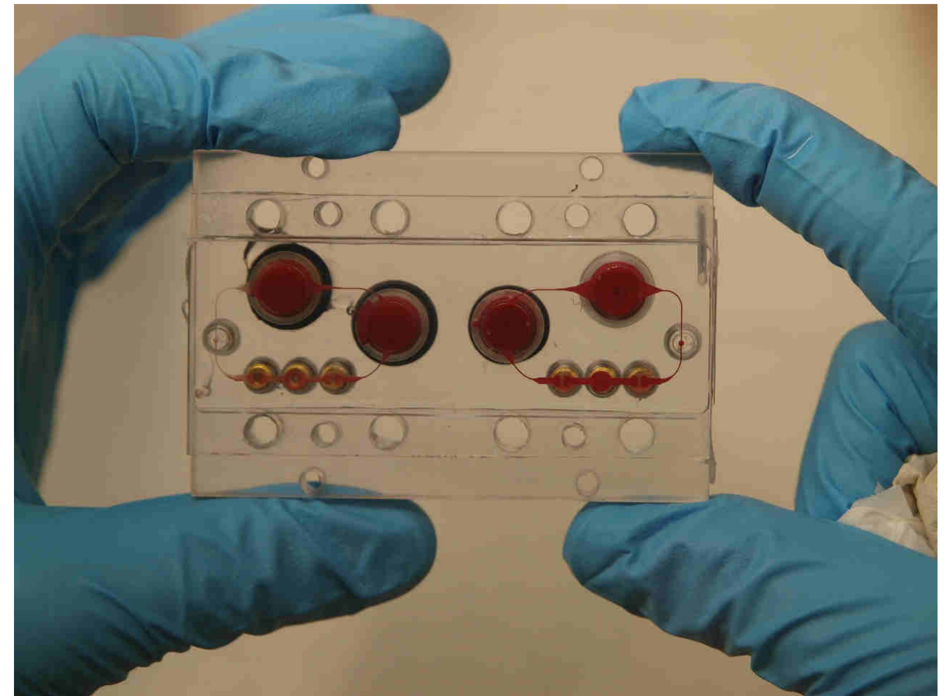
http://molbiol.ru/forums/index.php?act=Attach&type=post&id=202120

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 antimycotics
    - FBS and L-glutamine maybe added
- Depending on the cell line replication time will vary.
- Once cells have reached a 85-95% confluent the cells can be split.
  - Things to avoid
    - Splitting cells to 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 many 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



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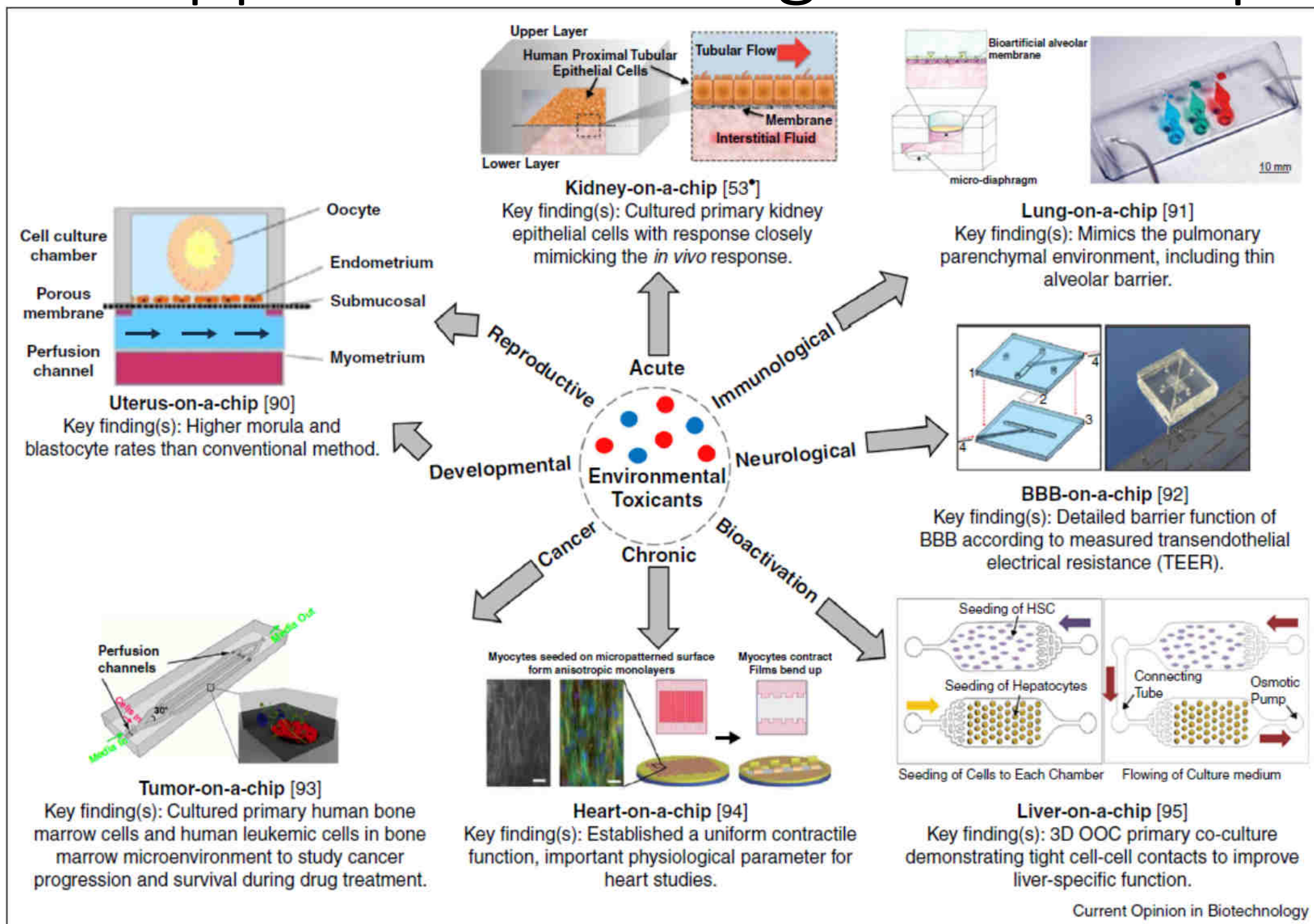
Applications of Organ-on-a-chip



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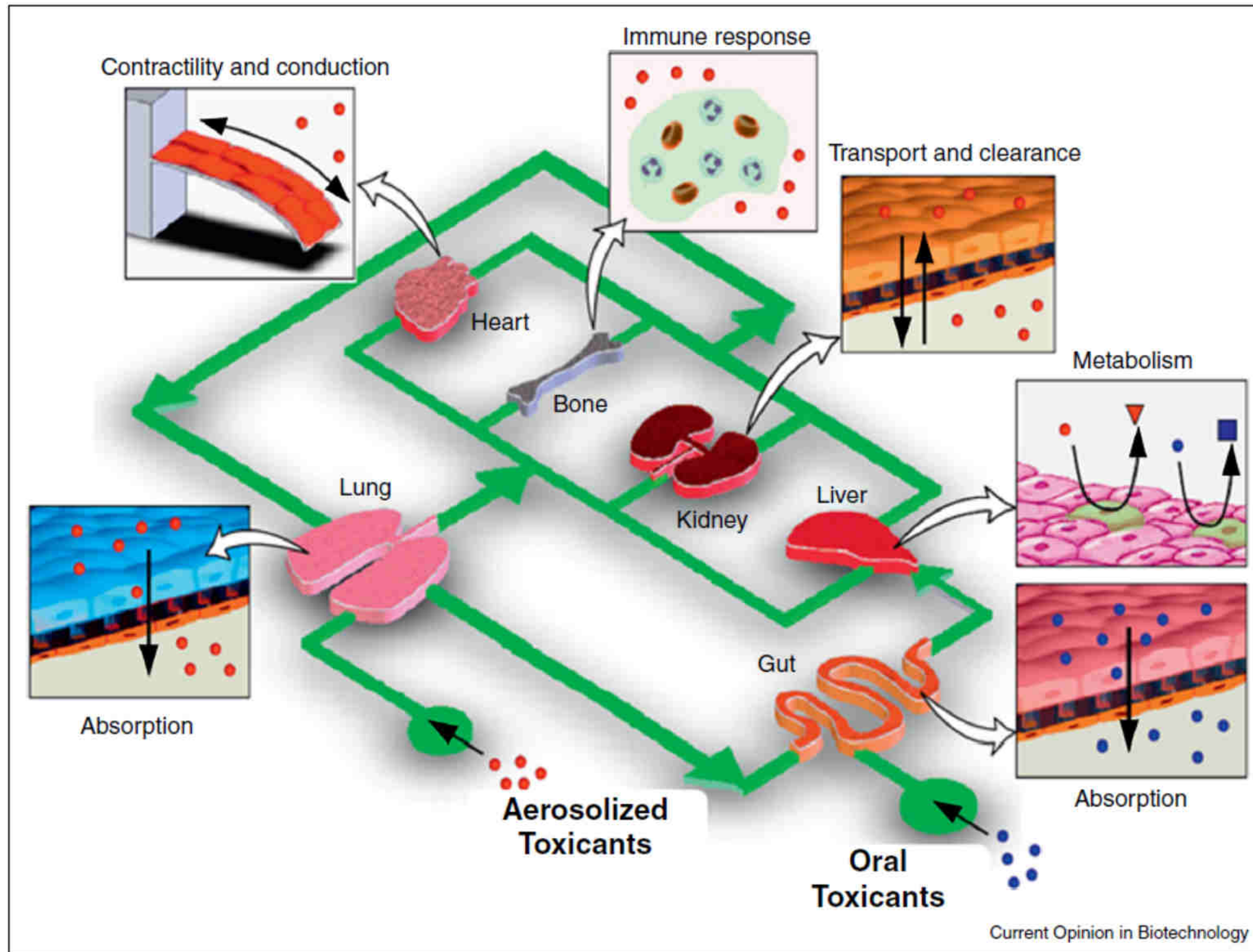
Human-on-a-chip Example



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