Biology 4141 – Current Topics and Methods in Cell Biology
Fall 2013 Course Outline

Calendar Description: Selected topics in cell biology, such as membrane dynamics, cell cycle control, apoptosis, signal transduction, and cellular rhythmicity. Presentation and critical discussion of recent research papers, emphasizing current methods and experimental design. Three lecture hours. One term. Three credits.
Prerequisite: SC/BIOL 3130 3.00.
Students without pre-requisite must request permission from the instructor. Permission will only be granted if the student has adequate background knowledge.

Time and location: Tues & Thurs 10:00-11:30, ACW 303

Course Director: Dr. Pat Lakin-Thomas, 005 Farquharson, x33461
Office hours: Tues & Thurs 11:30 – 1:00 or by appointment
E-mail: clocklab@yorku.ca I will try to respond within one working day, or answer your question at the next class meeting if appropriate.

Website: on Moodle

Learning Objectives
On completing this course, students should be able to:
1. describe recent developments in a selected set of topics at the frontiers of cell biology research.
2. suggest appropriate methods for answering questions about cells and evaluate the pros and cons of current methodologies for investigating cell structure and function.
3. suggest and evaluate experimental designs in cell biology research.
4. critically read original research papers in cell biology.
5. deliver a presentation of recent research at a professional level.

Structure
Five current topics will be covered. Each will be introduced by a recent review followed by several original papers relevant to the topic. Students will give presentations summarizing the papers and the course director will lead critical discussions on aspects of the papers.

Assessment
Presentation 30%, one midterm 30%, final exam 40%
Presentation topics and dates will be signed up on the first day of class, first come/first served, or assigned at random by the instructor. If there are more presentation dates than students, there will be an opportunity for students to volunteer to give a second presentation and use the better grade.
NOTE: Final course grades may be adjusted to conform to Program or Faculty grade distribution profiles.

Important Dates
First and Last Class Meetings: Sept 10 – Dec 5
Co-Curricular (Reading) Week: Oct 30 – Nov 1
Midterm date: Oct. 15, in class
Drop Date: Nov. 8

Exam format: Short answers and a choice of paragraph-length essay answers. The exams are open-book and open-note: You may bring the papers and your notes to the exam. You may not use computers during the exam. It is therefore essential for you to have printed copies of the papers.

Sample exam questions:

1. What is the experimental evidence to support a particular conclusion?
2. Given a particular experimental approach, suggest some controls that should be included in the experimental design and explain why they are useful.
3. What techniques would be appropriate to investigate a particular question, and what information would these techniques provide?
4. For a particular figure from a paper, explain the experimental design and explain what information was gained from the results.
5. From a particular review, what did the authors suggest are the most important unanswered questions on this topic?

Late policy

Presentations will not be accepted after the assigned date unless you have a well-documented excuse, in which case the presentation will be given at the first opportunity. If the midterm is missed due to a documented excuse, the weight will be assigned to the final exam. If the final exam is missed, the Biology Department's deferred exam policy will apply. A doctor's note is not sufficient for deferring the final exam; you must fill out an Attending Physician's Statement and have it signed by your doctor. The APS can be found in the Academic Petitions package:
http://www.registrar.yorku.ca/petitions/academic/index.htm

Academic Integrity

Students are expected to be familiar with and follow York University's policies regarding academic integrity. Please consult the website below for more details:
http://www.yorku.ca/academicintegrity/students/index.htm

Accommodation Statement

Students who feel that there are extenuating circumstances that may interfere with their ability to successfully complete the course requirements are encouraged to discuss the matter with the Course Director as soon as possible. Students with physical, learning or psychiatric disabilities who require reasonable accommodation in teaching style or evaluation methods should consult with the Office for Persons with Disabilities (OPD) and ensure that requests for appropriate accommodations are arranged with the Course Director early in the term.
The religious accommodation policy can be found at this website:
https://w2prod.sis.yorku.ca/Apps/WebObjects/cdm.woa/wa/regobs

Required Text

Gillen, C.M. (2007) *Reading Primary Literature*, Pearson Benjamin Cummings.

This short pamphlet is an excellent introduction to critical reading and experimental design (and how science works). Copies are for sale in the bookstore and also on reserve at Steacie.

Finding papers

All papers for this course are available to download from the journals' own websites. You do not need to photocopy paper journals, and there are no copies of the papers on reserve. Please do not copy from the original journals, to save wear-and-tear on the bound volumes. Papers are not posted on the

course website, for two reasons: 1) Posting may violate copyright laws. 2) You need to practice how to find papers online. Go first to York Library eResources and find by periodical title, then find the paper by volume and page number. (You need to be connected via a York computer, or know how to get access from your own computer using Passport York.) If not listed in York's holdings, then go directly to the journal's own website for access. (Google the name of the journal in quotes.)

Be sure to download the pdf version (not full text/html) for printing. Use the html version for high-resolution figures (to use in your presentation) and to see colour figures. Also be sure to check for and download any supplemental files: many papers have additional information that is not included in the printed version and is only available online. (You may need to go to the journal's own website to get the supplements, not through an intermediary like Scholar's Portal.) Many papers require color for some of the figures; if you don't print in color, you may need to look at the figures in the electronic version to understand them.

Finding information on methods

(Include your sources in your presentation, at the bottom of the slide and/or a final references slide.)

1) For basic methods, go first to your cell biology textbook (Alberts), and the index. Try other textbooks such as molecular biology or genetics texts.
2) Look in Sambrook for basic molecular methods (reference listed below).
3) Try Current Protocols Online: Select "Current Protocols in Cell Biology" (or another topic) and use the search function with a keyword (website listed below).
4) For methods specific to your paper, go to the earlier papers that are referenced as sources for the methods. This may send you on a long chain of references to earlier and earlier papers.
5) Try websites of companies named in the paper that manufacture kits or supply reagents. This is a good source for proprietary (patented) kits and methods.
6) Wikipedia is surprisingly good for background information but don't use it as a primary reference-look at the bibliography at the bottom of the Wikipedia page and find those references. There is a Wiki project for molecular and cell biology: http://en.wikipedia.org/wiki/Portal:MCB
7) You can Google the name of the method but I don't recommend it; this is inefficient and sometimes unreliable. Check the reliability of the websites: often they will not be primary (reliable) sources, but rather someone's lecture notes.

General References

Alberts, B. et al. (2008) *Molecular Biology of the Cell*, Fifth Edition (on reserve at Steacie)
Very useful for background information and basic methods. CD has good figures to use in presentations.

Current Protocols Online:
http://www3.interscience.wiley.com/browse/?type=CURRENT_PROTOCOL
Online resource that describes methods in cell biology, cytometry, molecular biology, and other titles.

Sambrook, J. & Russell, D.W. (2001) *Molecular Cloning: A Laboratory Manual, Third Edition*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA. [Three volumes, on reserve at Steacie, QH 442.2 M26 2001]
The standard reference for molecular biology methods.

Methods In…
Steacie holds several series of publications with this title, including Enzymology (covering far more than enzymes), Cell Biology, Molecular Biology, etc. Each volume is on a specific topic and is catalogued under its specific title. They are best located by doing a Library Catalogue search > search by keyword > "methods" plus the subject you want, such as "methods cell cycle".

Model Organisms and Cell Lines

www.ceolas.org/VL/mo/

Online catalog of websites with information about various model organisms.

www.nih.gov/science/models/

Information on model organisms for biomedical research from the NIH

www.atcc.org/

American Type Culture Collection: source for cell lines and information about them

Presentation of Papers

Time limit: Approx. 20-30 min plus questions. Practice and time your talk.

Evaluation: Your grade will be based on the criteria listed on the evaluation form. Students will provide evaluations of their peers to be taken into consideration by the professor, but the final evaluation will be the professor's subjective judgment.

Organization: Start with a brief summary of the introduction: Why was this research done? Why is it important? How does it fit into the general topic we are studying? Next summarize the methods, then the results, and the final conclusions from the work as stated in the discussion.

Methods: Present a detailed description of methods, with appropriate diagrams to illustrate techniques. Explain how the method works and what information it provides. Concentrate on the methods unique to your paper but mention other important methods used in the paper. Most of these are listed for each paper on the course outline, but don't limit yourself to that list if you believe other methods should also be discussed.

Results: Present and explain the most significant results from the paper (using figures from the paper). You don't need to cover all the figures if there are too many. Summarize the conclusions and explain how the results lead to those conclusions. Briefly summarize the major points in the discussion section of the paper. Don't critique the paper- leave that for class discussion.

Questions: Be prepared to answer questions about the subject of your presentation.

Visual Aids: Use PowerPoint slides, and/or document camera for hard copy, and/or chalkboard. I will provide a colour printout of the paper for you to use during your presentation if you want it. Supplemental movies will be available on a USB drive. **WARNING**: If you use PowerPoint, bring your presentation on a USB drive or a CD and come early to class to get it loaded onto the computer. If your presentation fails to run for technical reasons you will NOT be allowed to postpone it, so test it beforehand AND have a backup plan (email your presentation to yourself, or bring hard copy of your slides to show on the document camera in case the computer fails). I will edit presentations for accuracy and length and post them on Moodle after the presentation. If you use online slide presentation software, you MUST provide me with an EDITABLE copy to post on the course website.

Handouts: Handouts can sometimes be useful, but are not required. A handout could be a summary of the main points in the paper, a diagram or additional data/figures not in the paper. Please limit the length to one page. Do not bring copies of all of your slides to distribute.

Biology 4141- Fall 2013 Presentation Evaluation

Speaker: Date:
Paper:

Weight (%)		Mark (0-100)
50%	Did the presentation accurately summarize the important points from the paper, including background information from additional sources if necessary?	
25%	Was the material presented clearly and in a well-organized way?	
10%	Were the visual aids clear and readable and appropriate?	
5%	Was the level of explanation appropriate, not too difficult and not too simple?	
5%	Were the timing and pacing right, and was the speaker loud enough and understandable?	
5%	Did the speaker project enthusiasm and make the subject interesting?	

(Marking: 90-100 = A^+, 80-89 = A, 75-79 = B^+, 70-74 = B, 65-69 = C^+, 60-64 = C, 55-59 = D^+, 50-54 = D, 40-49 = E, 0-39 = F.)

Does the speaker have any mannerisms they should try to avoid?

Other comments:

Topics for Critical Discussion of Papers

We will not cover all these questions for every paper. The questions we will focus on are marked *. Read Gillen for further information about these questions.

Questions you should be able to answer about every paper:

1. Do the title and abstract accurately reflect the contents of the paper?

2. Does the introduction clearly state the question the authors are trying to answer and why it's important?

3. Are the methods described or referenced in sufficient detail to be able to reproduce the experiments?

4. Are the results presented in a clear and meaningful way? Are figures and tables appropriate and understandable? Are figure legends clear and complete?

*5. Was any evidence presented that the results are reproducible?

*6. Are the experiments causative studies or merely correlational?

7. Does the discussion adequately put the results in the context of other work?

Questions requiring more background or critical analysis:

8. Does the introduction provide adequate background and historical perspective? Have the authors referenced the work of others or do they cite only their own work?

*9. What are the strengths and limitations of the methods? What are the advantages/disadvantages of the choice of organism/cell type?

*10. What controls were run, and why? Were all the appropriate controls included?

11. Were the statistical tests appropriate for the data?

*12. Are the conclusions adequately supported by the data?

*13. Are the results physiologically relevant? (Do they tell us how real live cells behave?)

14. Have the authors discussed the limitations of the methods? Have the authors considered alternative explanations for the results?

15. What questions are left unanswered? What new questions have been raised? Have the authors suggested further experiments?

16. What does the paper tell us that is genuinely novel?

17. Do the authors have any conflicts of interest that might have influenced their work?

BIOL 4141 Fall 2012, Schedule

Methods: The listed methods are not the only methods in these papers. They are the methods that are important to the paper or are listed because the paper is the first time we will see those methods in this course. You should concentrate on the listed methods in your presentation but also mention the other important methods.

Sept 10, 12
Introduction, methods review

Golgi
Sept 17
Review:
Pfeffer, S. R. (2007) Unsolved mysteries in membrane traffic. Annu. Rev. Biochem. 76:629-645.
(concentrate on Introduction, Unsolved Mysteries, Hierarchy of Interactions, Transport through the Stack, and Vesicles Provide Important Clues.)

Cosson, P., Ravazzola, M., Varlamov, O., Söllner, T.H., Di Liberto, M., Volchuk, A., Rothman, J.E. Orci, L. (2005) Dynamic transport of SNARE proteins in the Golgi apparatus. Proc. Nat'l. Acad. Sci. USA 102:14647-14652.
Methods: CHO, NRK and HeLa cells, immunogold labeling for electron microscopy, quantification of EM images, transfection of cells, SNARE + protein A hybrid protein.

Sept 19
Malsam, J., Satoh, A., Pelletier, L., Warren, G. (2005) Golgin tethers define subpopulations of COPI vesicles. Science 307:1095-1098.
Methods: Purification of Golgi membranes from rat liver, in vitro vesicle formation and purification, tethering of vesicles to glass, immunoprecipitation, immunofluorescence microscopy of YFP-fusion proteins in live cells, microinjection, VSV-G transport assay, Sar-1^{DN} inhibition of ER export.

Sept 24
Losev, E., Reinke, C., Jellen, J., Strongin, D.E., Bevis, B.J., Glick, B.S. (2006) Golgi maturation visualized in living yeast. Nature 441:1002-1006.
Methods: yeast as model system, 4-D confocal microscopy, Golgi marker proteins with fluorescent tags, alpha-factor, radioactive pulse-chase

Sept 26
Ori-McKenney, K., Jan, L. and Jan, Y-N. (2012) Golgi outposts shape dendrite morphology by functioning as sites of acentrosomal microtubule nucleation in neurons. Neuron 76:921-930.
Methods: *Drosophila* neurons as model system, GAL4-UAS system for gene expression, EB1-GFP for microtubule dynamics, ManII-mCherry as a Golgi marker, purification of Golgi vesicles, immunostaining, inhibition of microtubule nucleation with a blocking antibody

Cell Size
Oct 1
Review:

Yang, X. & Xu, T. (2011) Molecular mechanism of size control in development and human diseases. Cell Research 21:715-729.

Schnittger, A., Weinl, C., Bouyer, D., Schöbinger, U., and Hülskamp, M. (2003) Misexpression of the cyclin-dependent kinase inhibitor ICK1/KRP1 in single-celled *Arabidopsis* trichomes reduces endoreduplication and cell size and induces cell death. Plant Cell 15: 303-315.

Methods: *Arabidopsis* as a model organism, trichomes as a model single-cell system, transgenic plants, double-mutant analysis for protein/protein interactions in vivo, DAPI for DNA content (focus on cell size, not cell death results)

Oct 3

Jorgensen, P., Nishikawa, J. L., Breitkreutz, B.-J. and Tyers, M. (2002) Systematic identification of pathways that couple cell growth and division in yeast. Science 297: 395-400.

Methods: *S. cerevisiae* as a model system, ORF deletion strains, synthetic genetic array, epistasis analysis, coulter counter for cell size assays, FACS analysis for DNA content, elutriation, alpha-factor resistance

Oct 8

Echave, P., Conlon, I. J., and Lloyd, A. C. (2007) Cell size regulation in mammalian cells. Cell Cycle 6: 218-224.

Methods: Rat Schwann cells and fibroblasts as model systems, immunopanning, cell culture and serum-free medium, cell volume by confocal sectioning and Coulter counter, BrDU for cell cycle timing, Phalloidin for actin staining, mathematical model

Oct 10: Review
Oct 15: Midterm

Apoptosis and Cancer
Oct 17
Review:

Meng, X. W., Le, S.-H. and Kaufmann, S. H. (2006) Apoptosis in the treatment of cancer: a promise kept? Current Opinion in Cell Biology 18: 668-676.

Conradt, B. and Horvitz, H. R. (1998) The *C. elegans* protein EGL-1 is required for programmed cell death and interacts with the Bcl-2-like protein CED-9. Cell 93: 519-529.

Methods: *C. elegans* as a model organism, nematode genetics, epistasis analysis for order of genes in a pathway, gene cloning strategy, in vitro protein binding assays, yeast 2-hybrid analysis

Oct 22

Deng, Y., Lin, Y. and Wu, X. (2002) TRAIL-induced apoptosis requires Bax-dependent mitochondrial release of Smac/DIABLO. Genes & Develop. 16: 33-45.

Methods: human colon cancer cells, overexpression and knockout cell lines, apoptosis assays, GFP fusion proteins, immunoprecipitation, subcellular fractionation, caspase inhibitors

Oct 24

Li, L., Thomas, R. M., Suzuki, H., De Brabander, J. K., Wang, X., Harran, P. G. (2004) A small molecule Smac mimic potentiates TRAIL- and TNFα-mediated cell death. Science 305: 1471-1474.

Methods: HeLa cells, human glioblastoma cells, synthetic chemistry, fluorescence polarization assay for molecular binding, GST-tagged proteins and biotin tags for protein-protein interaction assays, native PAGE for protein complex formation, assays for apoptosis and caspase activation

Oct 29

Petersen, S.L., Peyton, M., Minna, J.D., Wang, X. (2010) Overcoming cancer cell resistance to Smac mimetic induced apoptosis by modulating cIAP-2 expression. Proc. Nat'l. Acad. Sci. USA 107:11936-11941

Methods: cancer cell lines, DNA fingerprinting to identify cell lines, small molecule Smac mimetic, luminescent cell survival assay, siRNA knockdown, immunoprecipitation, chemical inhibitors of signaling pathways

Oct 31: No class (Co-curricular week)

Circadian Clocks

Nov 5

Review:

Panda, S., Hogenesch, J.B. & Kay, S.A. (2002) Circadian rhythms from flies to human. Nature 417:329-335.

Fan, Y., Hida, A., Anderson, D. A., Izumo, M. and Johnson, C. H. (2007) Cycling of CRYPTOCHROME proteins is not necessary for circadian-clock function in mammalian fibroblasts. Current Biology 17: 1091-100.

Methods: rat fibroblasts and human kidney cells, knockout mouse fibroblasts, transient transfection, cell-permeant proteins, His tag and protein purification, luciferase reporter genes and luciferase assay, Ca^{2+} assay with fura-2, period and phase calculations for rhythm assays

Nov 7

Harrisingh, M. C., Wu, Y., Lnenicka, G. A. and Nitabach, M. N. (2007) Intracellular Ca2+ regulates free-running clock oscillation in vivo. J. Neurosci. 27: 12489-12499.

Methods: *Drosophila* locomotor rhythms, GAL4/UAS and cell-specific drivers, Ca^{2+} imaging, Ca^{2+} buffer protein, calmodulin and CaMKII mutants, CaMKII inhibitor

Nov 8: Drop Deadline

Nov 12

O'Neill, J. S., Maywood, E. S., Chesham, J. E., Takahashi, J. S. and Hastings, M. H. (2008) cAMP-dependent signalling as a core component of the mammalian circadian pacemaker. Science 320: 949-953.

Methods: mouse brain slices in vitro, clock mutant mice, NIH3T3 cells, luciferase reporter for gene expression rhythms, cAMP assays, cAMP pharmacology (agonists and antagonists), running wheel assays for mouse activity rhythms

Nov 14

Zhang, E.E., Liu, A.C., Hirota, T., Miraglia, L.J., Welch, G., Pongsawakul, P.Y., Liu, X., Atwood, A., Huss III, J.W., Janes, J., Su, A.I., Hogenesch, J.B., Kay, S.A. (2009) A genome-wide RNAi screen for modifiers of the circadian clock in human cells. Cell, 139:199-210.

Methods: human osteosarcoma cell line, siRNA knockdowns, luciferase reporter genes, robotic high-throughput system, Q-PCR, period and amplitude measurements, protein interaction database, small molecule inhibitors of insulin signaling pathway

Stem Cells
Nov 19
Reviews:
1) Power, C., Rasko, J.E.J. (2011) Will cell reprogramming resolve the embryonic stem cell controversy? A narrative review. Annals of Internal Medicine 155:114-121.
2) Check Hayden, E. (2011) Stem cells: The growing pains of pluripotency. Nature 473: 272-274.
3) Mayhall, E.A., Paffett-Lugassy, N. and Zon, L.I. (2004) The clinical potential of stem cells. Current Opinion in Cell Biology 16:713-720. [Optional review with more background on stem cells]

Takahashi, K. and Yamanaka, S. (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126: 663-676.

Methods: isolation of mouse embryonic and adult fibroblasts, embryonic cell culture on feeder cells, retroviral transduction, selection for pluripotency using Fbx15 gene, chromatin immunoprecipitation assays, karyotyping, genetic fingerprinting, promoter methylation assay by bisulfite sequencing, teratomas and embryoid bodies, chimeric mice, other assays for pluripotency

Nov 21
Polo, J.M., Liu, S., Figueroa, M.E., Kulalert, W., Eminli, S., Tan, K.Y., Apostolou, E., Stadtfeld, M., Li, Y., Shioda, T., Natesan, S., Wagers, A.J., Melnick, A., Evans, T., Hochedlinger, K. (2010) Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. Nature Biotechnology 28: 848-855.

Methods: inducible lentivirus, reprogrammable mice, iPSC production, assays for pluripotency, qPCR and microarrays, assays for epigenetic marks (promoter methylation, histone acetylation and methylation), embryoid formation, hematopoeitic differentiation assay

Nov 26
Brennand, K.J., Simone, A., Jou, J., Gelboin-Burkhart, C., Tran, N., Sangar, S., Li, Y., Mu, Y., Chen, G., Yu, D., McCarthy, S., Sebat, J., Gage, F.H. (2011) Modelling schizophrenia using human induced pluripotent stem cells. Nature 473: 221-225.

Methods: production and characterization of human iPSCs, neuronal differentiation, rabies virus tracing, neurite and synaptic counting, electrophysiology, calcium dye for spontaneous transients, antipsychotic drugs

Nov 28
Tachibana, M. et al. (2013) Human embryonic stem cells derived by somatic cell nuclear transfer. Cell 153: 1228–1238.

Methods: human oocyte donation, cell synchronization, enucleation and nuclear transfer, embryo culture, microsatellite genotyping, karyotyping, mtDNA genotyping, cardiac differentiation, teratoma assay, ARMS-qPCR assay for mtDNA

(For discussion) Cyranoski, D. (2013) Fallout from hailed cloning paper. Nature 497: 543-544.

Dec 3: no class

Dec 5: Review

Tips on Presentations

- Make an outline first of the points you want to make and the pictures/graphics you will need to illustrate those points. Try to have an illustration for every important point. A picture is worth a thousand words.
- Start with the title of your presentation and your name.
- Practice and time your presentation. You should try for 25 min, no less than 20 and no more than 30. You will lose points if it's too short. You might get cut off if you run on too long.
- Speak up and make eye contact with the audience. Don't talk to the computer or screen.
- Don't read a script. Use notes if you need them but put only a few words on the notes to remind you what to say.
- Acknowledge your sources. Give credit for pictures you download from a website or copy from a published source by putting the reference at the bottom of the slide. Add a final references slide for your sources of information.

Tips on visual aids and PowerPoint

- Choose a simple design with high contrast between text and background. Black text on a pale background or white or yellow text on dark blue work well.
- Use large font sizes, at least 24 point. Use 28 or 32 for text and 36 to 44 for titles.
- Choose a clean, standard font like Arial or Times New Roman. Do not use many different fonts, or unusual fonts that might not be found in all standard computer installations.
- Don't put too much on one slide. Slides don't cost anything so you don't have to be frugal with them. One idea per slide is a good rule.
- Don't write whole sentences and then read them out word-for-word. Use a few words or a phrase to communicate the key words and ideas.
- Don't use fancy transitions and moving text. It is not helpful, just distracting. There are rare times when a simple animation can help to make a point, but usually it is a waste of your time and the audience's patience.
- Put a black slide after your last slide so you don't drop out of the presentation if you advance after the last slide. Add some extra slides after your last slide with information you might use to answer questions from the audience.
- Check your presentation on a computer similar to the one you will be presenting on. (This is a warning to Mac users like me: Sometimes Windows versions of PowerPoint don't display your slides the way you see them on your Mac. Movies are a particular problem.)

Lecture Outline: General Methods

A. Cell culture
    1. Primary cultures vs. cell lines
    2. Growth media
B. Microscopy
    1. Light Microscopy
    2. Electron microscopy
        a. Transmission EM (TEM)
            Thin section, Shadowing, Freeze-fracture
        b. Scanning EM (SEM)
C. Fluorescence
    1. Fluorescence microscopy
    2. Confocal microscopy
    3. Fluorescence-activated cell sorting (FACS) (flow cytometry)
D. Antibodies
    1. Types
        Polyclonal vs. Monoclonal
    2. Uses of antibodies
        a. Unconjugated
            Immunoprecipitation, Co-immunoprecipitation
        b. Conjugated
            Immunofluorescence, Immunogold, Enzyme-linked
        c. Secondary antibodies
E. Molecular methods
    1. DNA cloning
    2. Polymerase chain reaction (PCR)
    3. Blotting techniques
        a. Southern (DNA blot)
        b. Northern (RNA blot)
        c. Western (Protein blot, Immunoblot)
    4. Microarrays
    5. *in situ* methods
        a. *in situ* hybridization
        b. Immunohistochemistry (Immunolocalization)
    6. Transgenic methods
        a. Transfection methods
            Biochemical, Physical, Viral
        b. Transfection results
            Transient vs. Stable
        b. Gene manipulation
            i. *in vitro* mutagenesis
            ii. Ectopic promoters
            iii. Reporter genes
                LacZ, GFP, luciferase
            iv. Epitope tagging