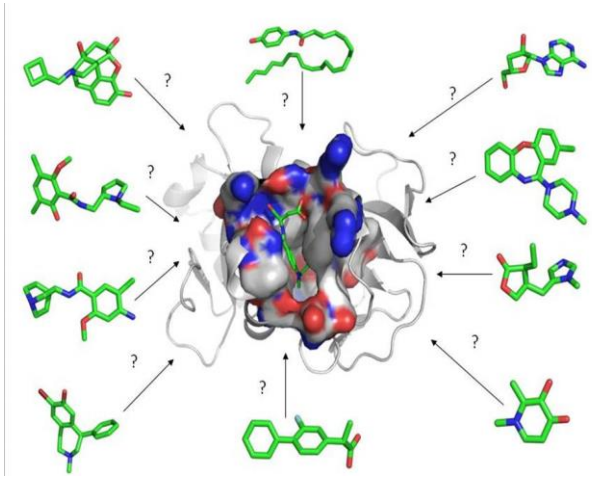
Virtual Drug Screening (VDS) Stream

5th Class – Fall 13



Freshman Research Initiative (FRI)

Dr. Jon Robertus - PI (principal investigator)

Dr. Josh Beckham – RE (research educator)

Outline

Early Class

- Dr. B slides
- Journal Club
  - none
- Research Presentations
  - Jacky & Kevin
- Technique Presentations
  - Priya
  - Katherine V.
- Dr. B slides

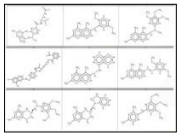
Late Class

- Dr. B slides
- Journal Club
  - none
- Research Presentations
  - Grace & Young
- Technique Presentations
  - Kavya
  - Grace
- Dr. B slides

Your VDS project in a nutshell

Virtual Screening

Library 1 | **Library 2** | **Library 3…**



Run 1 | **Run 1** | **Run 1**

Run 2 | **Run 2** | **Run 2**

Combined Run

Filter for Lipinski's Rule

Obtain top ligands

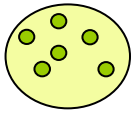
Test inhibitors against enzyme

Wet Lab

Amplify target gene With Overlap PCR

DH5α

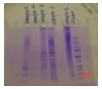
Clone into expression vector



Transform in BL21(DE3) Expression Cells

BL21(DE3)

Express protein with IPTG



Purify protein and characterize protein

Test in enzyme assay

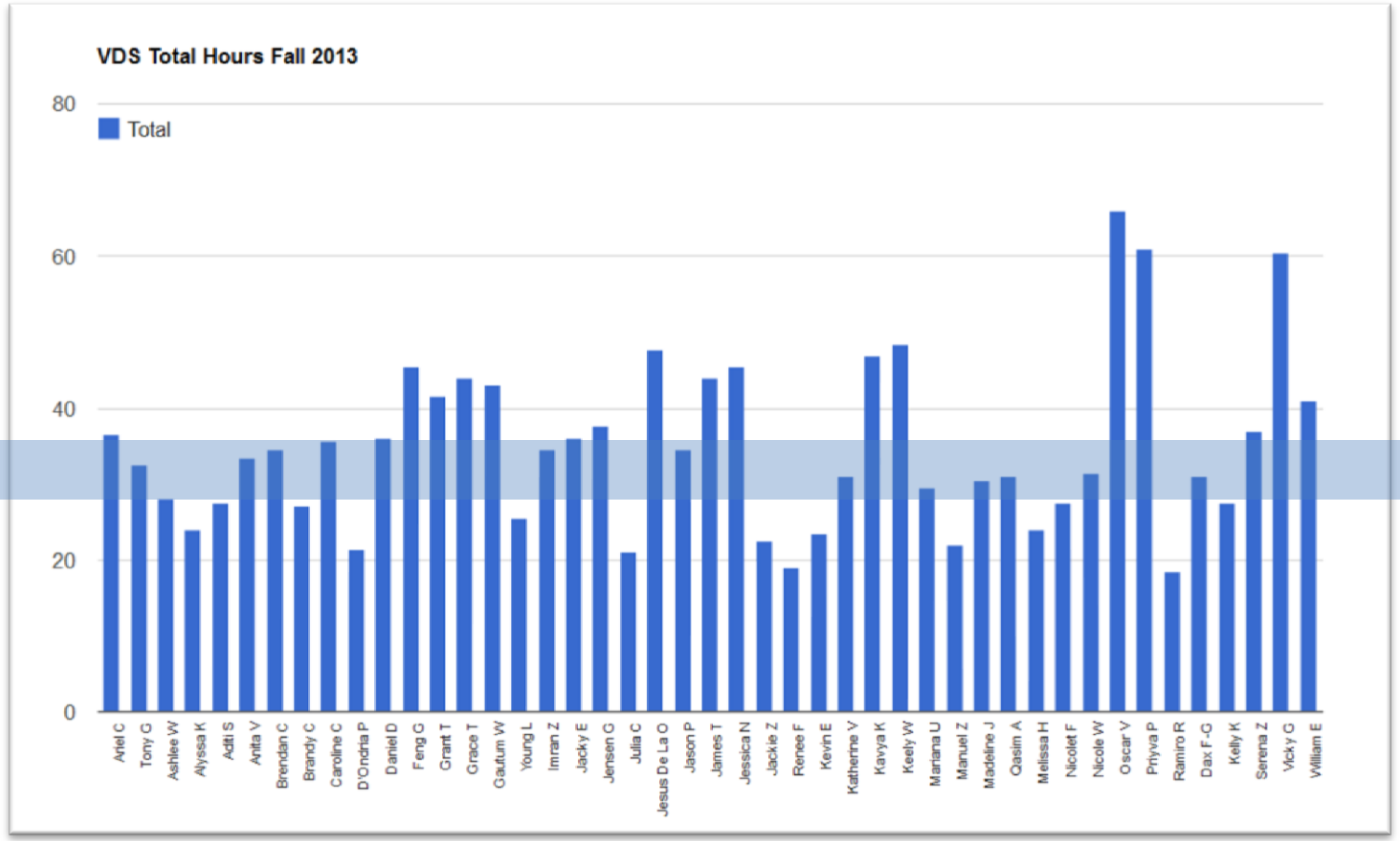
Timeline

- Veterans
  - Cloning should be done…… late Sept
  - Protein expression by …….Early October
  - Enzyme assays….Late Oct.
- Springers
  - Cloning should be done…… Early October
  - Protein expression by ….Late Oct.
  - Enzyme assays….Early Nov

Hours are nice, but progress is key

You are ultimately responsible for your progress…
not sure what to do next? – ask a mentor or Dr. B

- 4th Week = 3.5 weeks = at least 28 hrs - 35 hrs

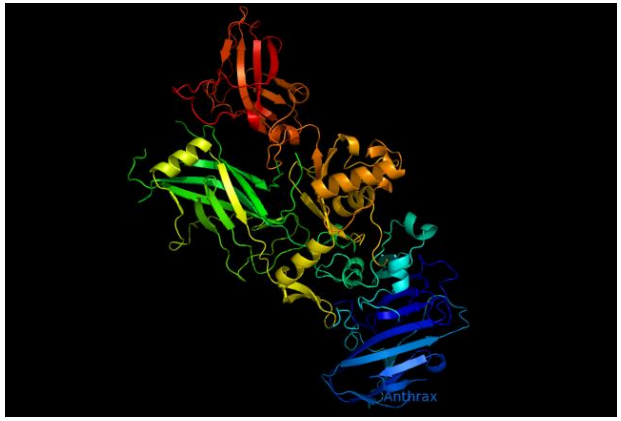


Research Report on Target

- Due: **Sunday Oct. 6th**

- Guidelines for Report will be posted to Google Docs
  - Background on Disease and Target
  - What methods you propose to use to find inhibitors

- You will use part of this as the Intro section of your final report



Research Report on Target

- **A lot of details can be recycled from your Target page and converted into a report format!**

Format for Individual Target pages (copy this list to new Target page and then fill in for your target):

*Target (protein/gene name):
*NCBI Gene # or RefSeq#:
*Protein ID (NP or XP #) or Wolbachia#:
*Organism (including strain):
Etiologic Risk Group (see link below):
*Background/Disease Information (sort of like the Intro to your Mini Research Write up):
Link to TDR Targets page (if present):
Link to Gene Database page (NCBI, EuPath databases -e.g. TryTryp, PlasmoDB, etc - or PATRIC, etc.)
Essentiality of this protein:
Complex of proteins?:
Druggable Target (list number or cite evidence from a paper/database showing druggable in another organism):

*EC#:
Link to BRENDA EC# page:
-- Show screenshot of BRENDA enzyme mechanism schematic
Enzyme Assay information (spectrophotometric, coupled assay ?, reagents):
-- link to Sigma (or other company) page for assay (see Sigma links below)
-- -or link (or citation) to paper that contains assay information
-- links to assay reagents (substrates) pages.
--- List cost and quantity of substrate reagents, supplier, and catalog #

Structure Available (PDB or Homology model)
-- PDB # or closest PDB entry if using homology model:
-- For Homology Model option:
---- Show pairwise alignment of your BLASTP search in NCBI against the PDB
---- Query Coverage:
---- Max % Identities:
---- % Positives
---- Chain used for homology:

Current Inhibitors:
Expression Information (has it been expressed in bacterial cells):

Research Report on Target

- There will not be a **Results** section
  - because you don't have any yet!

- Cite at least 3 papers
  - one review paper and one experimental
  - ACS (American Chemical Society) bibliography format
  - PubMed, Web of Science
    - see Spring semester Lit Search Assignment

Research Report on Target

- Resources

  PDB

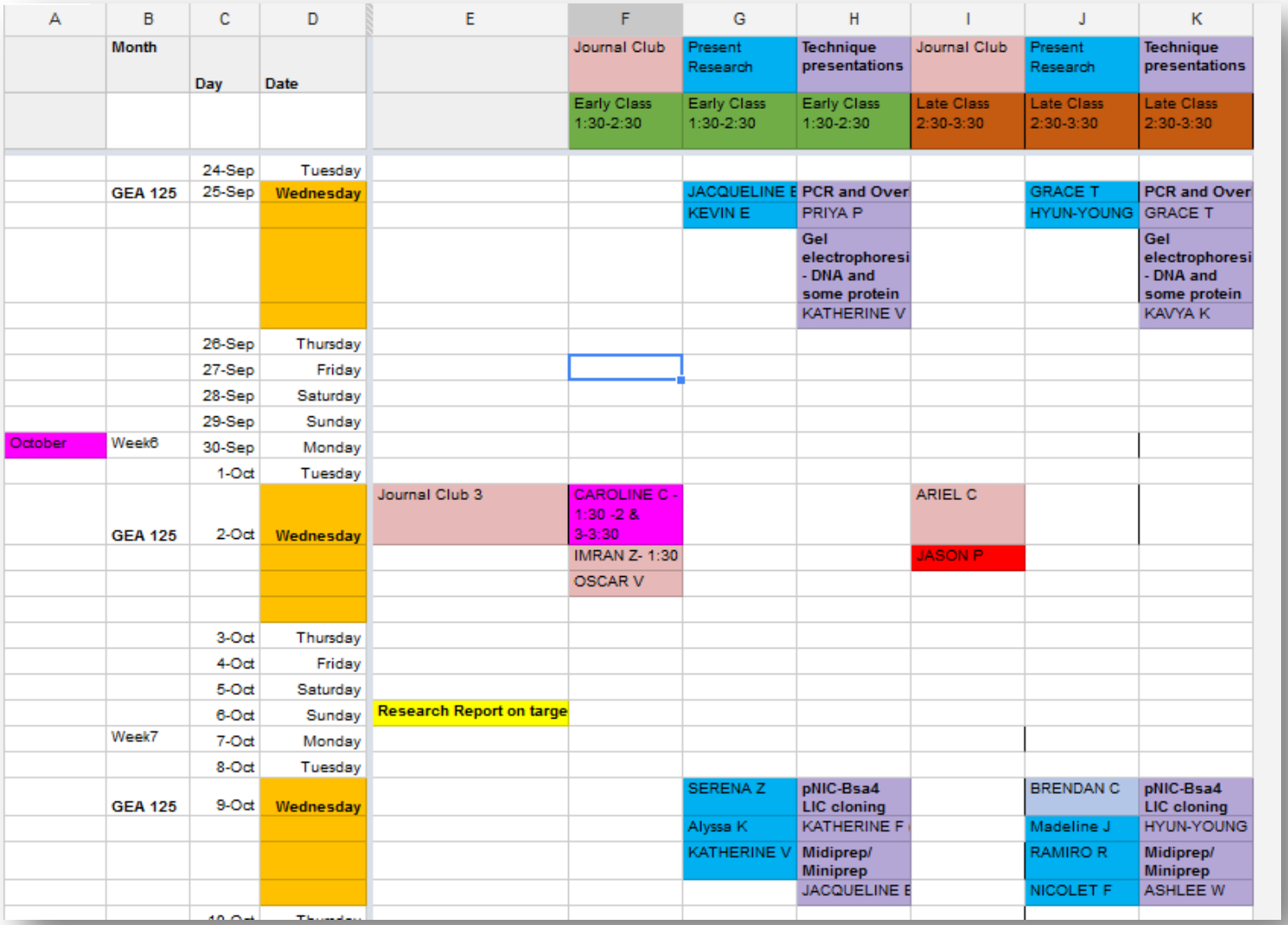
  BRENDA (http://www.brenda-enzymes.org/)

  EXPASY (http://ca.expasy.org/tools/protparam.html)

  A few other on Wiki Site – at bottom of Target Page

  you can add to the list if you find some good ones you want to share

Schedule of Presentations



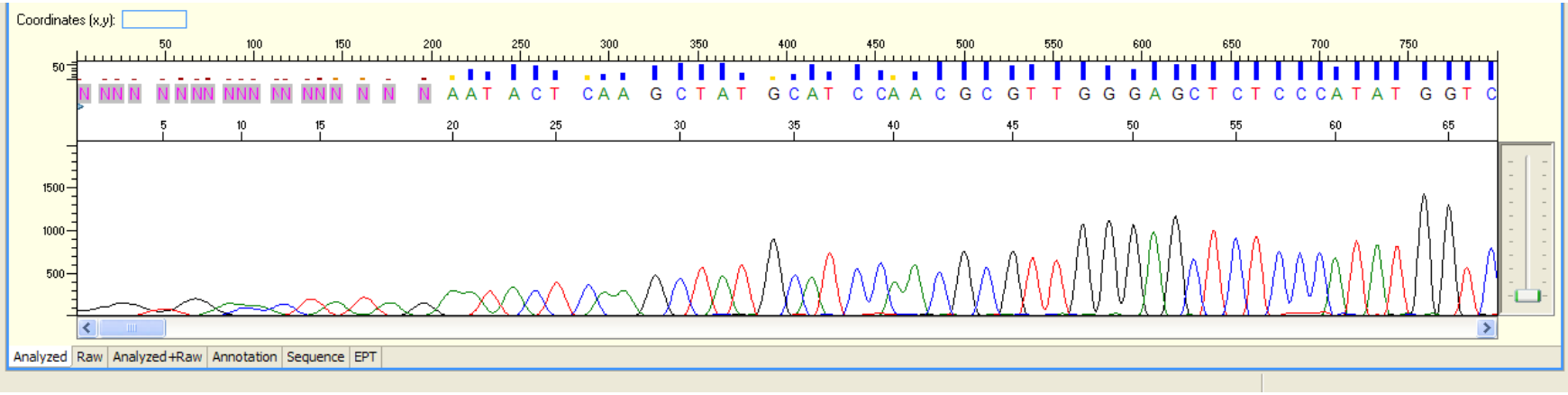
	Month	Day	Date		Journal Club	Present Research	Technique presentations	Journal Club	Present Research	Technique presentations
					Early Class 1:30-2:30	Early Class 1:30-2:30	Early Class 1:30-2:30	Late Class 2:30-3:30	Late Class 2:30-3:30	Late Class 2:30-3:30
		24-Sep	Tuesday							
	GEA 125	25-Sep	Wednesday			JACQUELINE E	PCR and Over		GRACE T	PCR and Over
						KEVIN E	PRIYA P		HYUN-YOUNG	GRACE T
							Gel electrophoresi - DNA and some protein			Gel electrophoresi - DNA and some protein
							KATHERINE V			KAVYA K
		26-Sep	Thursday							
		27-Sep	Friday							
		28-Sep	Saturday							
		29-Sep	Sunday							
October	Week6	30-Sep	Monday							
		1-Oct	Tuesday							
	GEA 125	2-Oct	Wednesday	Journal Club 3	CAROLINE C - 1:30 -2 & 3-3:30			ARIEL C		
					IMRAN Z- 1:30			JASON P		
					OSCAR V					
		3-Oct	Thursday							
		4-Oct	Friday							
		5-Oct	Saturday							
		6-Oct	Sunday	Research Report on targe						
	Week7	7-Oct	Monday							
		8-Oct	Tuesday							
	GEA 125	9-Oct	Wednesday			SERENA Z	pNIC-Bsa4 LIC cloning		BRENDAN C	pNIC-Bsa4 LIC cloning
						Alyssa K	KATHERINE F		Madeline J	HYUN-YOUNG
						KATHERINE V	Midiprep/ Miniprep		RAMIRO R	Midiprep/ Miniprep
							JACQUELINE E		NICOLET F	ASHLEE W

Research Presentations

Technical Presentations

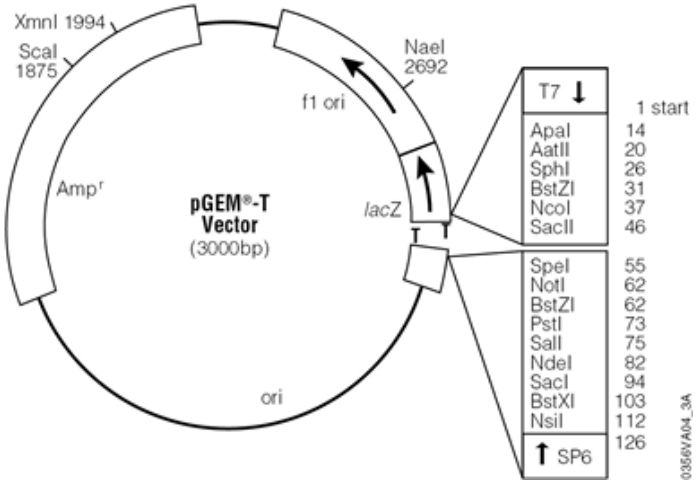
DNA Sequencing

- What is this called?
  - Electropherogram

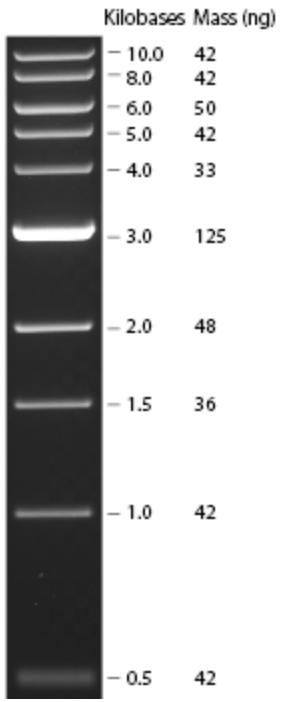


Our test plasmid: pGEM-gbr22

- pGem T vector backbone
- Size is 3,708 bp

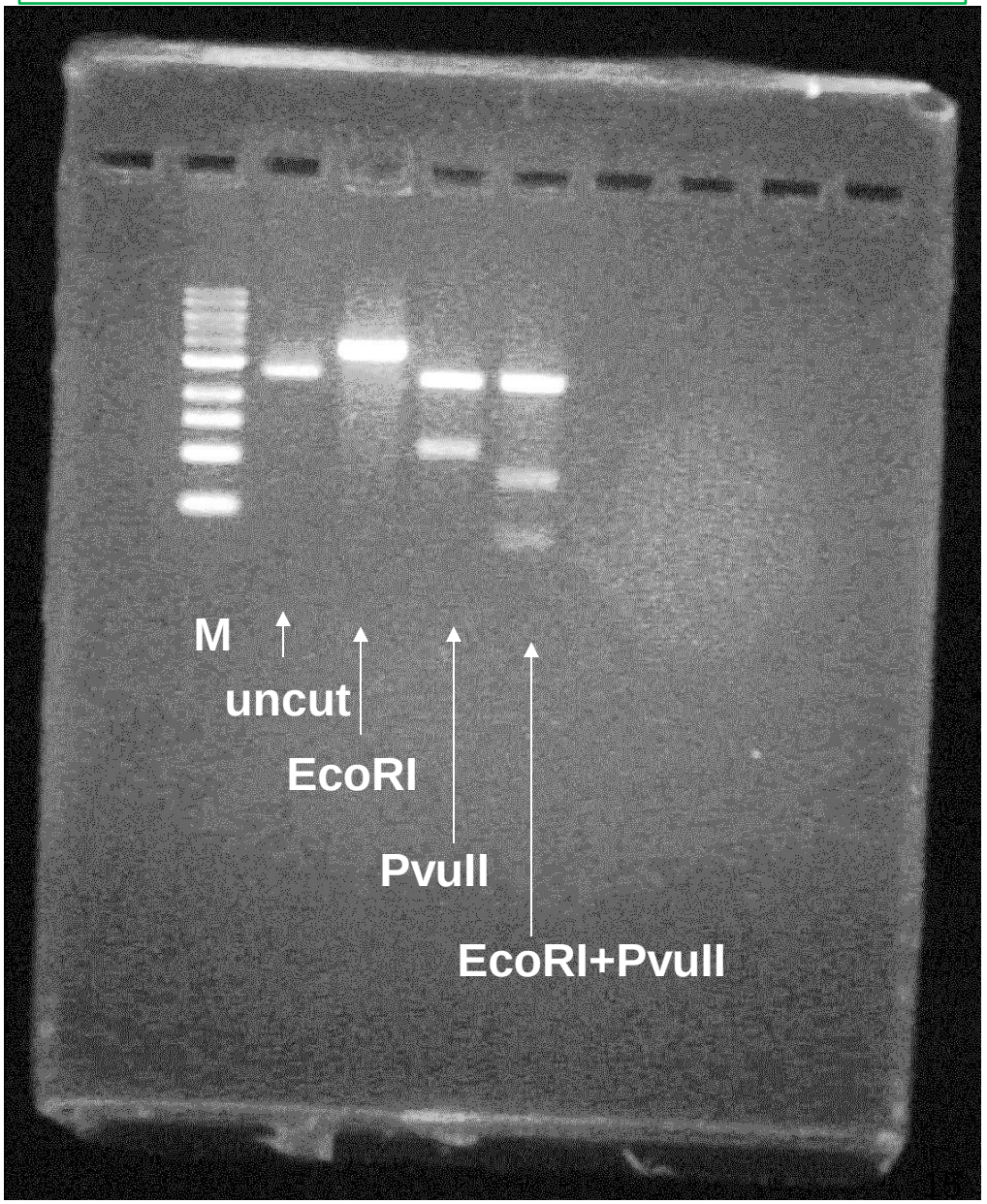


pGBR22 gel w/ EtBr



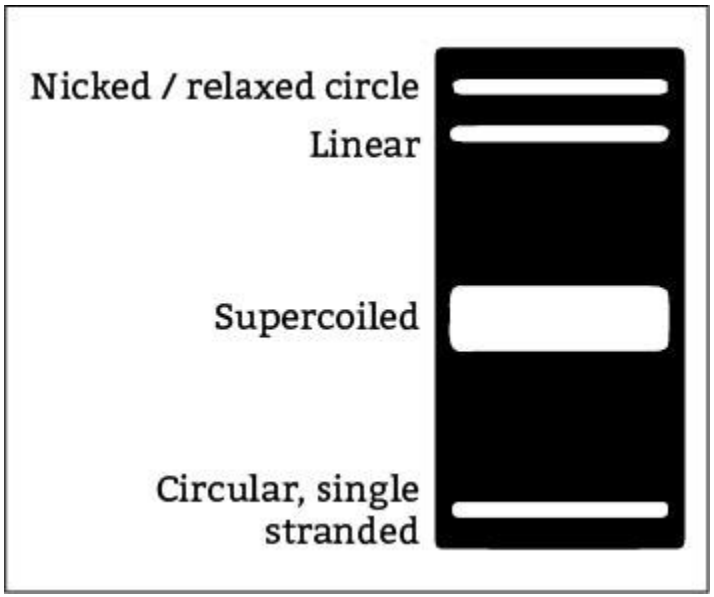
#	Ends	Coordinates	Length (bp)
1	PvuII-PvuII	111-2674	2564
2	PvuII-EcoRI	2675-3488	814
3	EcoRI-PvuII	3489-110	332

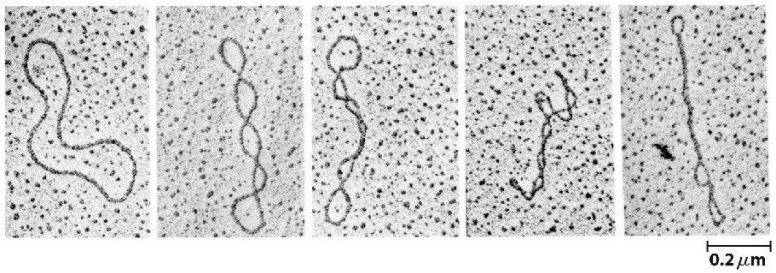
Why are the lower bands less bright?



Why is Uncut plasmid higher than Cut plasmid?

Relative Positions of Different DNA Forms of a Plasmid on a Tris-Acetate Agarose Gel



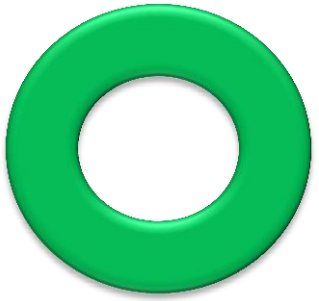


Circular ⟶ **Supercoiled**

Cloning

- Insert our gene of interest (G.O.I.) into an plasmid so that we can make more copies

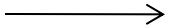
- How do you get the ‘gene’ though
  - We will piece it together (polymerize - PCR)
  - Vs. cutting it out of something else

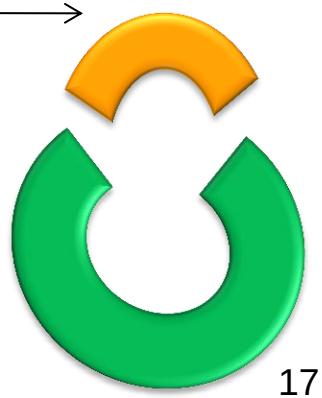


Plasmid ‘vector’

Open plasmid

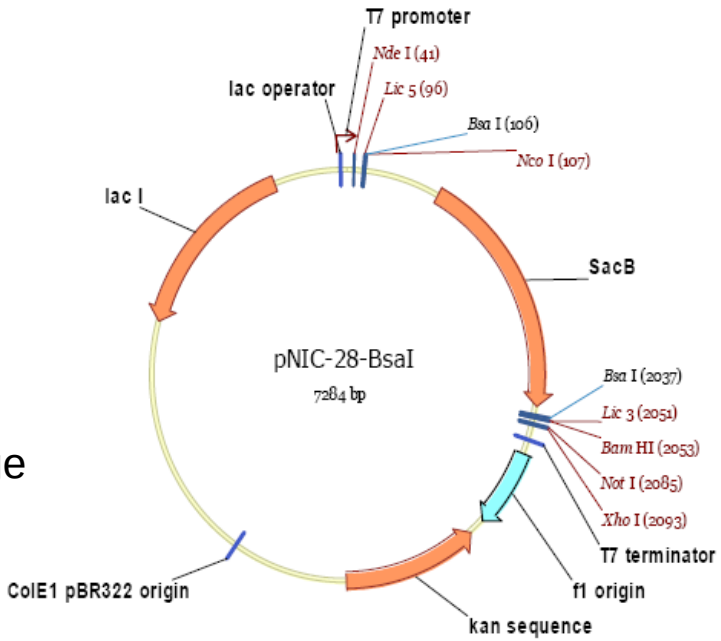
Gene





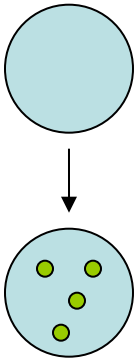
Vector from SGC (Structural Genomics Consortium)

- Expression vector
  - Make protein
- Need lots
- LB agar plates
  - transformation
- LB media
  - To grow up large batch



Transformation of expression vectors

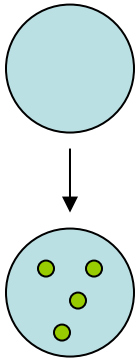
pNIC-Bsa4 into DH5alpha



KANAMYCIN plates

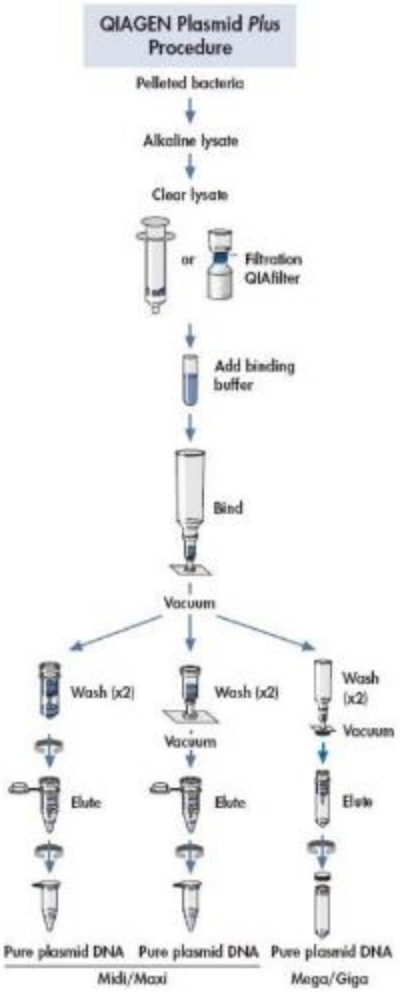
For DNA MidiPrep

Your Gene in pNIC-Bsa4 into **BL21(DE3)**



KANAMYCIN + SUCROSE plates

For Protein Expression



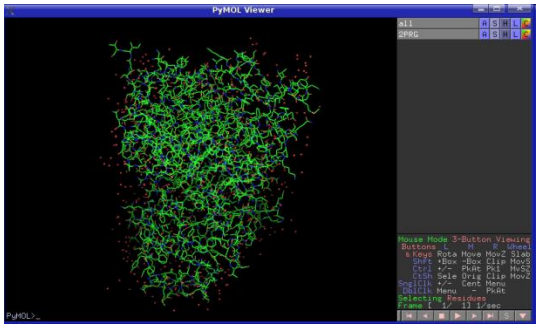
Midi- Prep

- Extract plasmids DNA from bacterial cells
  - Get rid of lipids, proteins, RNA, and other junk

NOTE:
post your Nanodrop concentrations of pNIC-Bsa4 from MidiPrep on the Google Docs spreadsheet
- in /Misc/PlasmidConcentrations.

QIAGEN®

Pymol Refresher



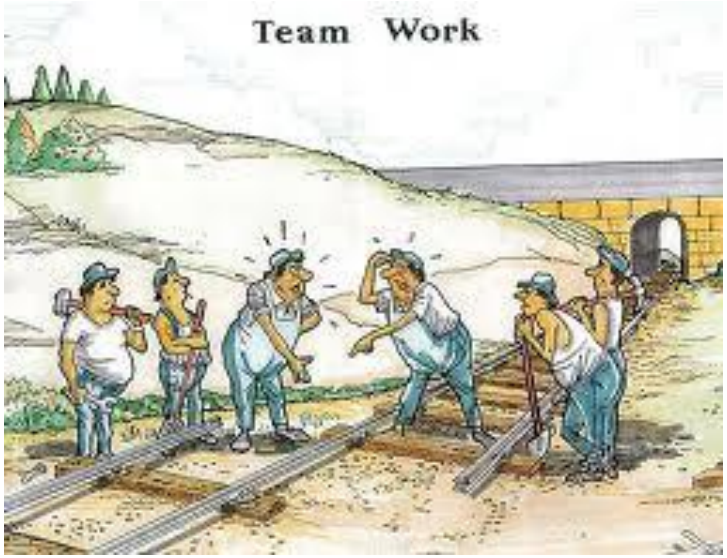
- Purpose: to get reacquainted with PyMol
- Target: *T cruzi* dihydrofolate reductase-thymidilate synthase (tcDHFR-TS)
- Due next week: **Saturday Sept. 28th**
  - for Returning Springers & Sidestreamers
  - Counts as 2 hrs of lab work

Virtual Screening

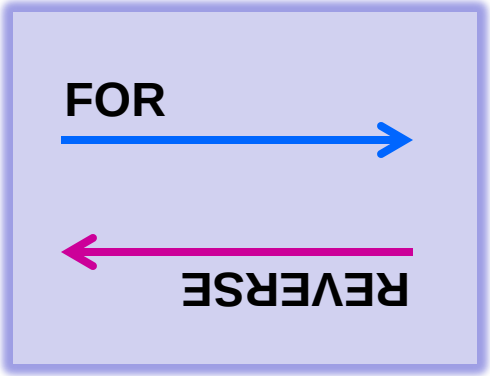
- Veterans
  - Homology models
  - Set up protein receptor in GOLD
  - Control Ligand docking run
- Protocol to be posted
- Help Sessions on Thursday and Friday

Working in Teams

- Some things can be done as teams
- But, if your ‘team’ is slowing you down – then move ahead on your own.



To Do list:



- Returning Spring and New VDSers
  - Primary (1^{o}) and Secondary (2^{o}) PCR
  - PyMol Refresher
  - Make LB media
  - Make LB plates
  - Transformation, grow up pNIC-Bsa4, cut it, etc.
- Veterans
  - Finish cloning of your gene into pNIC-Bsa4
  - Set up virtual screening and do Control Docking run

Lab Safety training

- Due: ASAP printout your results, put in binder
- Online training classes (TxClass)
  - CW 512 – recombinant DNA for non-exempt projects

- In person classes
  - FF 205 – Fire Extinguishing Training
  - 'Site Specific' Hazard Communication (OH 102)
    - Must repeat this semester
    - Show up at times with Aptamer mentor
      - ~~**Wed 9/18 4-5 pm**~~
      - ~~**Mon 9/23** from **3-4pm**~~
      - **Wed 9/25** from **1-2pm**
      - **Maybe more to be added….**

Journal Club – for next week

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Identification of new classes of ricin toxin inhibitors by virtual screening

Yan Bai [a], Beth Watt [a], Paul G. Wahome [b], Nicholas J. Mantis [b], Jon D. Robertus [a,*]

[a] Institute of Cellular and Molecular Biology, Department of Chemistry and Biochemistry, 1 University Station A5300, University of Texas, Austin, TX 78712, USA
[b] The Wadsworth Center, Division of Infectious Diseases, New York State Department of Health, Albany, NY 12208, USA

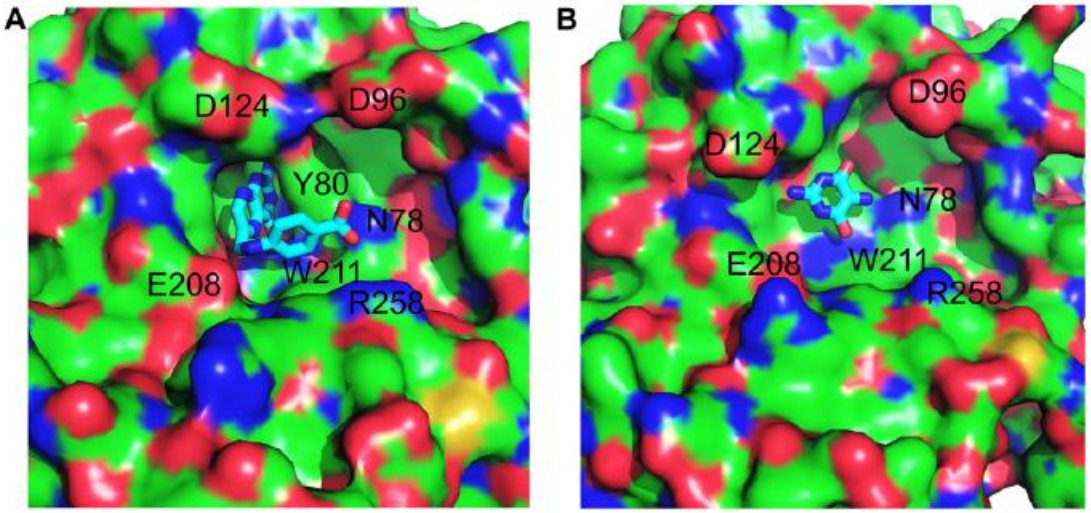
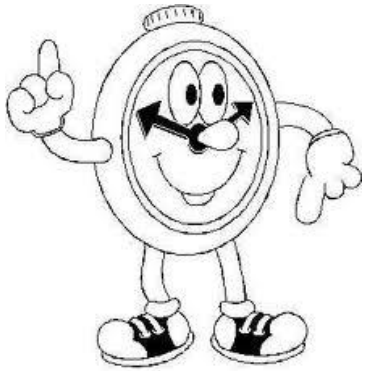


Fig. 1. Solvent-accessible surface structure of RTA. On the surface, oxygens are red, nitrogens blue, and carbons green. A. RTA in the open form with the inhibitor PTA (PDB:1BR6) occupying the adenine specificity pocket, and shown as a stick molecule. B. RTA in the closed form with the inhibitor 2,5-diamino-4,6-dihydroxypyrimidine (PDB:1IL5) shown in stick bonds. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

END



- Hours
  - Average 8 – 10 hrs of lab time
  - Makeups must be done the week after or week prior
  - Can’t cram everything into a few weeks!
  - All virtual work should also be in your lab notebook
    - E.g. PyMol refresher (can be small)