BCH714: Computational Systems Biology Minicourse 2024:

The Program:

28/03 Thursday	**08/04 Monday**	**09/04 Tuesday**
09:00 Peer grading of Assessment 1 JMR & DvN **11:00** Research lect. DvN **14:00** Introduction to Practical	**9:00** Group 1 (First set of experiments) Group 2 (work on seminar) **14:00** Group 2 (First set of experiments) Group 1 (work on seminar)	**9:00** Group 2 (Second set of experiments Group 2 (work on seminar) **13:30** Group 1 (Second set of experiments) Group 1 (work on seminar)
10/04 Wednesday	**11/04 Thursday**	**12/04 Friday**
9:00 Data analysis JMR **11:00** Redo experiments DvN	**09:00** Research lect. JMR **11:00** Data analysis JMR Redo experiments DvN	**09:00** Data analysis (whole day) JMR
15/04 Monday	**16/04 Tuesday**	
10:30 Data analysis (whole day) JMR	**09:30** Research lect. JLS Finish final report **Hand in final report (16/04/2024) at 23:59**	

GROUPS:

1A
- James Clarke
- Sophie de Villiers
- Elsa Pretorius

1B
- Courtney Campbell
- Amy Josephus
- Amogelang Mataboge

1C
- Haley Lewendal
- Suleiman Sungay
- Dom Beaumont

1D
- Brett Arnolds
- Faith Lekhu

2A
- Theo Willemse
- Katinka Louw
- Cayley Sampson

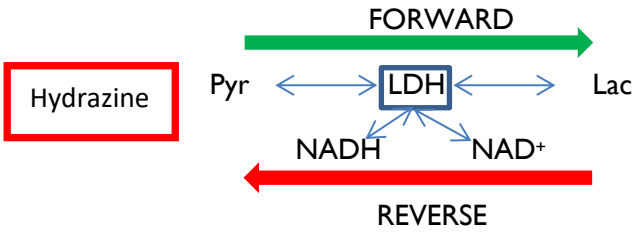
2B
- Conli Titus
- Cameron Bunch
- Marenique Smit

2C
- Le Roux Strydom
- Chrisilie Henning

2D
- Elna Kruger
- Kurauwone Marongwe

ABOUT THE PRAC:

(1) Characterise the enzyme:



(2) The experiments:

Experiment 1: Rate as a function of substrate in the presence of low F16BP [0.5 uM]

GROUP A
- Reverse direction
- Varying [NAD^+]
- Same [Lac] + [F16BP] + [CFE] + [Hydrazine]

GROUP B
- Reverse direction
- Varying [Lac]
- Same [NAD^+] + [F16BP] + [CFE] + [Hydrazine]

GROUP C
- Forward direction
- Varying [NADH]
- Same [Pyr] + [F16BP] + [CFE]

GROUP D
- Forward direction
- Varying [Pyr]
- Same [NADH] + [F16BP] +[CFE]

Experiment 2: Rate as a function of substrate in the presence of high F16BP [10 mM]

GROUP A
- Reverse direction
- Varying [NAD^+]
- Same [Lac] + [F16BP] + [CFE] + [Hydrazine]

GROUP B
- Reverse direction
- Varying [Lac]
- Same [NAD^+] + [F16BP] + [CFE] + [Hydrazine]

GROUP C
- Forward direction
- Varying [NADH]
- Same [Pyr] + [CFE] + [F16BP]

GROUP D
- Forward direction
- Varying [Pyr]
- Same [NADH] + [CFE] + [F16BP]

(3) An enzyme assay:

a- Pipette metabolites into the cuvettes, the reaction is initiated by the addition of the cell free extract (cfe), (which contains the enzyme of interest: LDH).

b- Once the cell extract is added, measure the absorbance readings for the reaction over a period of 3mins.

c- The types of data we expect to see- Forward reaction: decreasing Abs; Reverse reaction: increasing Abs

(4) What needs to be done:

Create a pipetting schema so that we investigate 10 different concentrations around the Km values. Take the following into consideration:

Km		Stock concentrations	
Km(NADH)	= 0.08mM	[NADH]	= 5mM
Km(NAD^+)	= 2.4mM	[NAD]	= 100mM
Km(Pyr)	= 1.5mM	[Pyr]	= 50mM
Km(Lac)	= 100mM	[Lac]	= 5M
		[F16BP]	= 0.1 mM, 100mM

b - Volumes:

- The total cuvette volume is 1 ml.
- If you are doing reverse direction experiments (only) 25 µl of the 1 ml is the Hydrazine.
- 100 uL of the total volume is cfe.
- The other volumes are of the substrate and co-factor which you calculated in the pipetting schema.
- Lastly, the remaining volume to make up this 1 ml volume is done by adding buffer to the cocktail mixture.

c - The ten substrate concentrations:

- The range you are going to investigate is between Km/10 and 10Km and the fixed substrates are usually at saturating condition i.e. 10 x Km. Use Excel to create an incremental pipetting schema.