BCH714: Computational Systems Biology Minicourse 2025:

The Program:

28/03 Friday	**07/04 Monday**	**08/04 Tuesday**
09:30 Research lecture DvN **11:00** Introduction to Practical JLS & KS	**9:00** Group 1 (First set of experiments) **13:00** Group 2 (First set of experiments) Group 1 (Data analysis) JLS & DvN	**9:00** Group 1 (Second set of experiments) Group 2 (Data analysis) JLS & DvN **13:00** Group 1 and 2 (Data analysis) JLS
9/04 Wednesday	**10/04 Thursday**	**11/04 Friday**
9:00 Group 2 (Second set of experiments Group 1 (data analysis) JLS & DvN **13:00** Data analysis / Redo experiments JLS & DvN	**09:30** Research lecture JLS **11:00** Data analysis / Redo experiments JLS & DvN	**09:30** Data analysis (whole day) / Redo experiments DvN
14/04 Monday	**15/04 Tuesday**	
9:30 Data analysis (whole day) DvN	**Hand in final report (15/04/2025) at 23:59**	

GROUPS:

1A
- Alexander Peters
- Chloe de Fondaumiere

1B
- Jahno Bester
- Skylah Esterhuizen

1C
- Ruan Heyns
- Luan Swart

1D
- Milla van der Merwe
- Zainab Osman

2A
- Cameron Crawford
- Noah Bauer

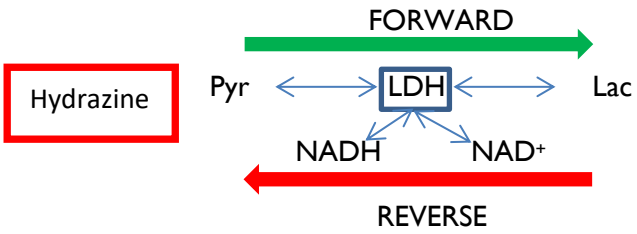
2B
- Likhona Mbuzi
- Amaya Lakmeeharan

2C
- Nazier Dodgen
- JC Shefferlie

2D
- Oliver Faure
- Hannah Catto
- Jano Bezuidenhout

ABOUT THE PRAC:

(1) Characterise the enzyme:



(2) The experiments:

Experiment 1:

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

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

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

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

Experiment 2:

GROUP A
- Product Inhibition: NADH
- Varying [NADH]
- Same [Lac] +[NAD^+] + [LDH] + [Hydrazine]

GROUP B
- Without Hydrazine
- Varying [Lac]
- Same [NAD^+] + [LDH]

GROUP C
- Product Inhibition: NAD^+
- Varying [NAD^+]
- Same [Pyr] + [NADH] + [LDH]

GROUP D
- Product Inhibition: Lac
- Varying [Lac]
- Same [NADH] + [Pyr] + [LDH]

(3) An enzyme assay:

a- Pipette metabolites into the cuvettes, the reaction is initiated by the addition of the enzyme, LDH.

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

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)	= 0.03 mM	[NADH]	= 1 mM
Km(NAD^+)	= 0.5 mM	[NAD]	= 20 mM
Km(Pyr)	= 1 mM	[Pyr]	= 20 mM
Km(Lac)	= 10 mM	[Lac]	= 500 mM

b - Volumes:

- The total cuvette volume is 1 mL.
- If you are doing reverse direction experiments (only) 100 µL of the 1 ml is Hydrazine (Stock is 25%, final concentration should be 2.5%).
- 50 µL for the forward direction and 100 µL for the reverse direction, of the total volume is LDH (Stock is 10 U/mL, final concentration in cuvette for the forward direction is 0.5 U/mL and reverse direction 1 U/mL.
- 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 10 x Km and the fixed substrates are usually at saturating conditions i.e. 10 x Km (unless instructed otherwise). Use Excel to create an incremental pipetting schema.