

BCH714: Computational Systems Biology Minicourse 2026:

The Program:

04/03 Wednesday 09:30 Peer grading of Assessment 1 JR & DvN 14:00 Introduction to Practical DvN & KS	05/03 Thursday 09:30 Research lecture JR 11:00 Group 1 (First set of experiments) DvN	06/03 Friday 9:00 Group 2 (First set of experiments) DvN Group 1 (Data analysis) JR 14:00 Group 1 (Second set of experiments) DvN Group 2 (Data analysis) JR
09/03 Monday 9:00 Group 2 (Second set of experiments) DvN Group 1 (data analysis) JR 13:00 Data analysis / Redo experiments JR & DvN	10/03 Tuesday 09:00 Data analysis / Redo experiments JR & DvN 14:00 Data analysis / Redo experiments JR & DvN	11/03 Wednesday 09:00 Data analysis / Redo experiments JR & DvN 14:00 Data analysis / Redo experiments JR & DvN
12/03 Thursday 9:30 Research lecture DvN 11:00 Data analysis (whole day) JR	13/03 Friday 09:00 Data analysis (whole day) JR	16/03 Monday Hand in final report (16/03/2026) at 23:59

GROUPS:

IA

- Dillon Williams
- Louis Potgieter
- Nabeelah Ismail

IB

- Kerryn Bosch
- Kate Biebuyck

IC

- Louis Barnard
- Jordan Hobbs

ID

- Josh Richardson
- Leah Geldenhuys

2A

- Florian Ruffler
- Megan Venter

2B

- Karissa Govender
- Khumo Shungo

2C

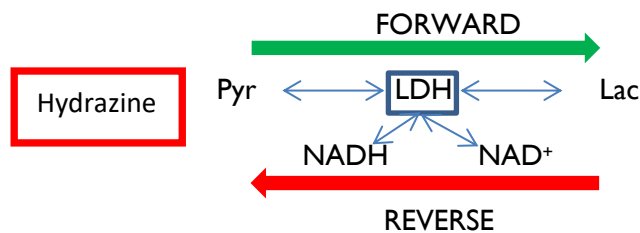
- Nathan Haupt
- Jonathan Mills
- Anifa Mabece

2D

- Clarissa Joubert
- Olaf Vries

ABOUT THE PRAC:

(1) Characterise the enzyme:



(2) The experiments:

Experiment 1: Rate as a function of substrate

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: Product Inhibition

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 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 K_m 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 μ L of the 1 ml is Hydrazine (Stock is 25%, final concentration should be 2.5%).
- 100 μ L of the total volume is LDH (Stock is 5 U/mL, final concentration in cuvette is 0.5 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 $K_m/10$ and $10 \times K_m$ and the fixed substrates are usually at saturating conditions i.e. $10 \times K_m$ (unless instructed otherwise). Use Excel to create an incremental pipetting schema.