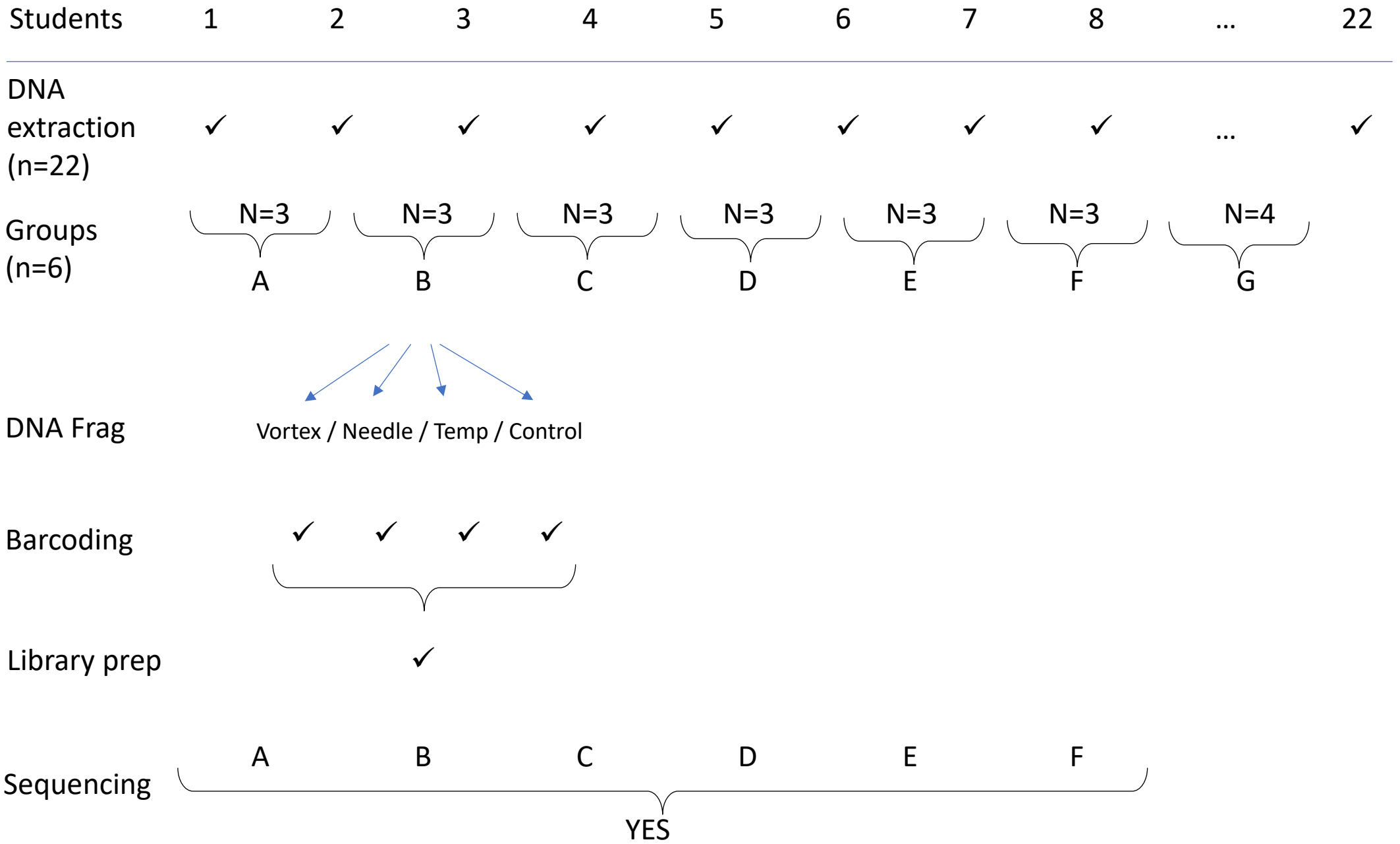
2026

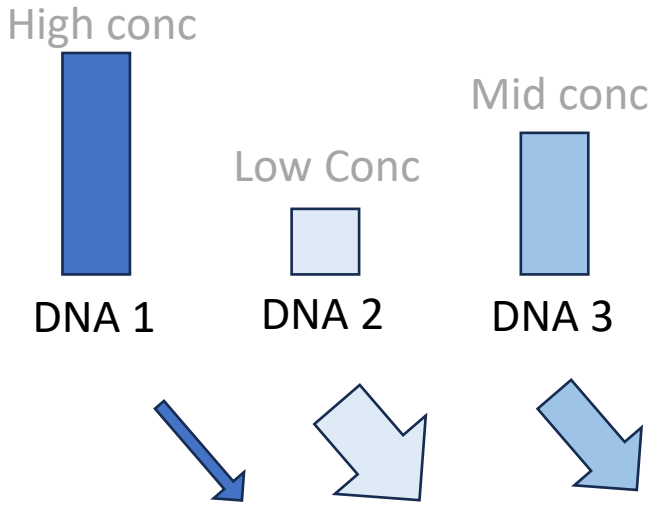

Students
1
2
3
4
5
6
7
8
…
22
DNA extraction (n=22)
Groups (n=6)
N=3
N=3
N=3
N=3
N=3
N=3
N=4
A
B
C
D
E
F
G
DNA Frag
Vortex / Needle / Temp / Control
Barcoding
Library prep
Sequencing
A
B
C
D
E
F
YES

Key points, Day 1

- Day 1 : extract DNA from blood, measure concentration with qubit
    - We will **re-quantify** after 3 days, run on gel and perform nanodrop.

Key points, Day 2

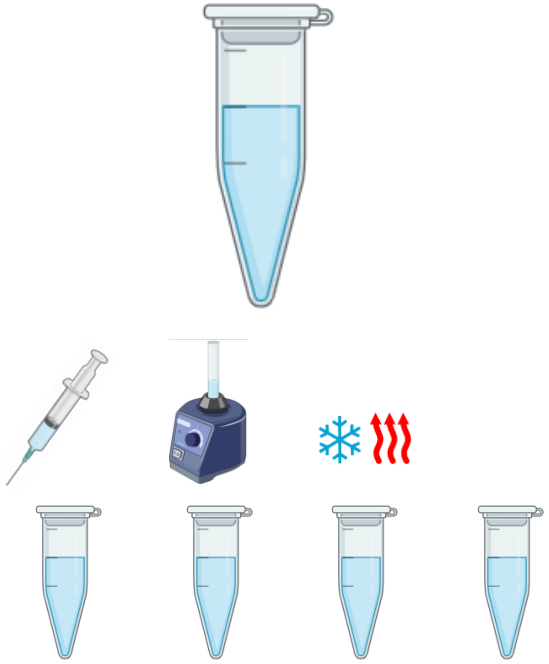
- Day 2 : using the re-quantify qubit values, within each group, combine an equal mass of DNA from each extraction (n=3) to make a final solution in water with a volume of 200ul and concentration of 60ng/ul.



200ul @ 60ng/ul = 12,000 ng DNA (12ug)

Key points, Day 2

- Divide this into four microfuge tubes as follows:
  - Needle shearing = 70ul
  - Vortexing = 70ul
  - Temperature extremes = 20ul
  - Control = 20ul



DNA Treatments

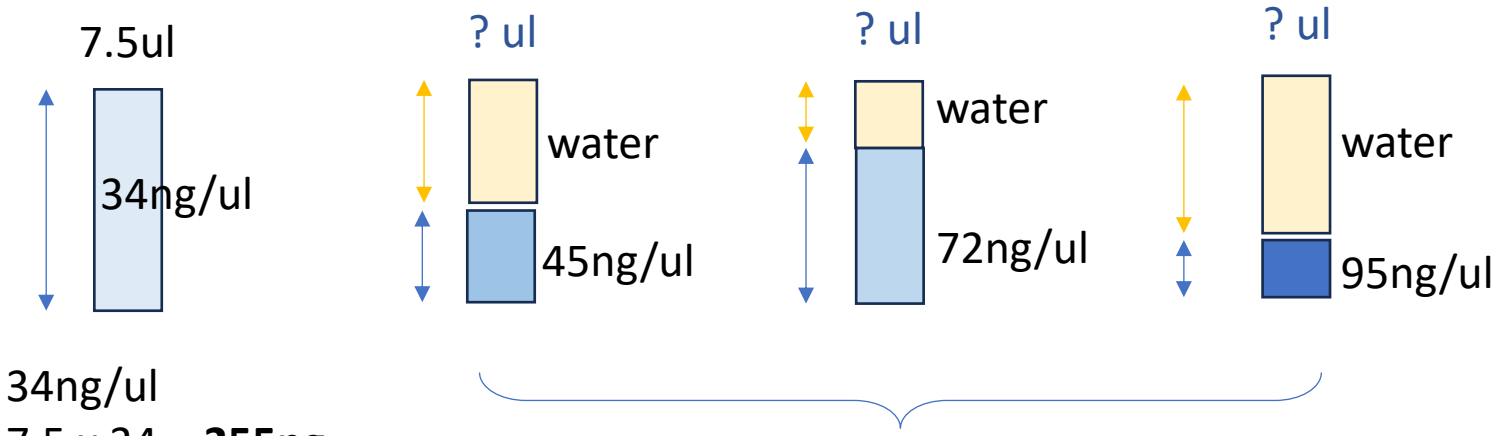
- **70ul** Needle shearing : pass DNA in solution up and down quickly through a blunt needle 30X.
- **70ul** Vortex : vortex DNA and metal bead in solution for 60 seconds at full speed.
- **20ul** Temp x-trem : Heat for 45s, freeze for 45s, repeat for total 4X.
- **20ul** Control : no treatment

Key points, Day 2 cont’

- Treated DNA will now be nick repaired and end-prepped, and then cleaned with beads and quantified (again!).

Key points, Day 3

- Calculate the total mass (ng) of DNA in 7.5ul of the treatment with lowest conc.
- For the three other treatments, calculate how much DNA and H2O to combine to give a final mixture of 7.5ul containing the same mass of DNA as the lowest conc treatment.



34ng/ul
7.5 x 34 = **255ng**

For each … ? ul water + ? ul DNA = **255ng** in 7.5ul

Key points, Day 3

- Each sample will receive a different barcode.
- Samples within group will be combined (pooled) to one.
- This will be cleaned, quantified and used as input to adapter ligation.
- Adapter ligation is done on one sample per group. Finish with quantification.

- Prior to loading, Mariann will combine equal mass from each group.
- Video on loading here…
  - https://www.youtube.com/watch?v=pfftqldRSbk

Dry lab intro