Single-cell mtDNA quantification protocol Summary

~~Doerg meeting Sept 5th~~

- Protocol steps are roughly as follows: Aim is to measure N and h from a single cell. We will use two separate qPCR reactions per cell to do this.

Step 1 Use flow cytometer to separate a single cell from a solution containing many.

- NB the initial solution may contain dead cells which have lysed, so there could be free mtDNA in the solution. However, Doerg thinks that the amount of liquid from the solution that gets passed through the cytometer with the cell is small, so he thinks we can neglect this.

96 well plate

Step 2 Digest the cell to liberate the DNA.

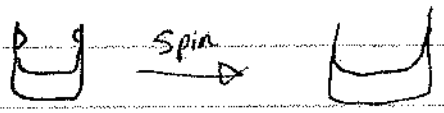
- There are multiple different digestion methods (different chemicals) which appeared to affect quantification during qPCR. Discuss later.

- 3 μL digestion protein buffer + 12 μL "qPCR stuff"
  (TRIS)

  ½ of contents

Step 3 Move ~~for~~ from 96 well plate to 384 well plate (so can put it all into the qPCR machine), and ½ to another 384 well plate. One for N, another for h

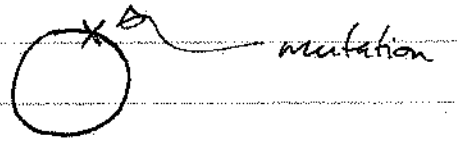
- NB to do this, the ~~96~~ plate is spun at 2000 rpm, so that any droplets on the side of the 96-well plate move into the main bit



but this could create a concentration gradient in mtDNA. ~~This is an issue for next step.~~

~~Step 4~~ · Extract ½ well contents

Step 4 Do qPCR on both plates. ~~If we see evidence~~
If the mtDNA looks like this:



then we have primers that look like this

Measures: m N

from which we can deduce heteroplasmy as $\frac{m}{N}$.

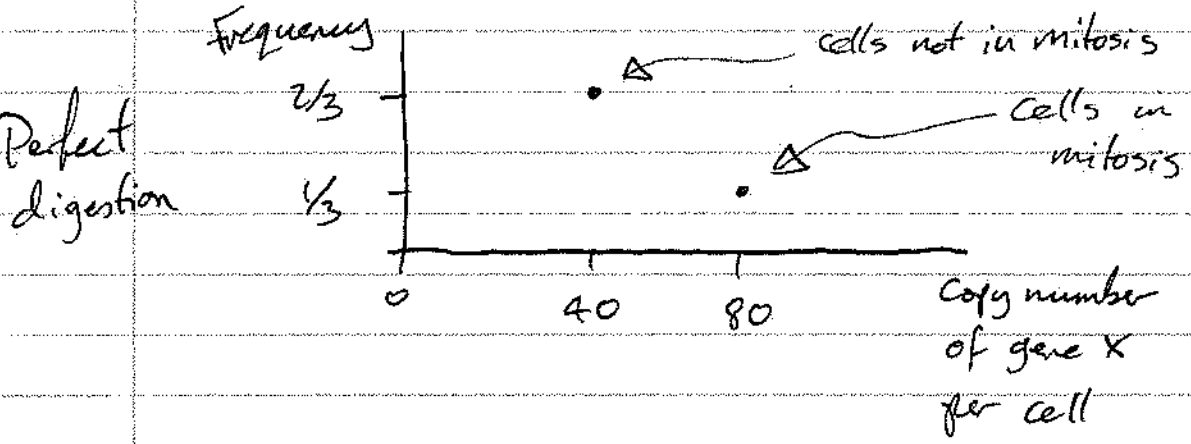
Things to think about

- Does the spinning at step 3 matter? Joerg says he can fix this issue by pipetting up and down, ~~the~~ before pipetting ~~the~~ to the 384 well plate, so it's well mixed
- Nuclear DNA contain something called NUMTS "nuclear mitochondrial DNA segment". Our qPCR for N is presumably affected by this, but m is not. How big is the effect? How can we sensibly account for it? Can we account for it in-silico, or do we need to ask Joerg to change protocol?
- Appropriate error model for pipetting. ±10% volume error, and a stochastic splitting of particles into each well.

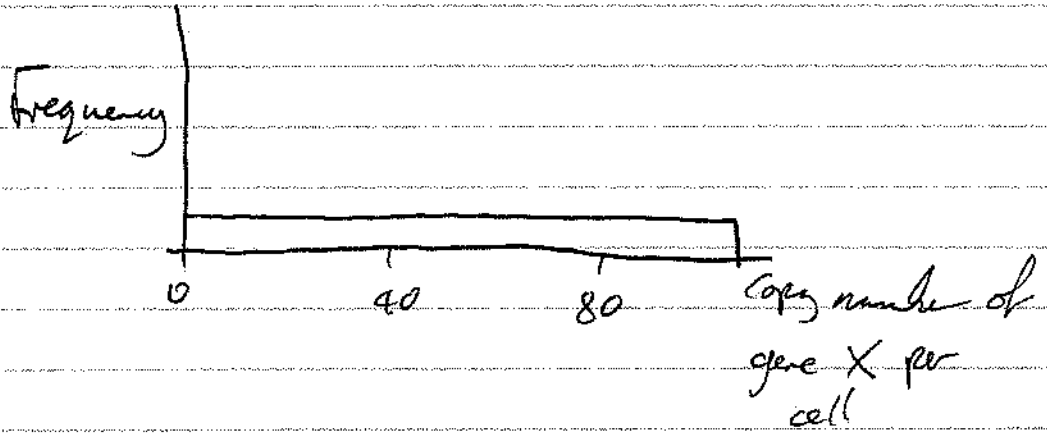
- DNA digestion. Proteins are sticky, and can interfere with the qPCR reaction. We can use a variety of chemicals to try and make the DNA available in the qPCR reaction. Joerg did the following experiment to understand how effective the DNA digestion is:

(gene X)

In the nucleus, there exists a gene which can be found with a copy number of 40. He did the above protocol a number of times (~20?). In a perfect scenario, we would get a distribution like this



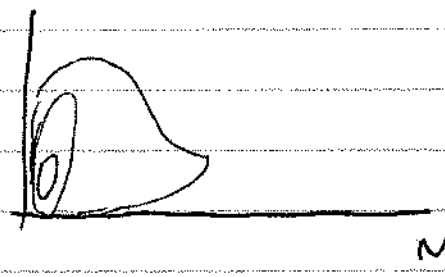
Instead, with some digestion buffers, he sees this



- We think this needs to be solved experimentally, perhaps by incubating for longer with the DNA digestion buffer?

Possible extensions

- For each cell, we will get a distribution for N and h. What's the best way to visualise such data for many IID cells? Perhaps a 2D ~~[illegible]~~ histogram?



- For data of this type, can we infer parametric heteroplasmy distributions, such as the Kimura distribution (see Wonnapinij (2008)) or other stochastic models (see Johnston (2015), Elite & Burgstaller)

↳ this is useful for comparing, say, two different sets of IID cells under different conditions and asking questions like, is heteroplasmy mean/variance different between these two conditions? There are other benefits of getting distributions for parametric distributions.

↳ posterior