Task 1
Sexual Development

Introduction

Plasmodium berghei is used as a rodent model of malaria. It is known that in the lab it can evolve to stop producing sexual stages (Figure 1). We want to try and use this observation to our advantage. If we can understand how the parasite switches to the sexual, transmissible stage, then we might better understand how to prevent this from happening and prevent the spread of malaria.

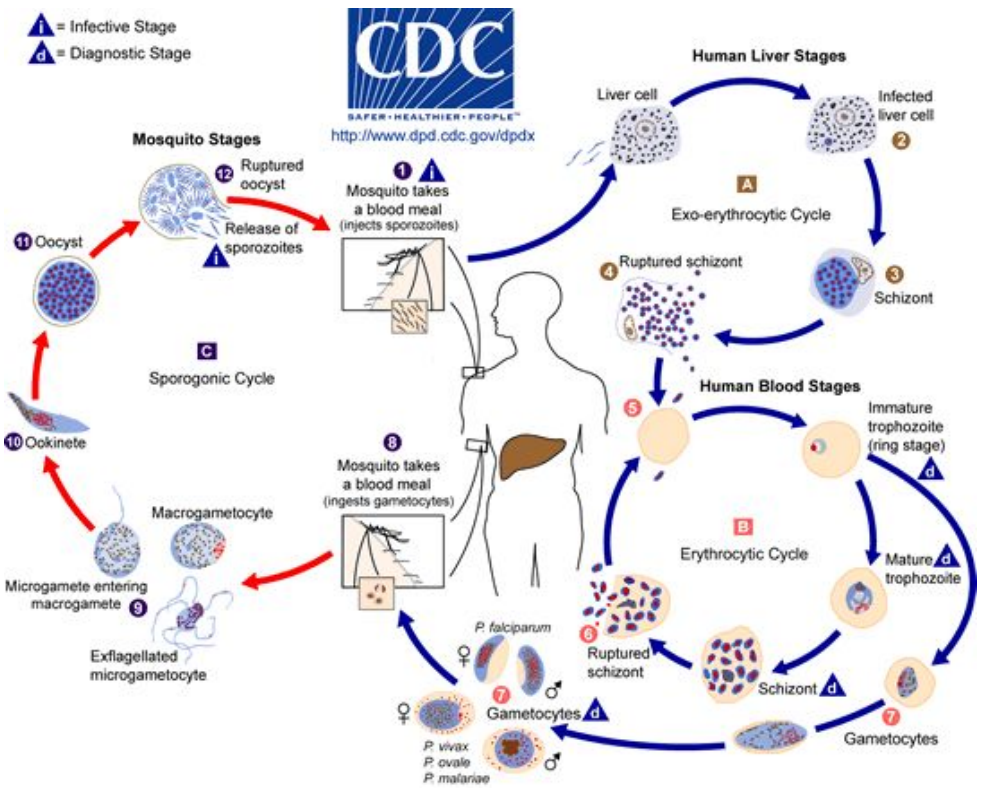

Figure 1. Malaria life cycle. The parasites must produce sexual forms called gametocytes in order to be transmitted from the intermediate mammalian host to the definitive mosquito host.

Our colleagues have grown several cultures of a transmissible strain in the lab continuously for several months (Figure 2). These have now all become gametocyte non-producers (GNPs). Our role in the project is to identify the mutations in these strains, which contribute to the GNP phenotype. Our hypothesis is that while each GNP strain will have many mutations compared to the parental strain, only one gene will be mutated in every strain and this gene will be a key regulator of gametocytogenesis. Luckily there is evidence from earlier work that the gene is located on chromosome 14, so we need only map to that one.

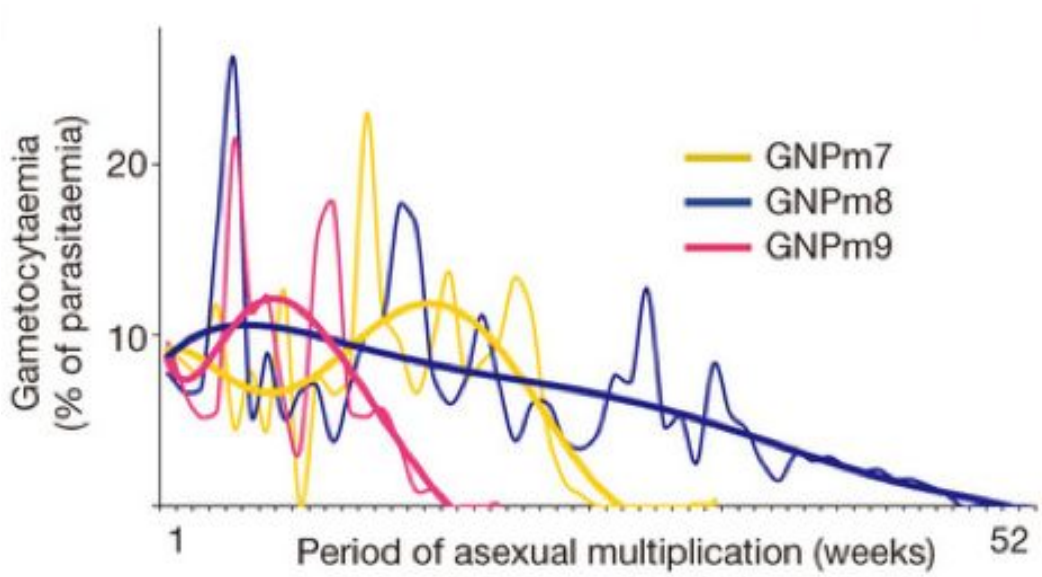

Figure 2. Parasites were passaged continuously in mice until they lost the ability to produce gametocytes. The bold lines show smoothed versions of the real data to even out fluctuations in gametocytemia. GNPm7-9 are different lines of parasites from the same parent which have independently lost the ability to produce gametocytes (transmissible sexual stages).

Step 1: identify the gene responsible for gametogenesis

- use Artemis to identify a gene with variants in each of your mutant strains that are capable of disrupting the coding sequence
- once identified, determine the likely function of this gene

A day passes… After we found the gene yesterday, one of our wet lab colleagues managed to knock it out, grow the mutant up, extract and sequence its RNA. She had to stay up quite late, but that sounds like two days wet lab work at most, right? The MiSeq runs really fast! This means we are able to examine the role of the gene and how it affects the transcriptional landscape of the parasite. Which transcripts are affected by the knockout of this gene? What does this tell us about the importance of the gene in the switch to sexual development? What could this gene list be useful for in future?

Sequencing reads, and reference sequence are available in the data directory **Task_1_SexualDevelopment** (please use this rather than download one). There are also files of genome annotation, product descriptions, GO terms and an R script for performing GO term enrichment. A full explanation is found in a README file in the directory.

Step 2: Determine the effect of the mutation on gene expression

- map RNAseq data to the genome and confirm the knockout phenotype
- Use Kallisto and Sleuth to identify differentially expressed genes
- Perform a Gene Ontology enrichment analysis (we have provided an R script to help with this)

Top tips

- all of the basic commands are in previous Modules
- make sure the sample names you use in the kallisto mapping are the same as those in the R script.