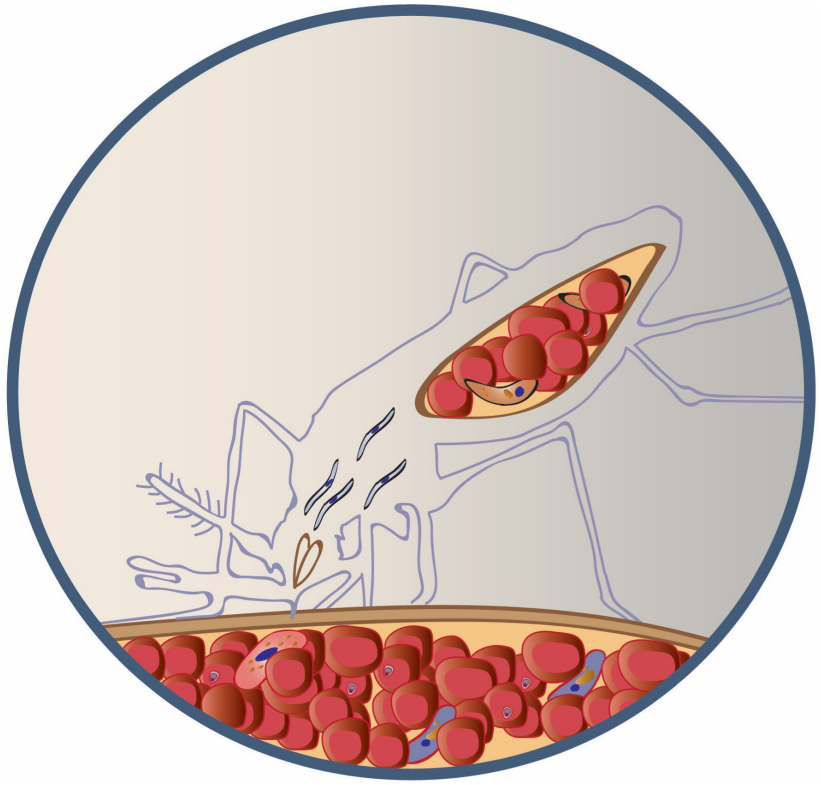
Single cell approaches in malaria: From whole organism Atlas to protein function

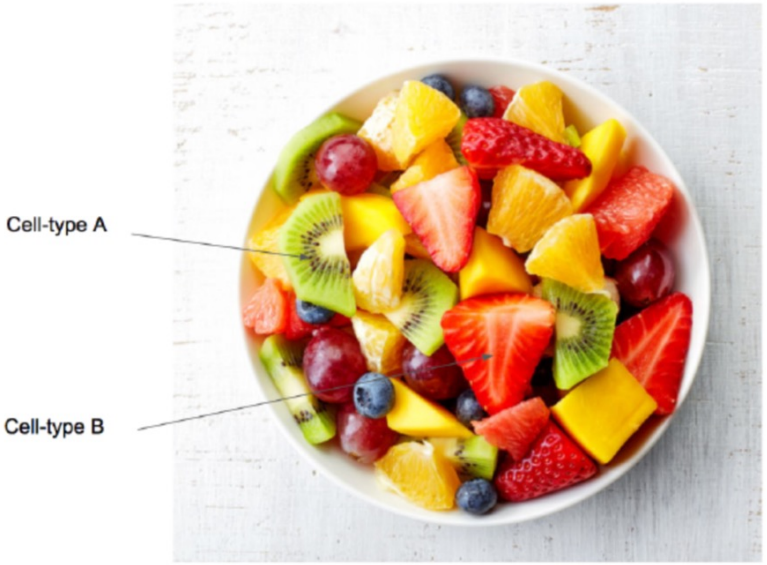


arthur.talman@ird.fr

9.11.23

Single RNAseq is capturing gene expression at the cellular level

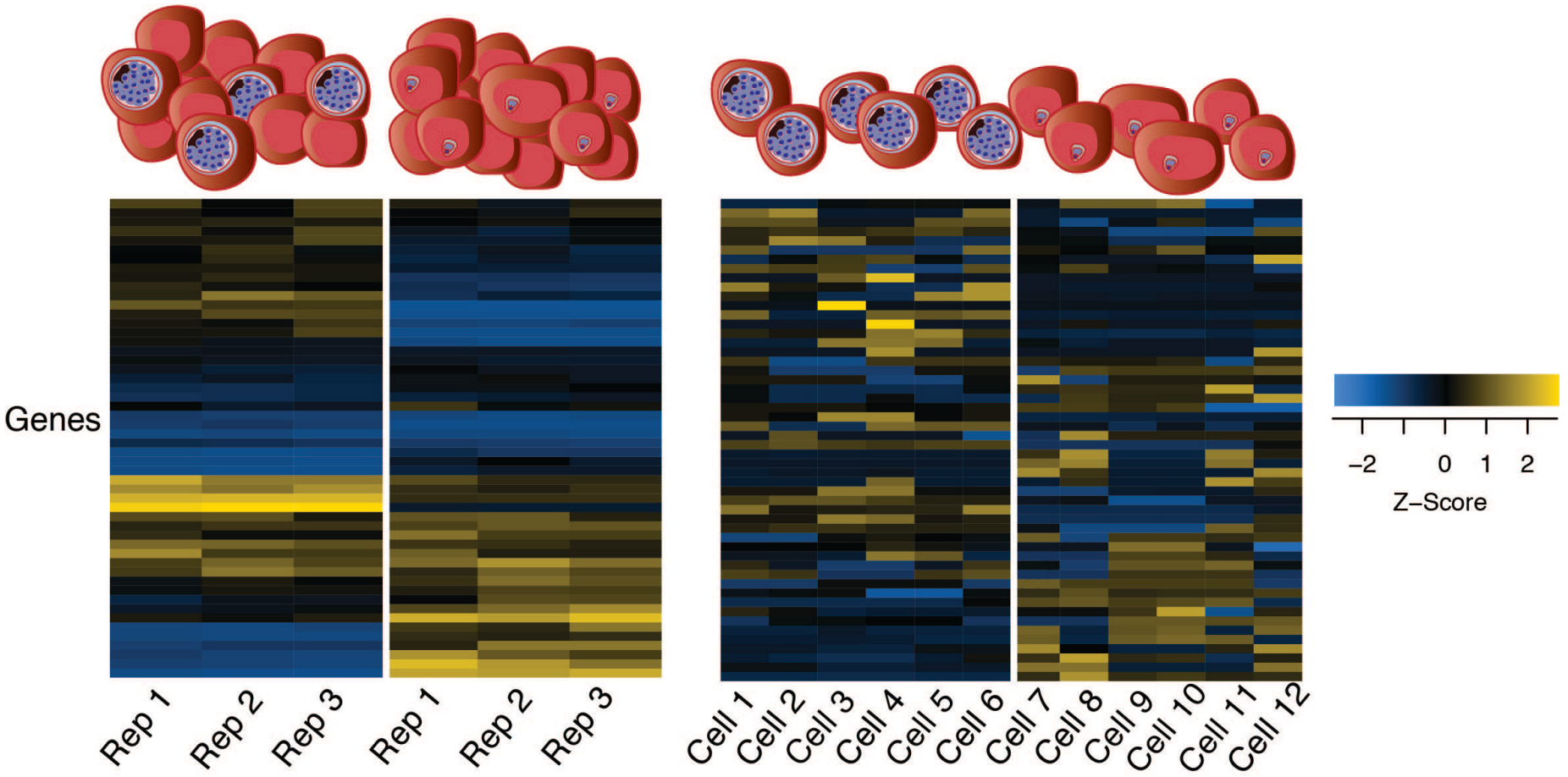
Single-cell



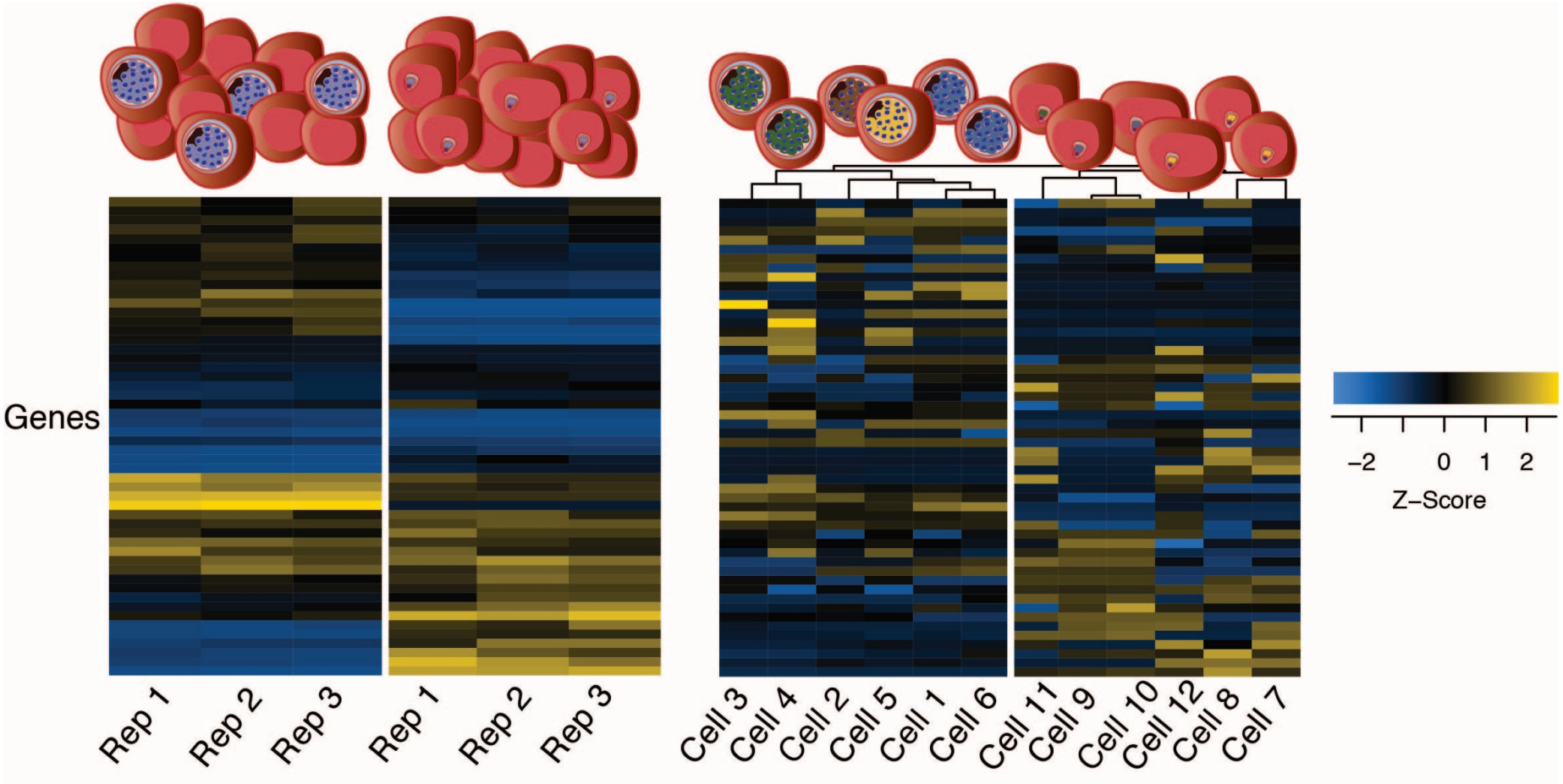
Bulk

Shalek and Regev (2016)

Single cell approaches: Better transcriptomes

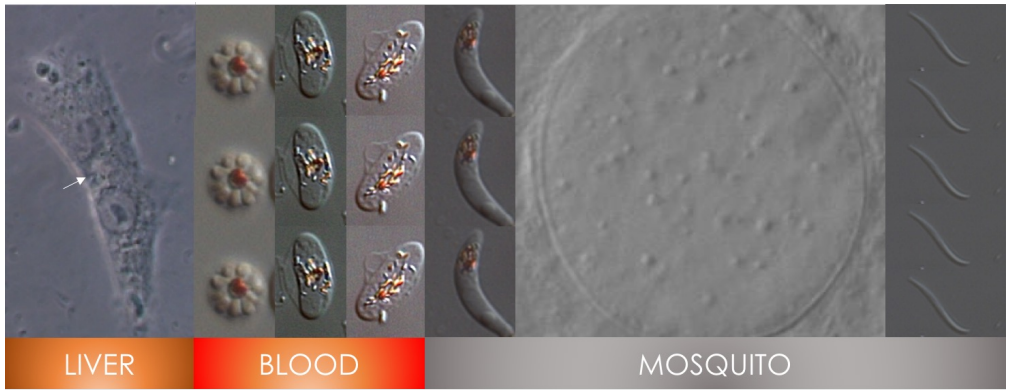


Single cell approaches: Cell-to-cell variability

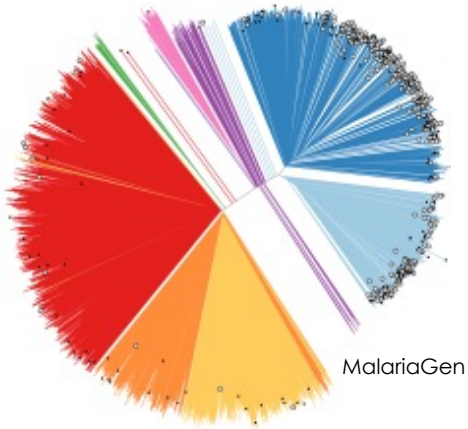


Multiple layers of single cell variation

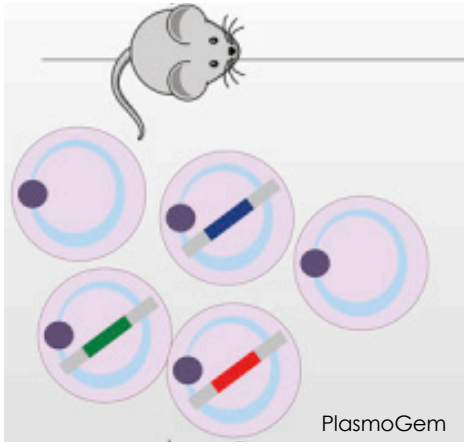
Developmental



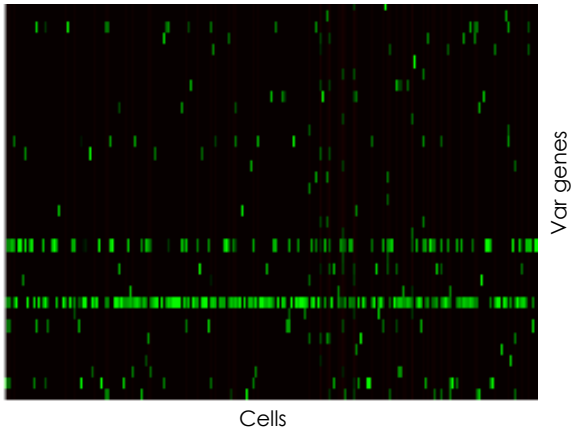
Genetic



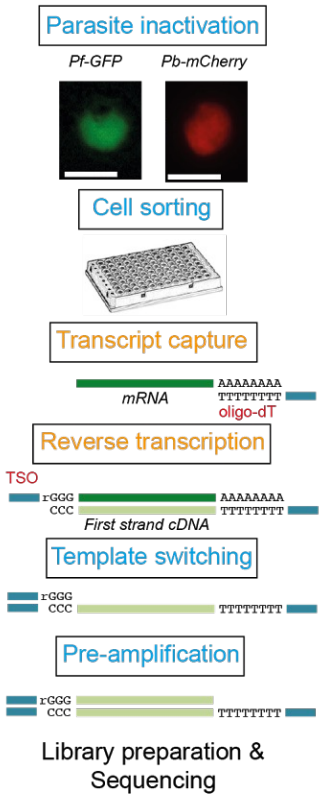
Functional/Phenotypic

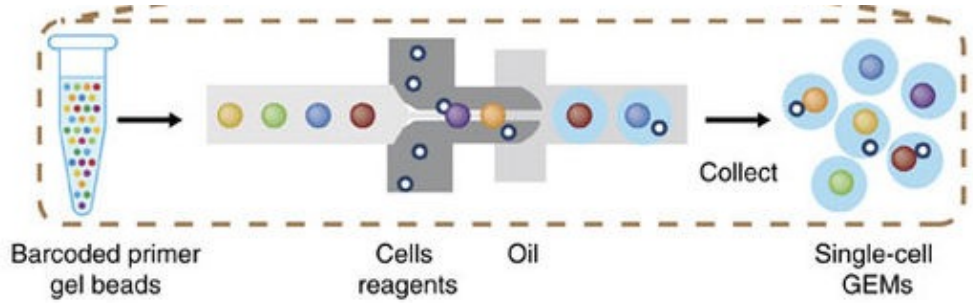


Contingency/Bet-edging



Single cell RNAseq methods





Reid*, Talman*, Bennett* *et al.* (2018) eLife

Single cell RNAseq analyses

Fastqs

Other things too

Splicing analysis
(Portcullis…)

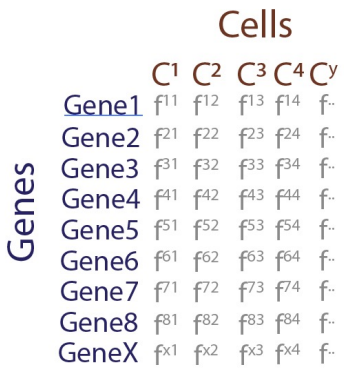
Genotype
(Souporcell…)

Transcriptomes

Triming
(trimgalore)

Mapping
(hisat2, minimap2, star)

Counting
(Feature counts, HTseq)



Comparison to reference

Cluster

Developmental ordering

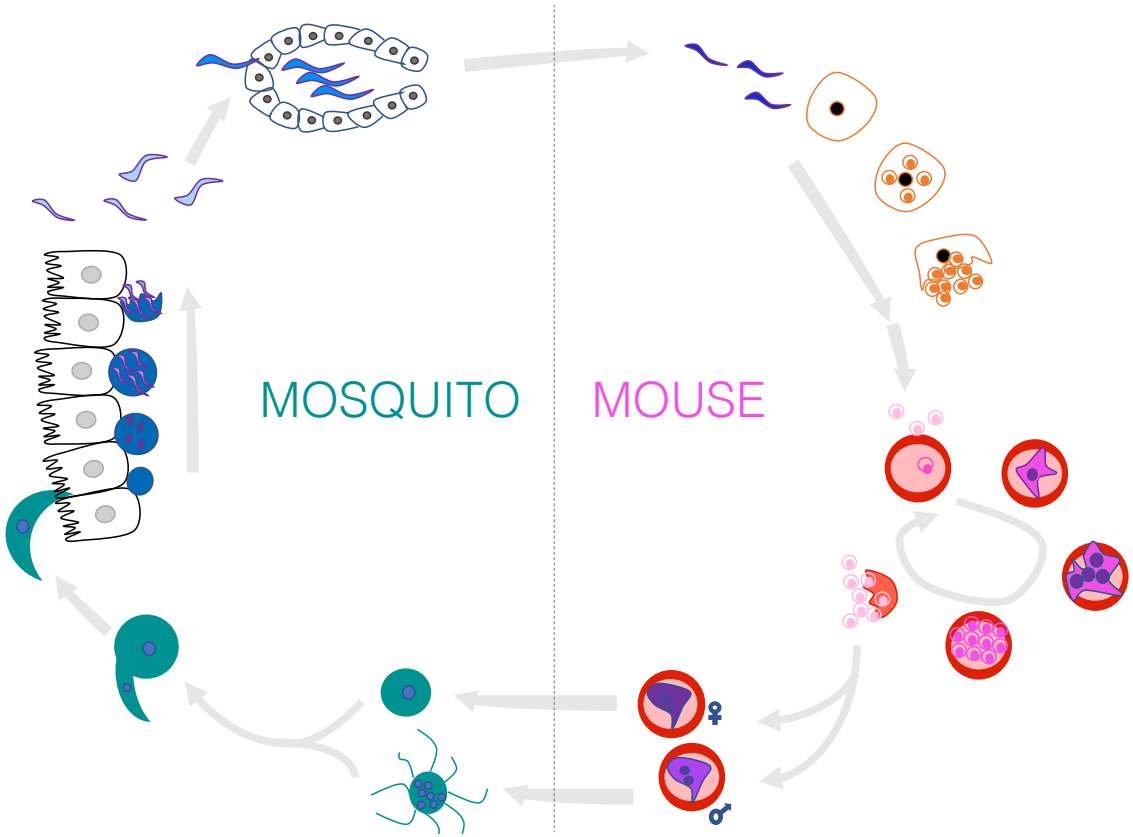
Differential expression

Gene clustering

Developmental ordering

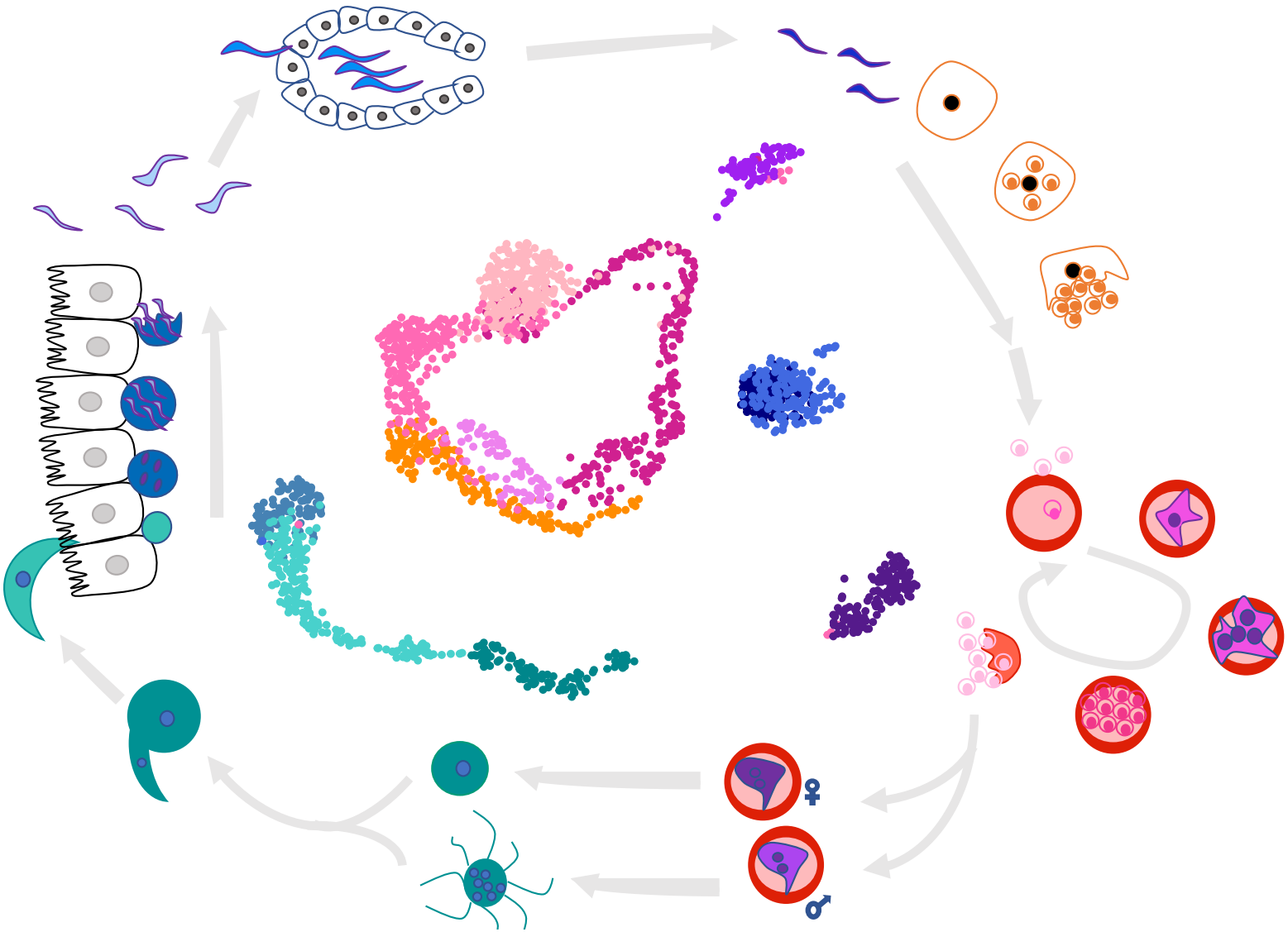
Pace of transcription

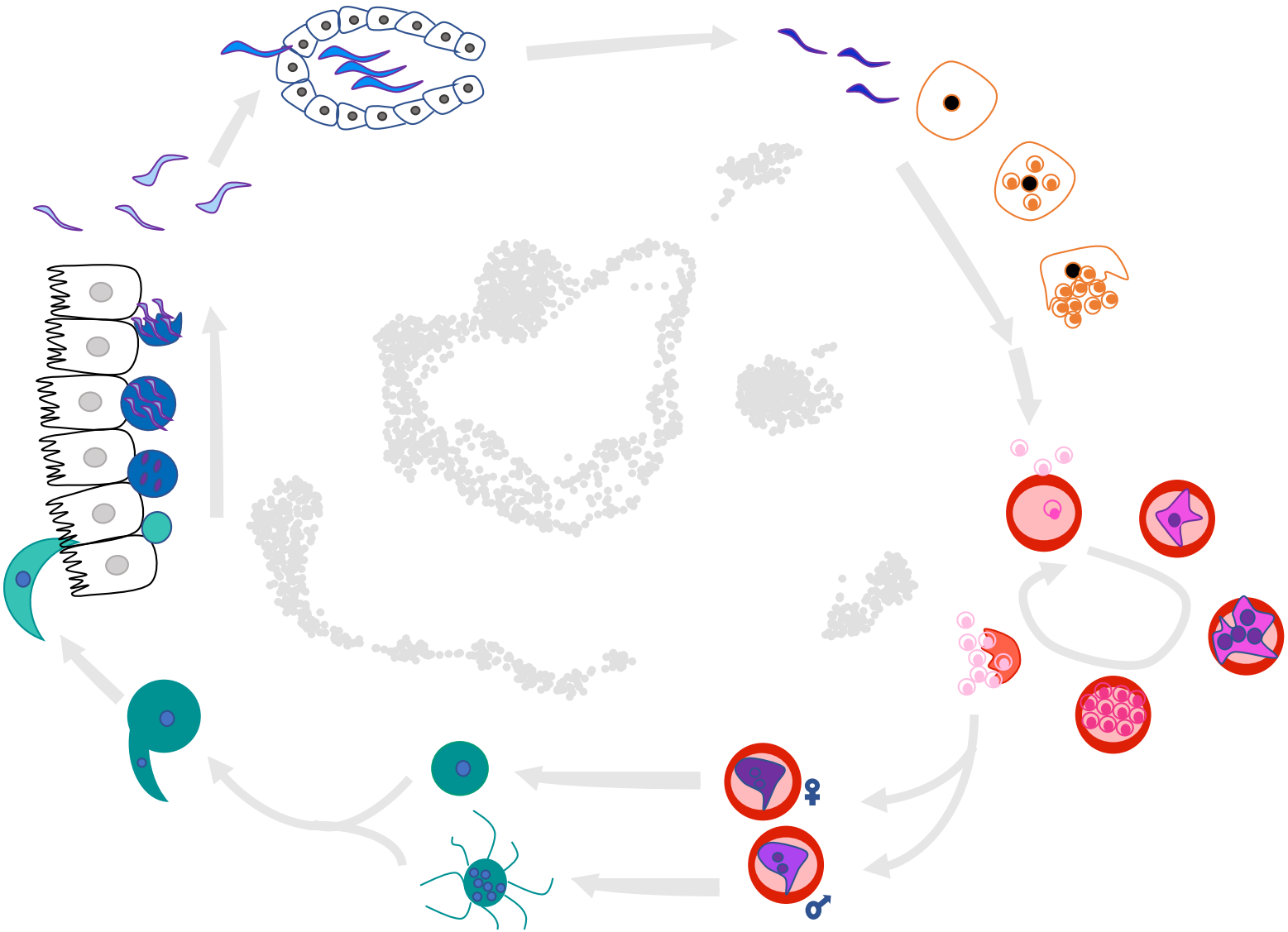
Splicing analysis

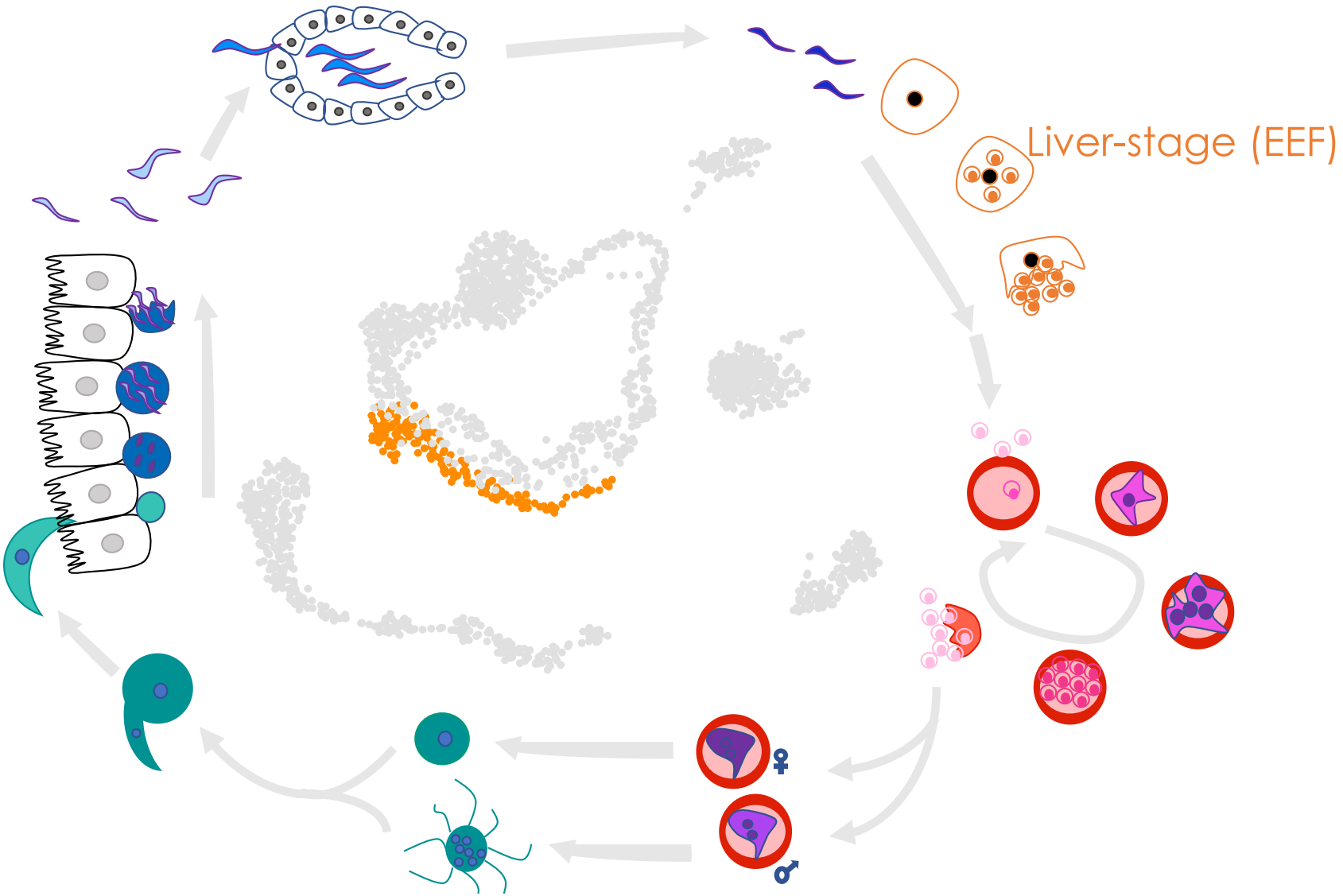


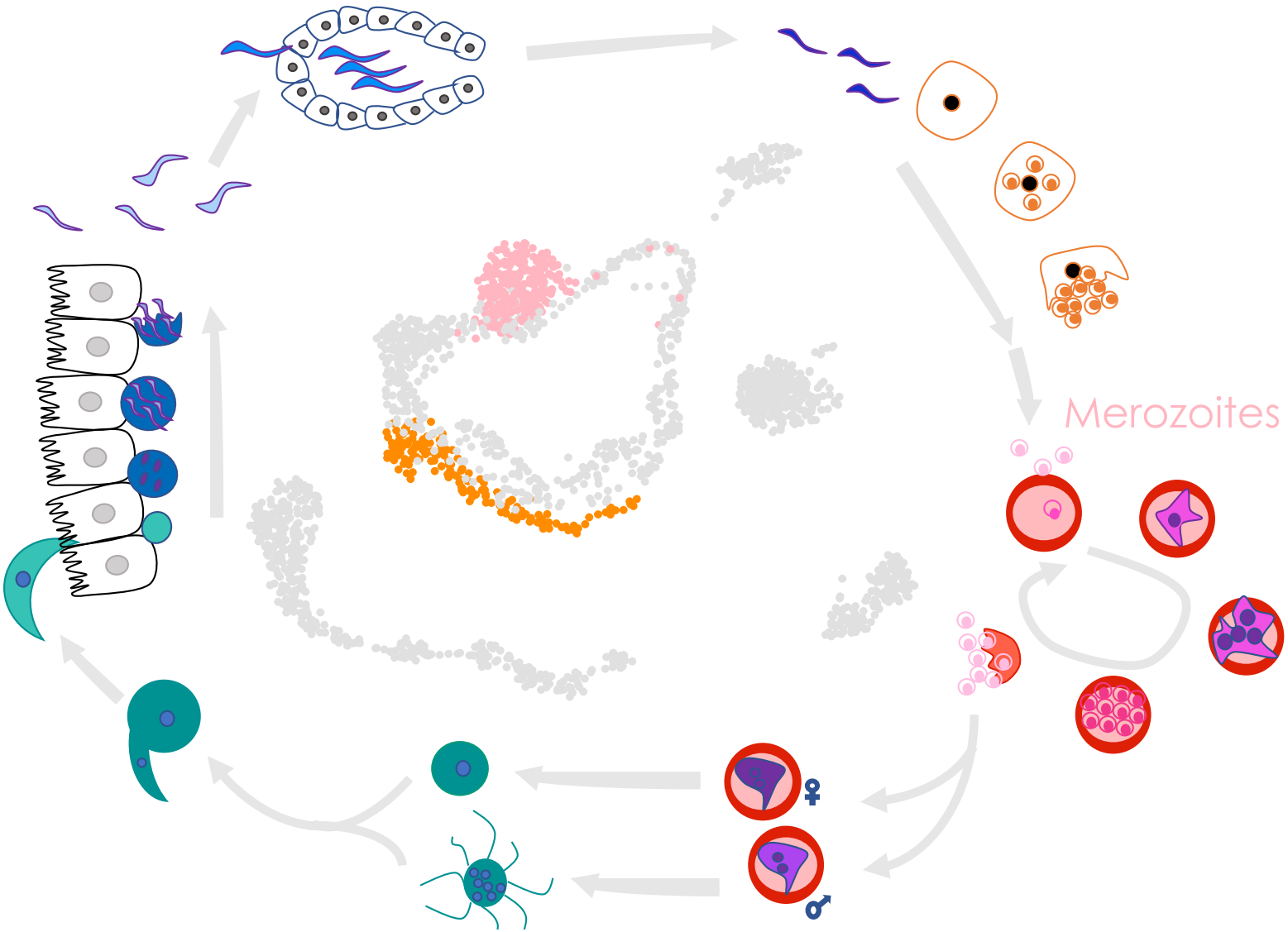
Howick*, Russell* *et al.* (2019) Science

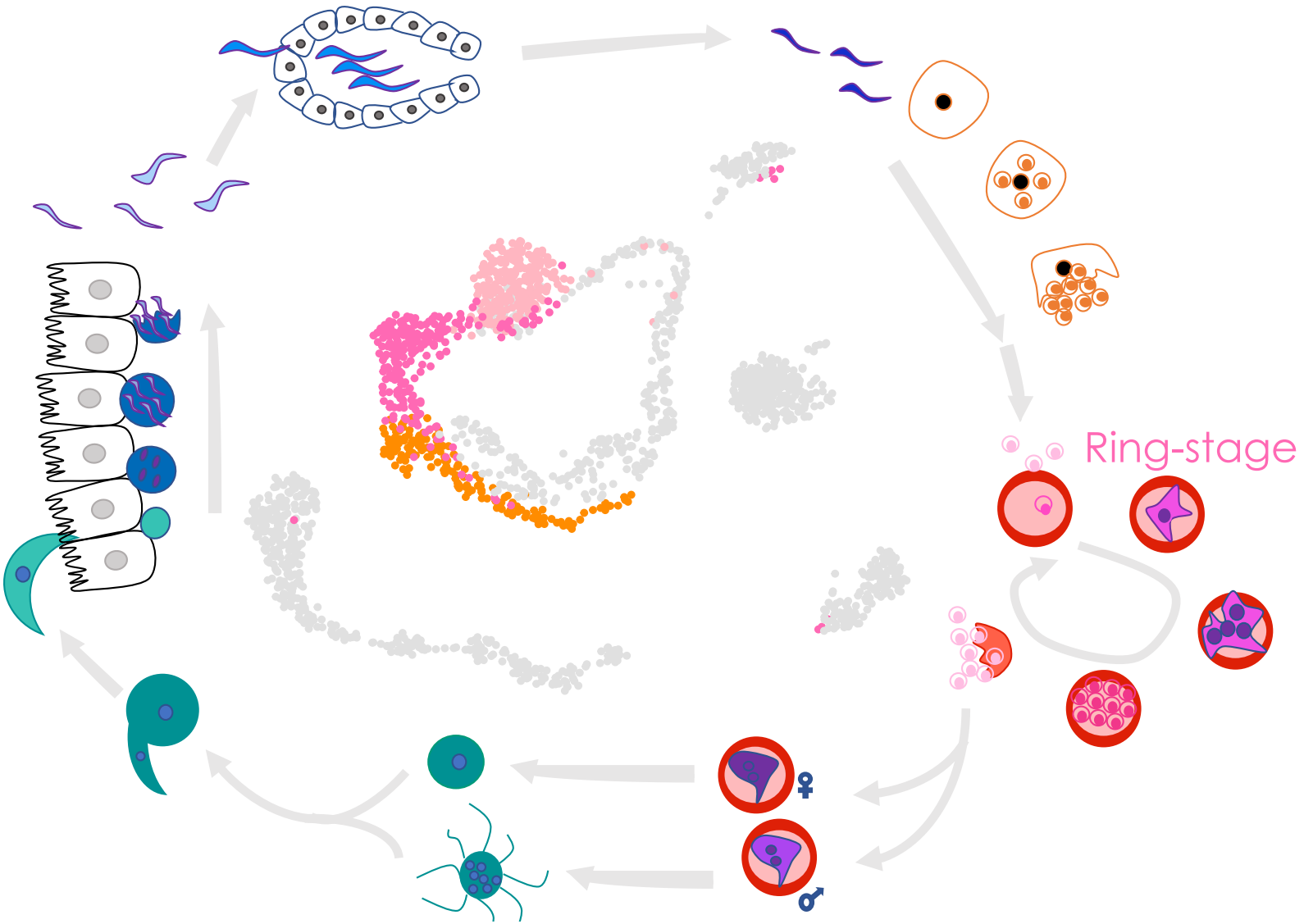
The Malaria Cell Atlas aims to provide a resource of single cell transcriptomic data across the full lifecycle of the parasite

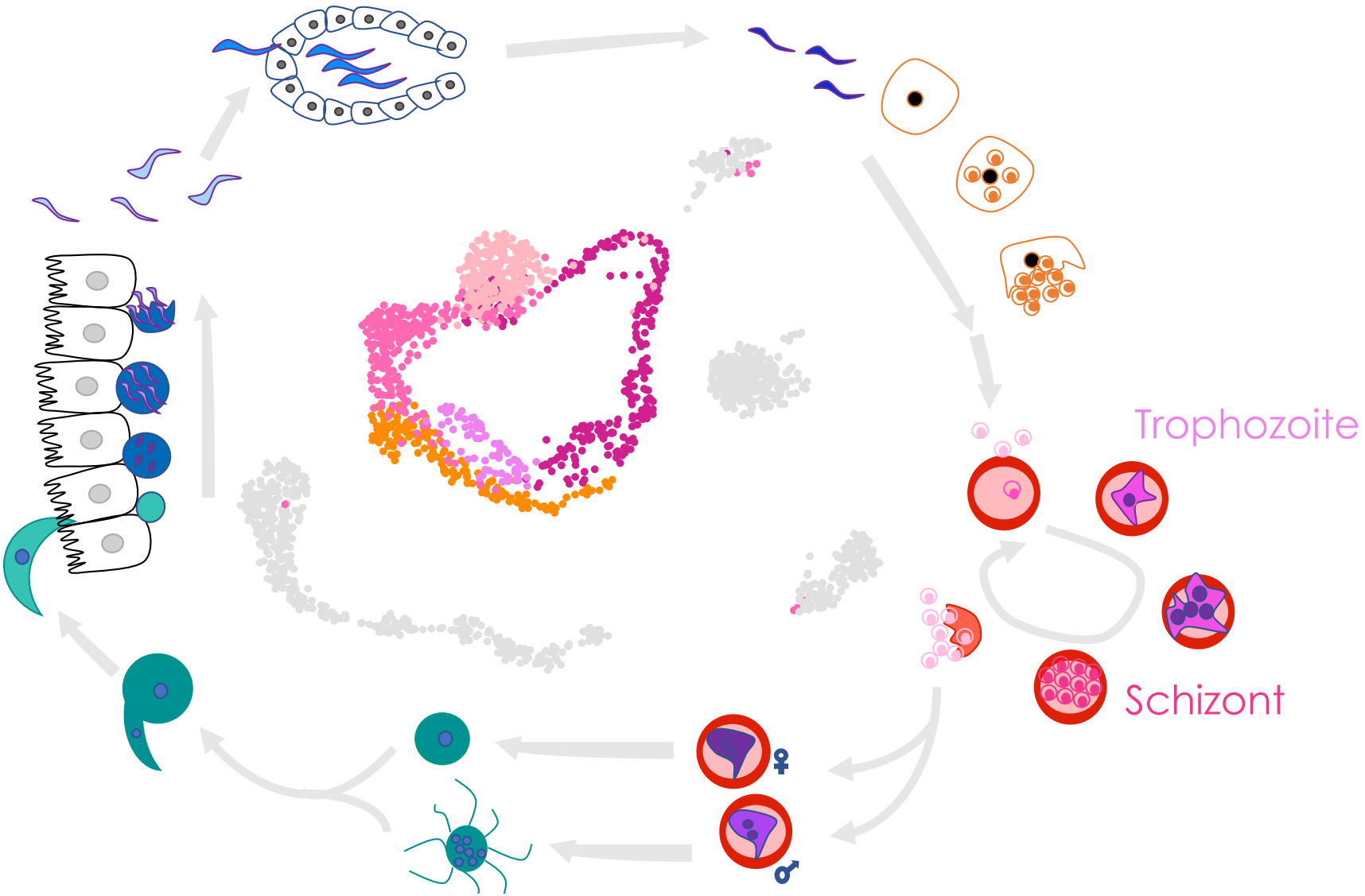


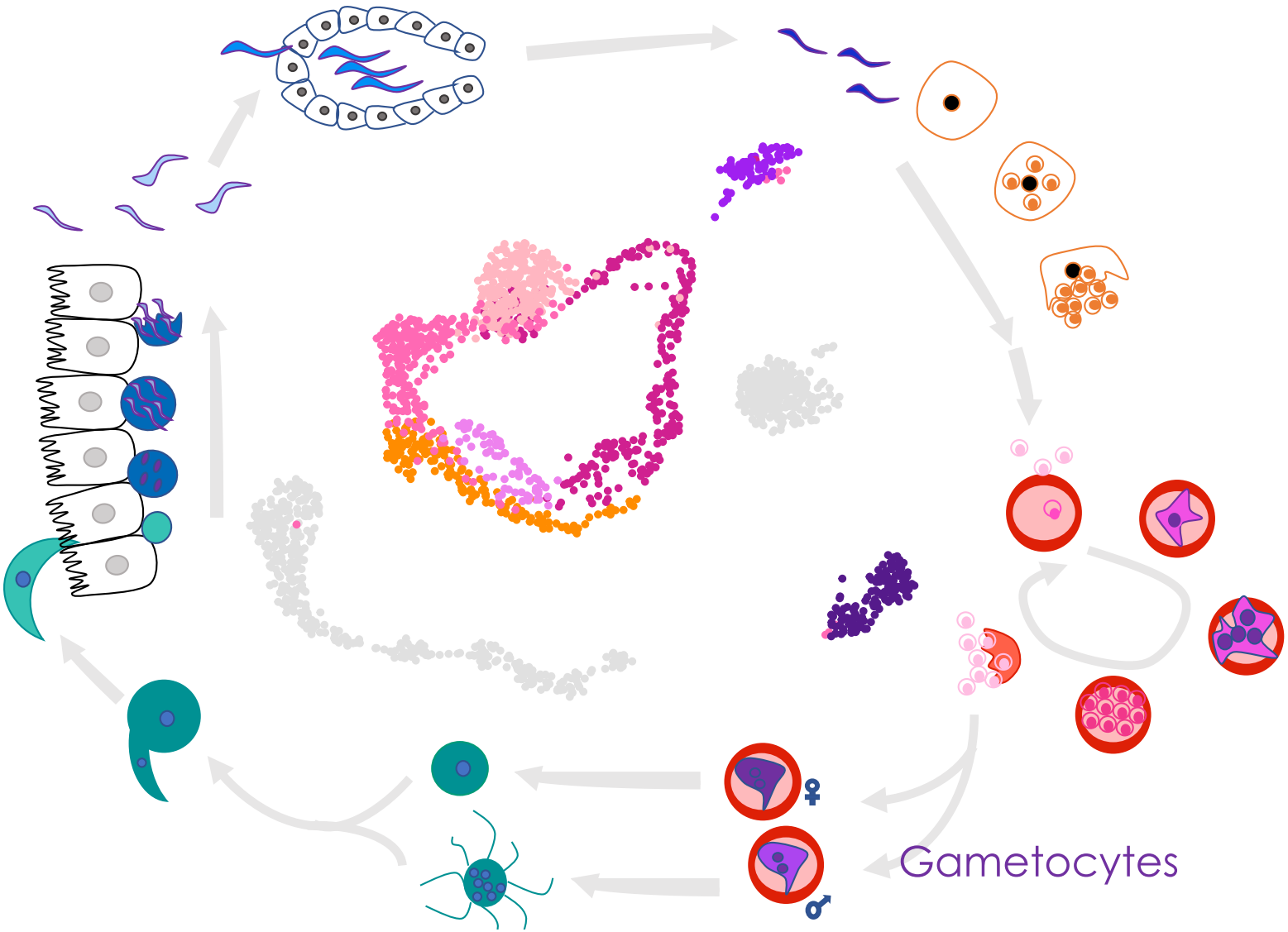


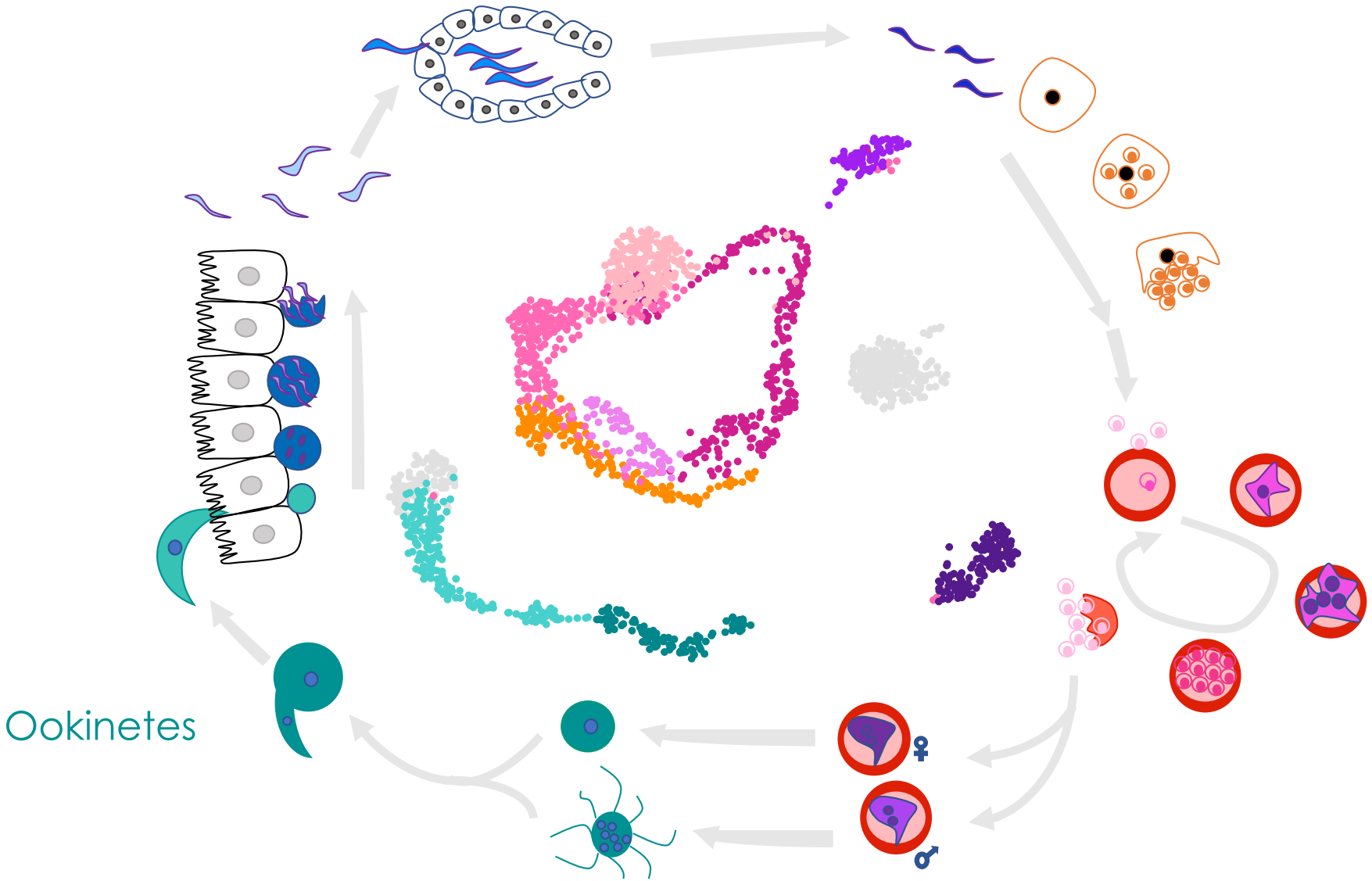

Liver-stage (EEF)
♀
♂


Merozoites

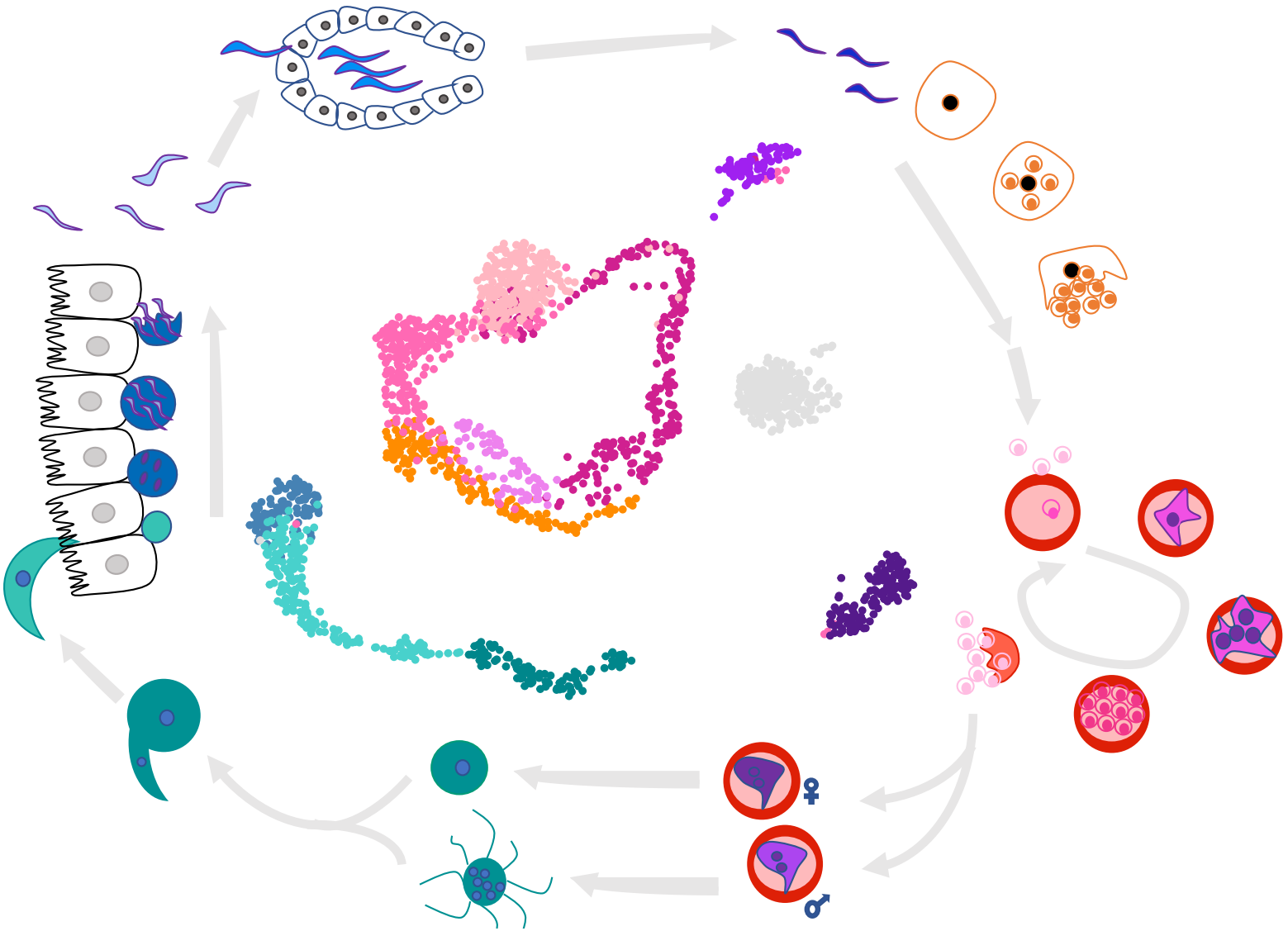

Ring-stage

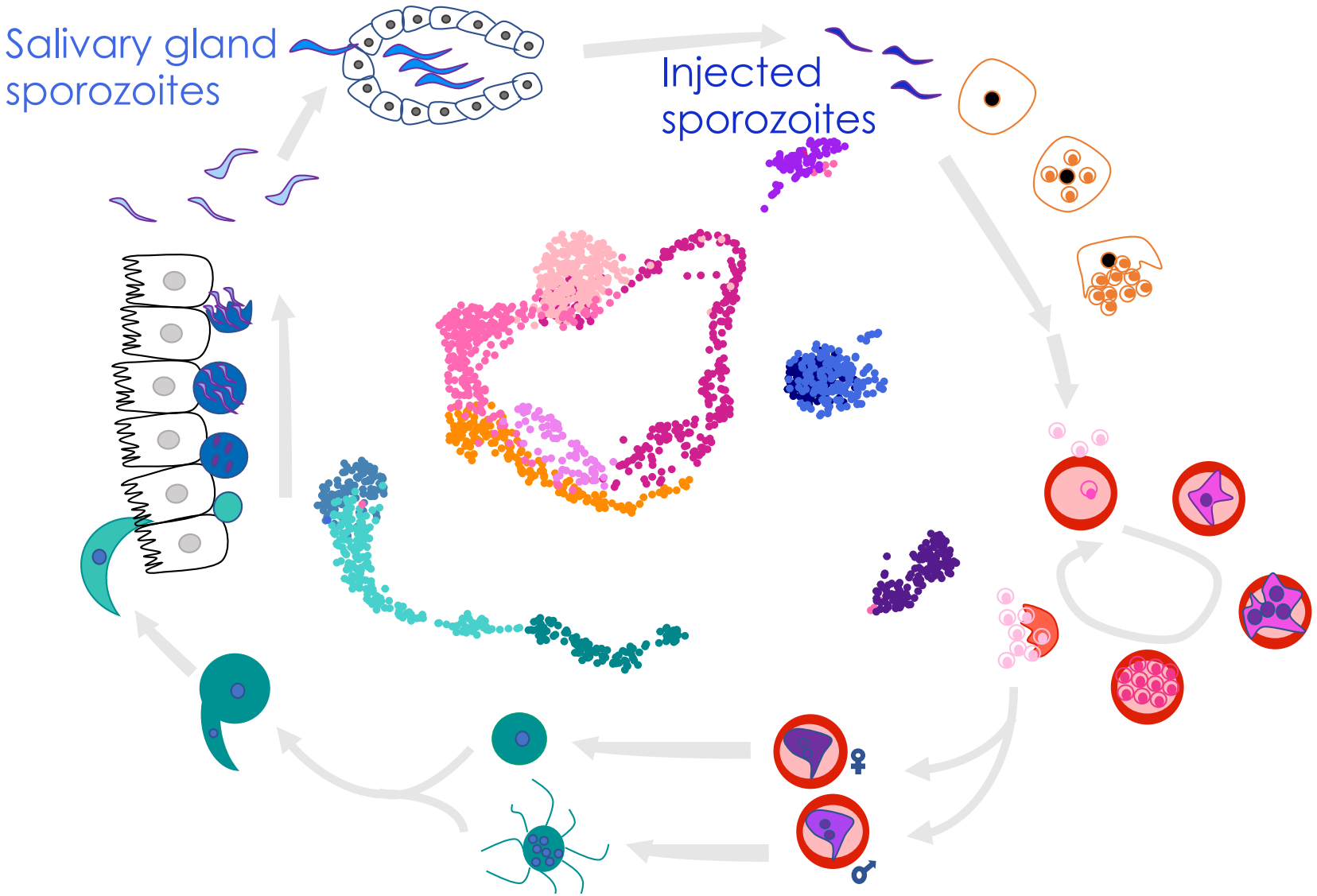

Trophozoite
Schizont

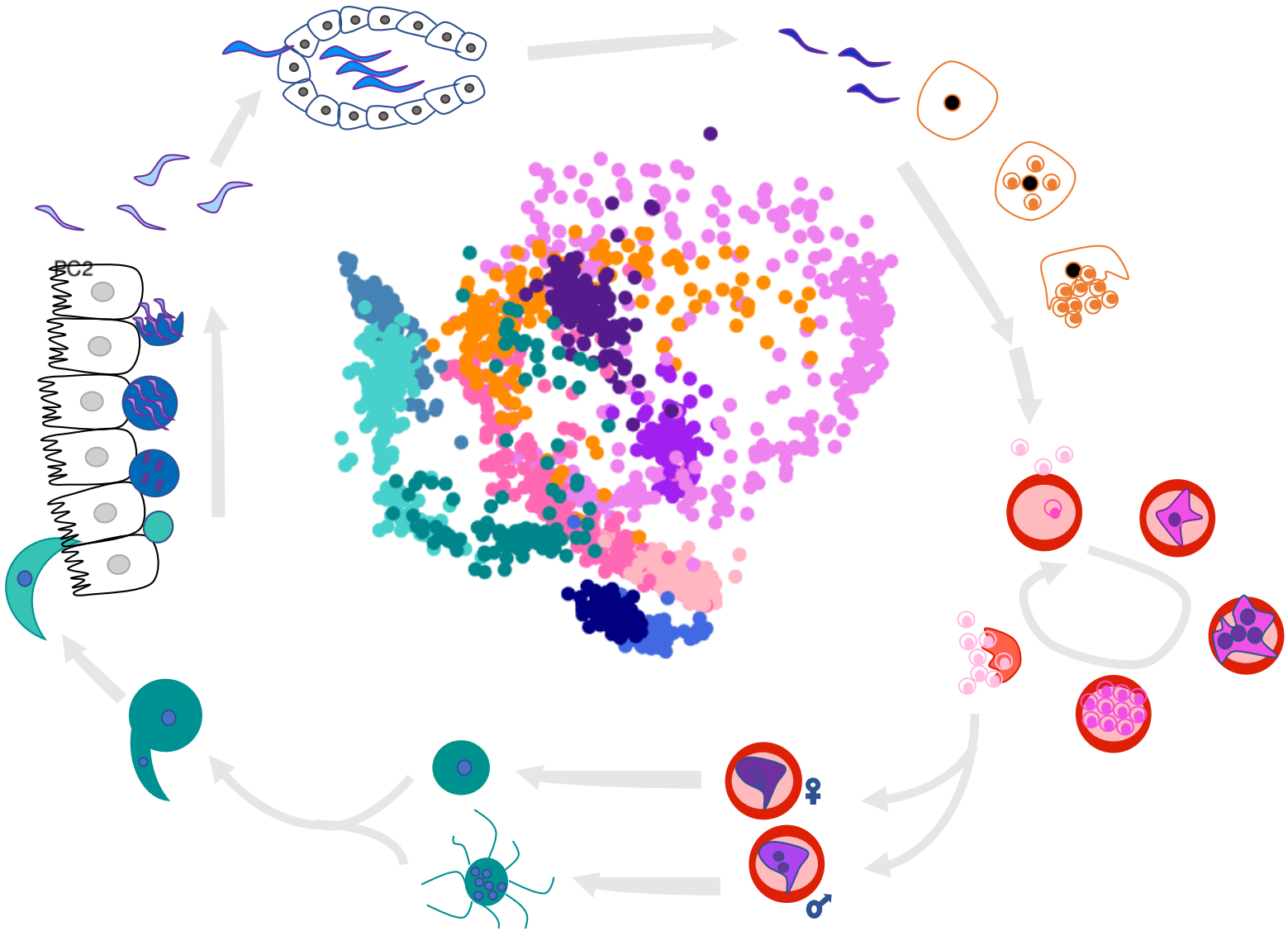

Gametocytes


Ookinetes

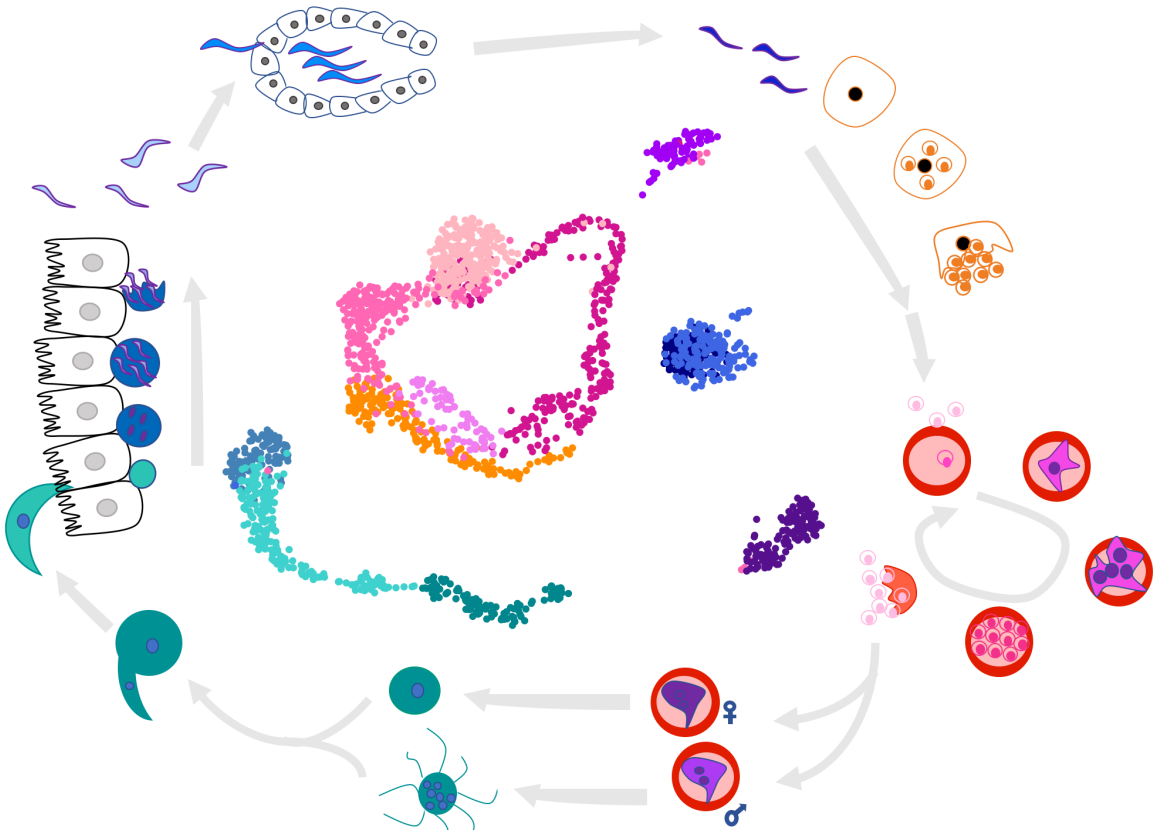
Oocysts



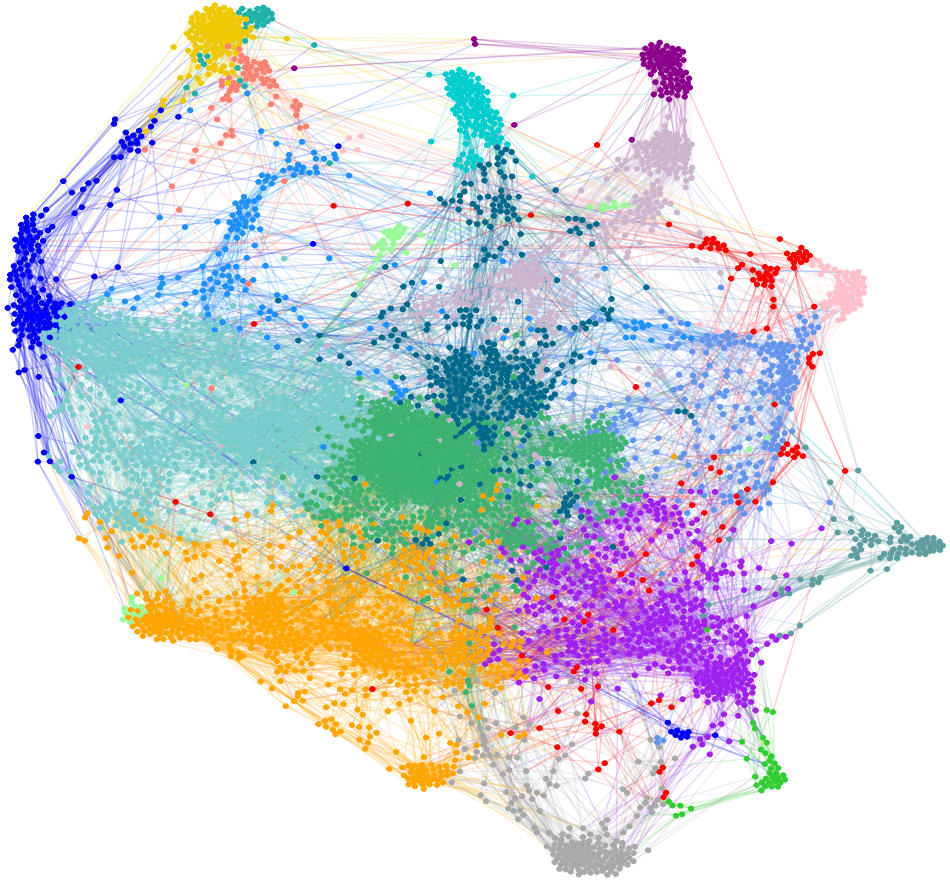

Salivary gland sporozoites
Injected sporozoites


PC2

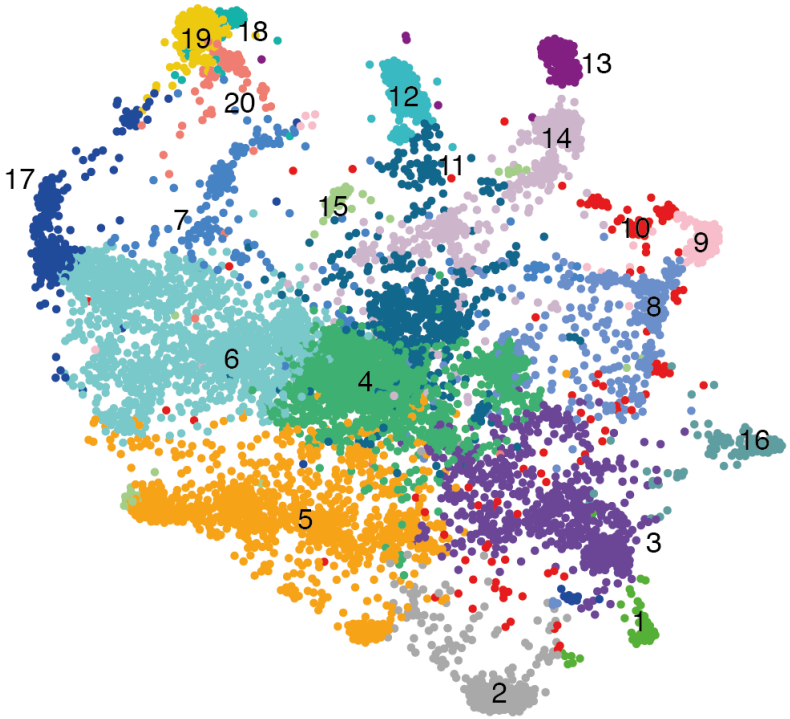
Global patterns of gene expression

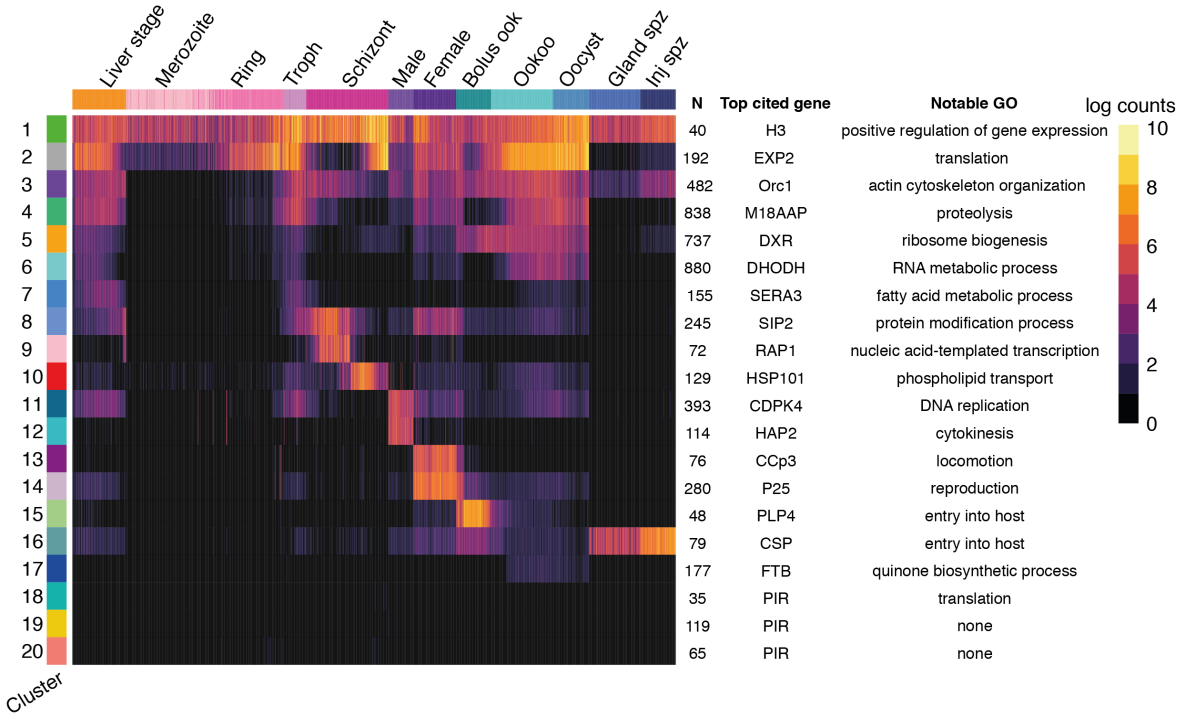


Global patterns of gene expression

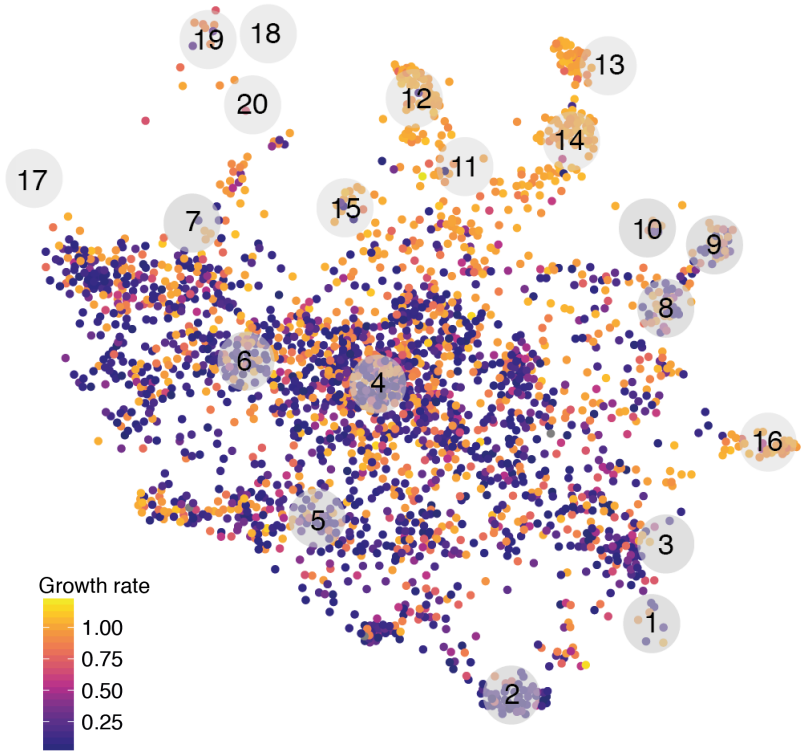


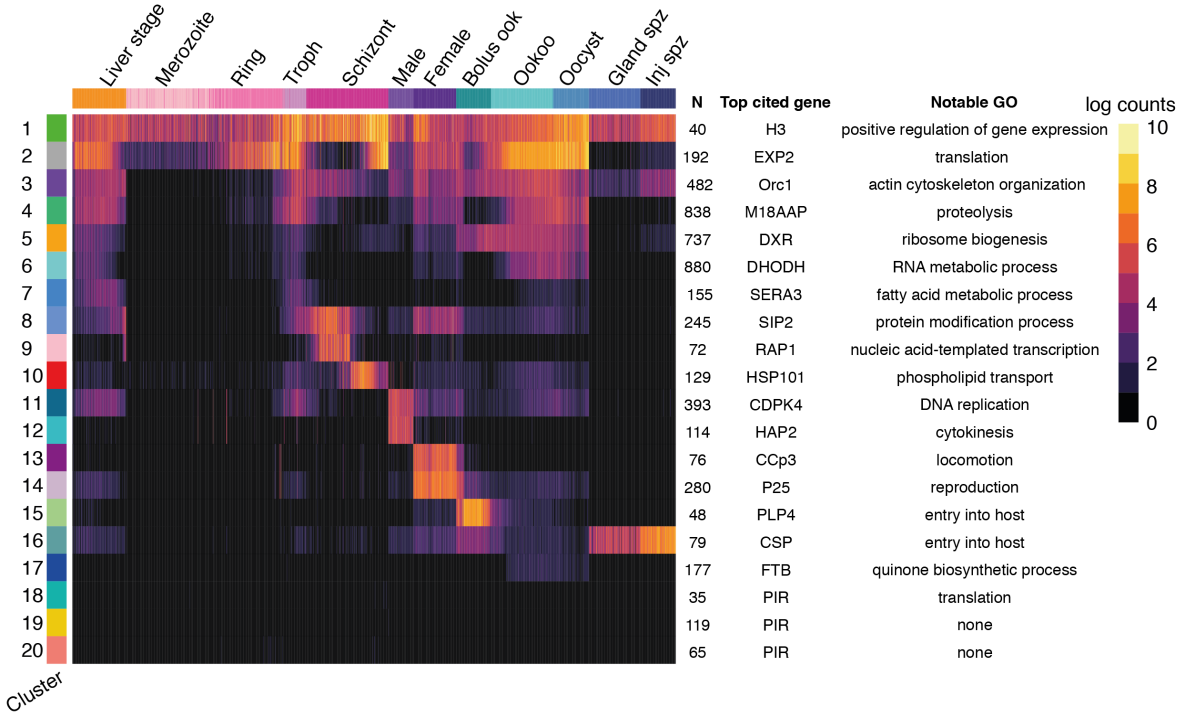
Global patterns of gene expression



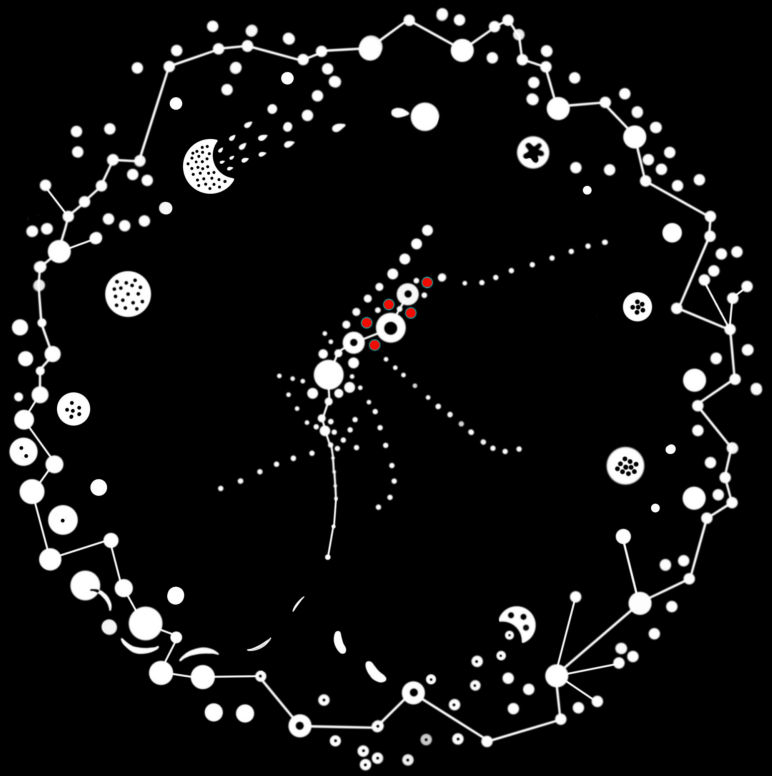


Global patterns of gene expression



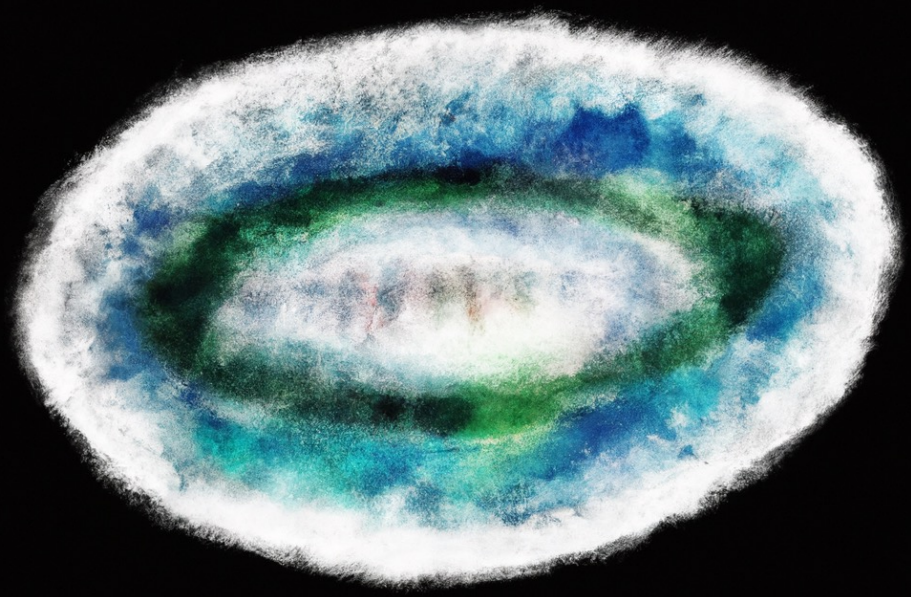


Howick*, Russell* *et al.* (2019) Science

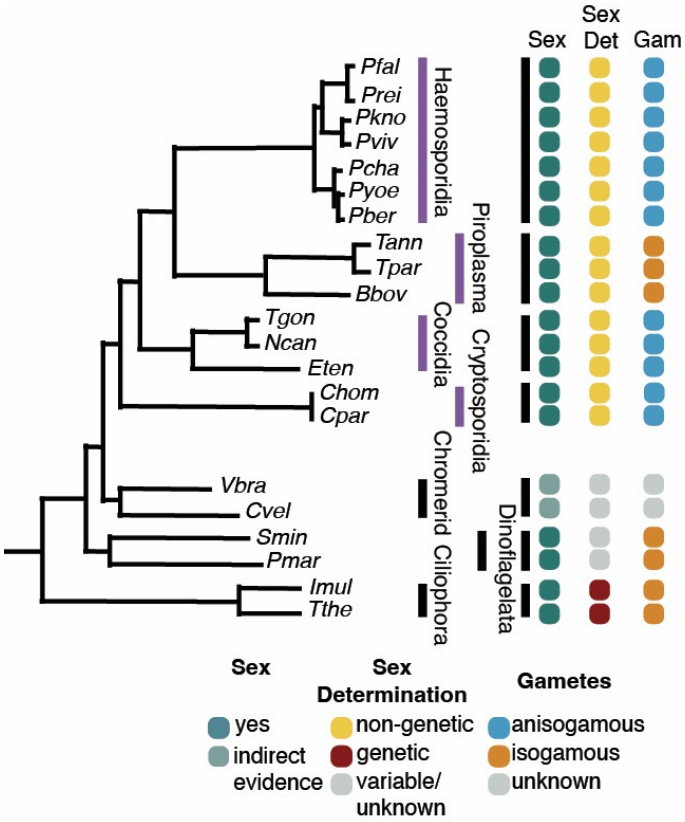


www.sanger.ac.uk/science/tools/mca/mca

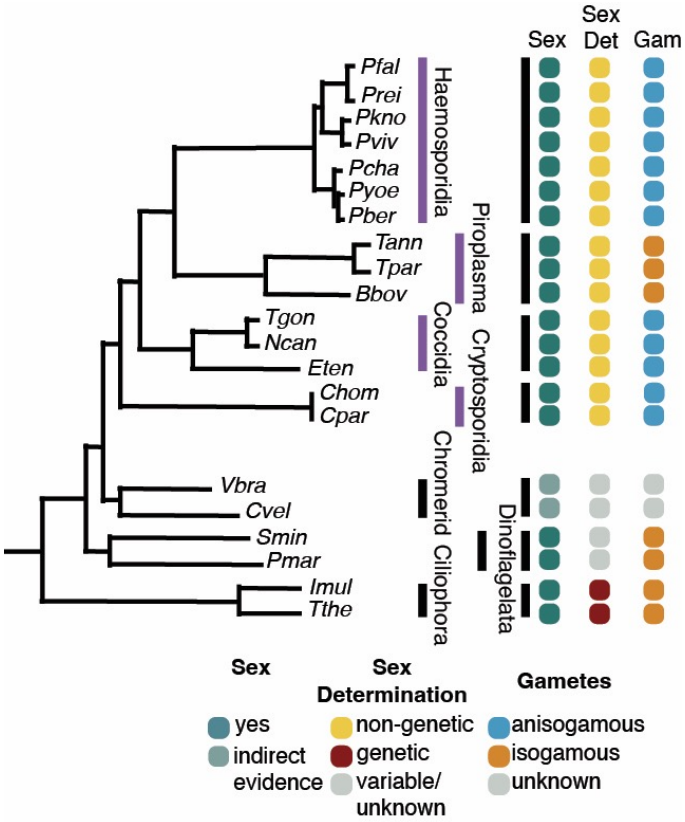
A transcriptional switch controls sex determination in *Plasmodium falciparum*



Sex determination in Alveolata



Sex determination in *Plasmodium*

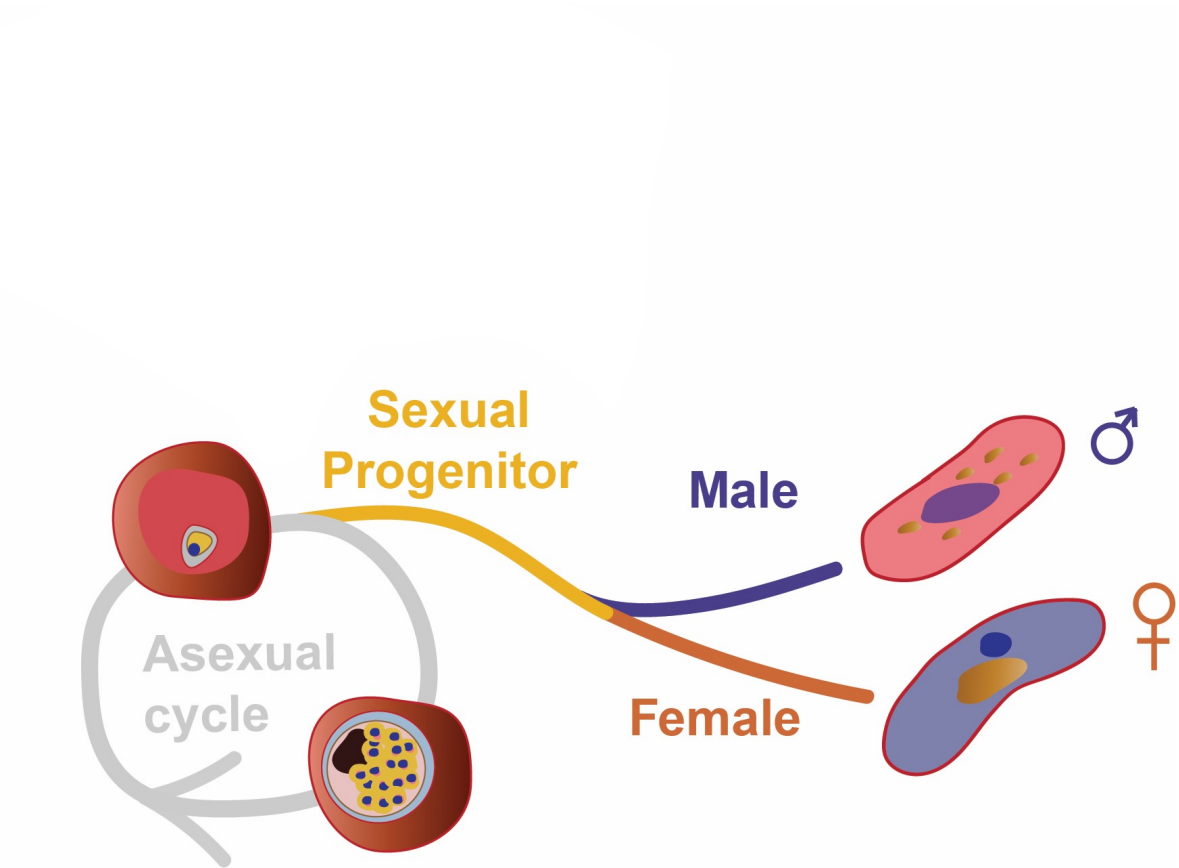


Plasmodium has a haplontic life cycle, is homothallic (mating can occur between genetically identical cells)

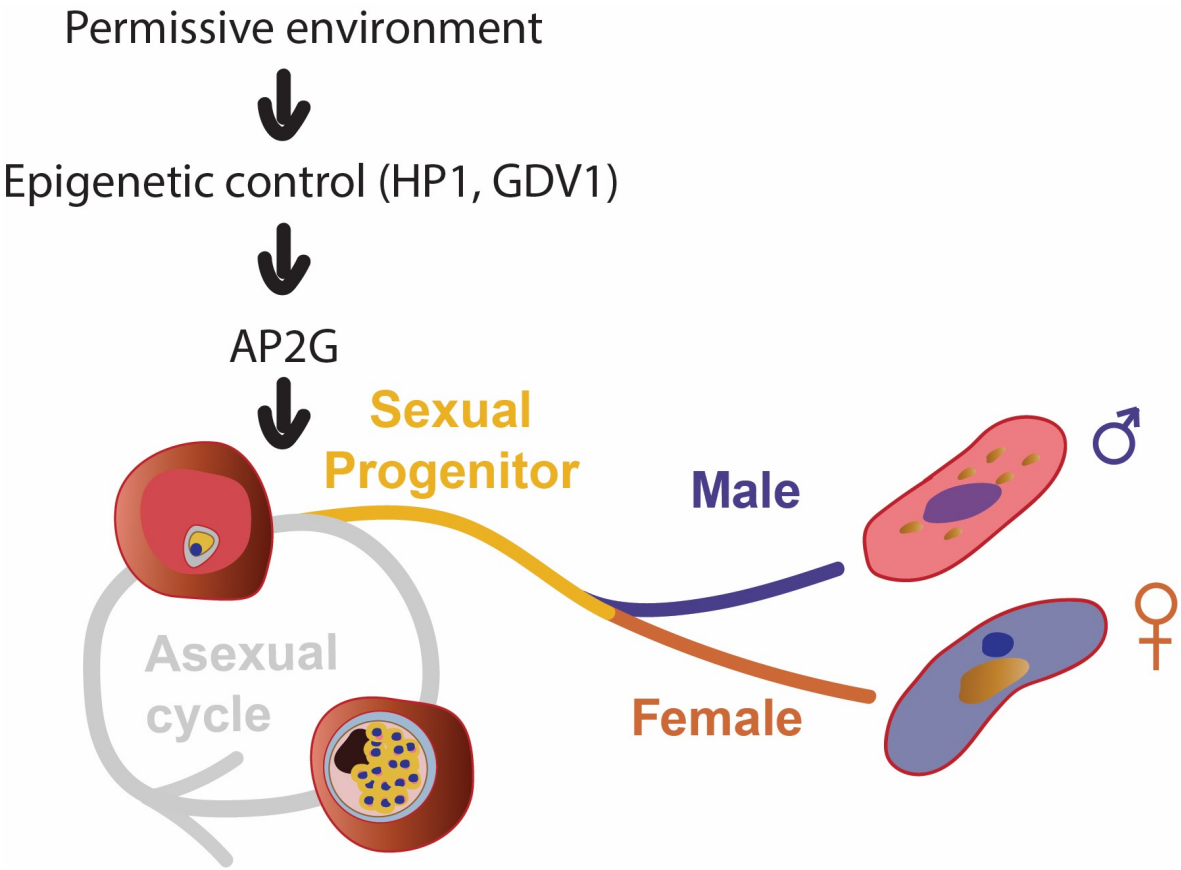
No sex chromosone or mating type locus = non-genetic sex determination

No conserved sex determination factors identified

Sex determination in *Plasmodium*

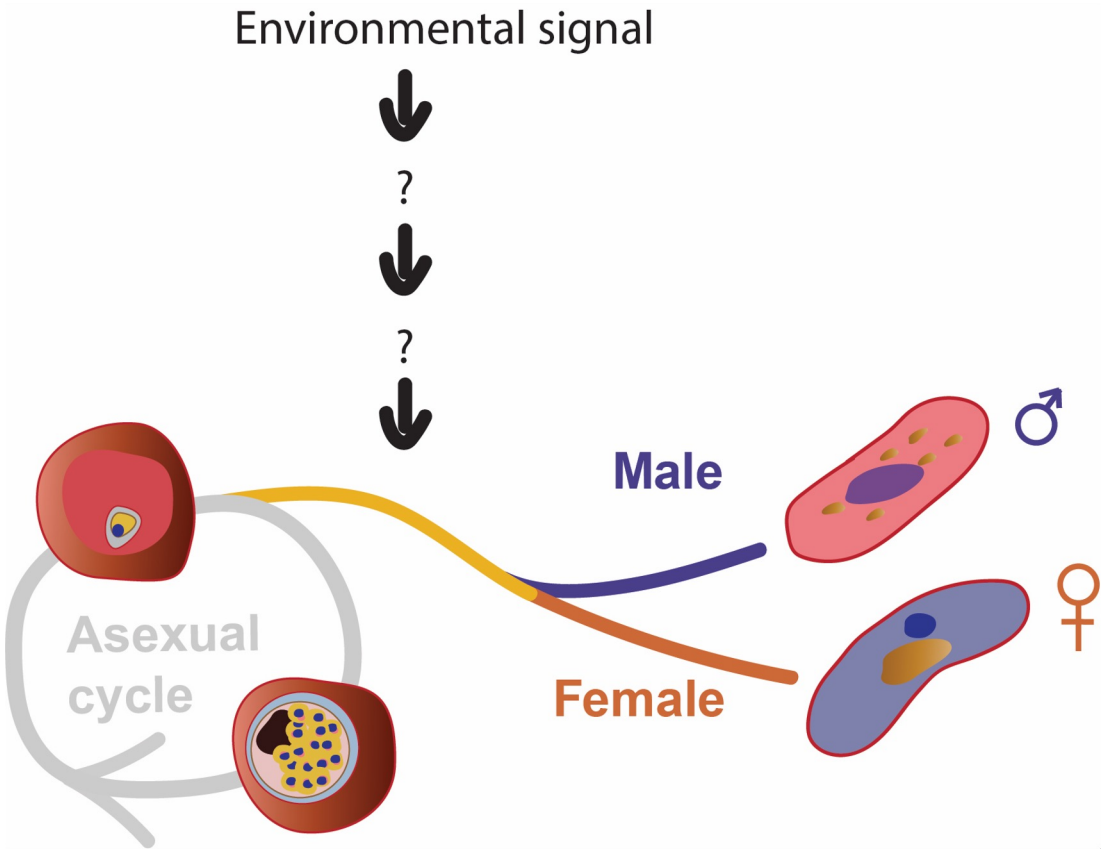


Sex determination in *Plasmodium*



Kafsack *et al.* 2014, Sinha *et al.*, 2014
Brancucci, Gerdt *et al.*, 2017
Kent, Modrzynska *et al.*, 2018
Filarsky *et al.*, 2018
Bancells *et al.*, 2019
Josling *et al.*, 2020

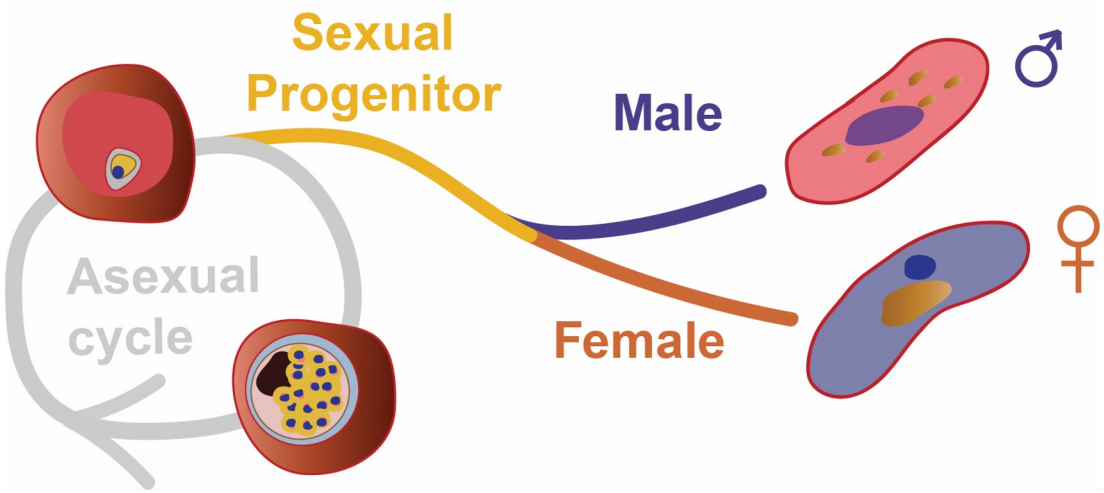
Sex determination in *Plasmodium*



Reece *et al.*, 2008
Schneider *et al.*, 2019

What is the mechanism of sex determination in *Plasmodium falciparum*?

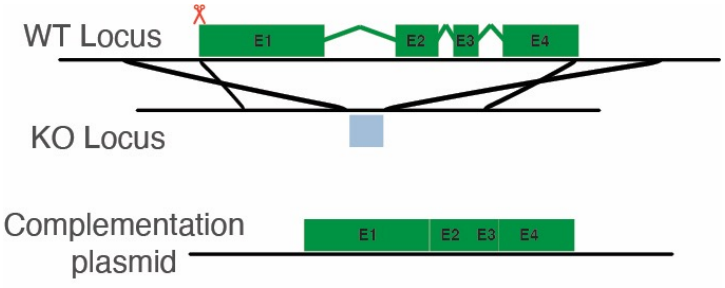
Identification of candidate sex determining effectors in *P. berghei*
(Russell†, Sanderson†, Bushell†, Talman† *et al., bioRxiv 2021*)

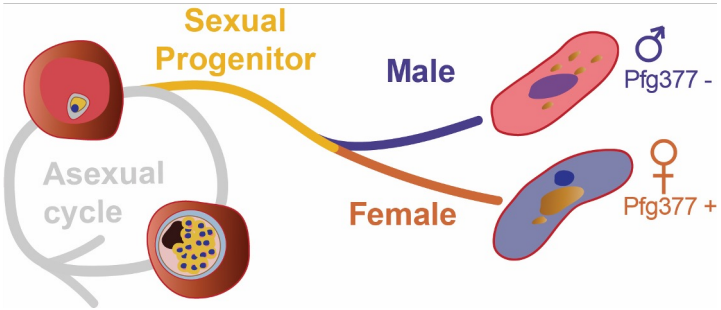


Ana Gomes

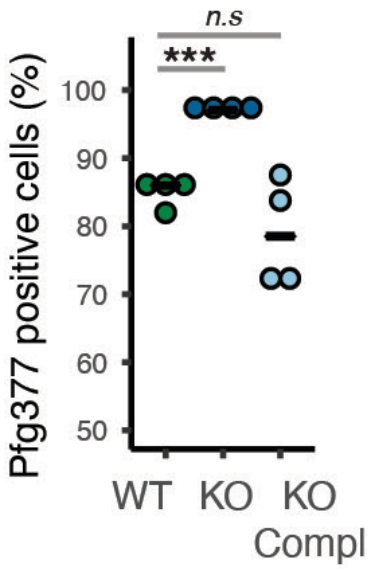
Celia Bardy

What is the role of *md1* in *P. falciparum* sex determination?

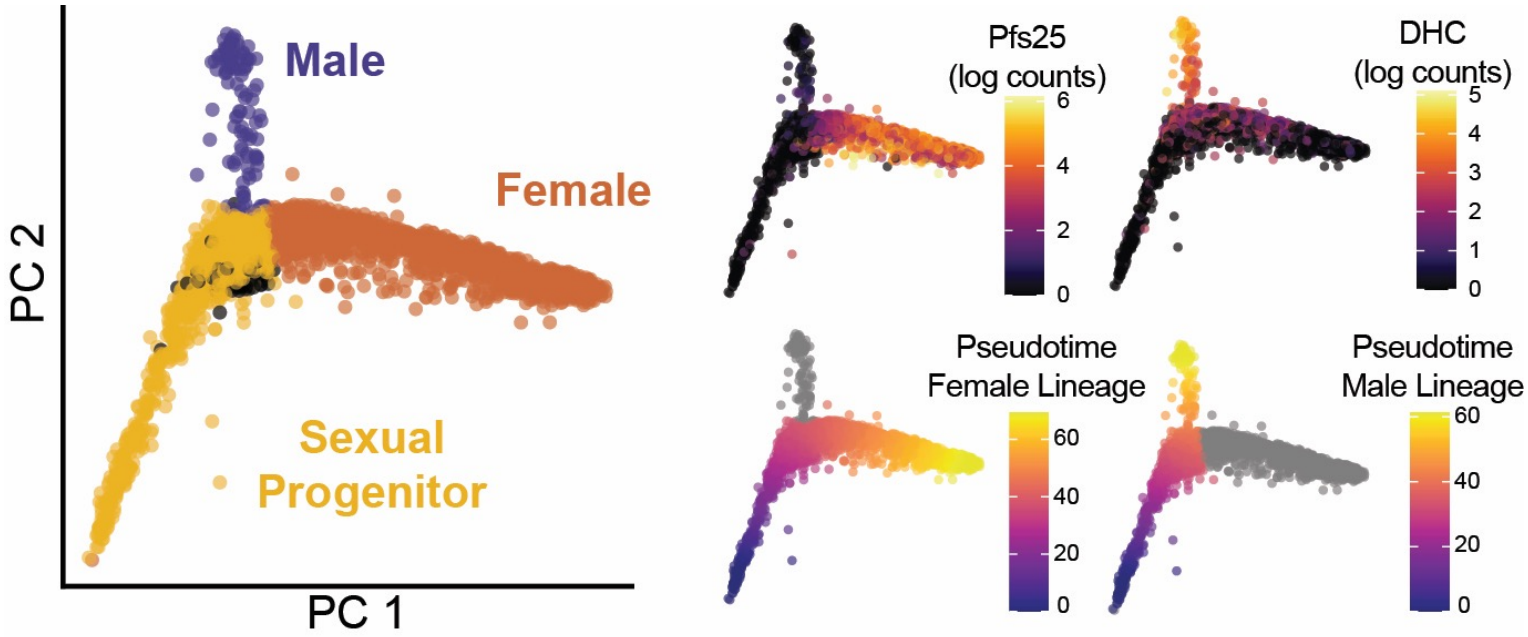




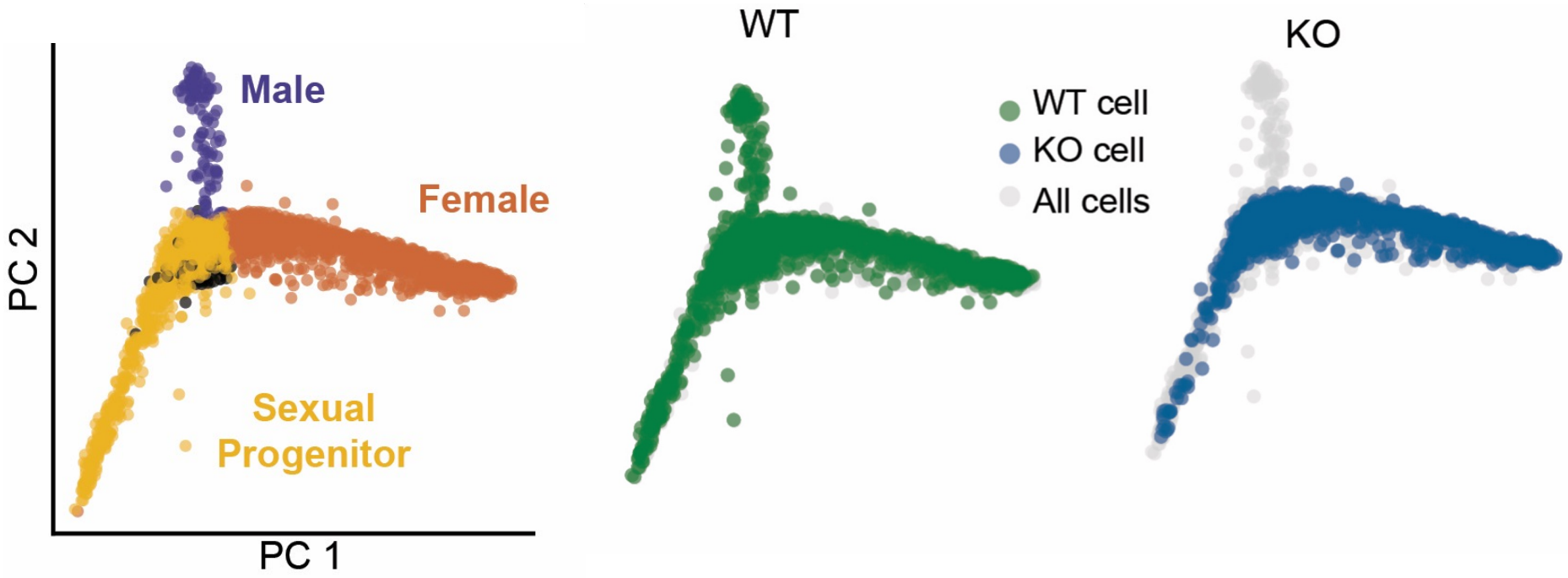
Absence of male cells?



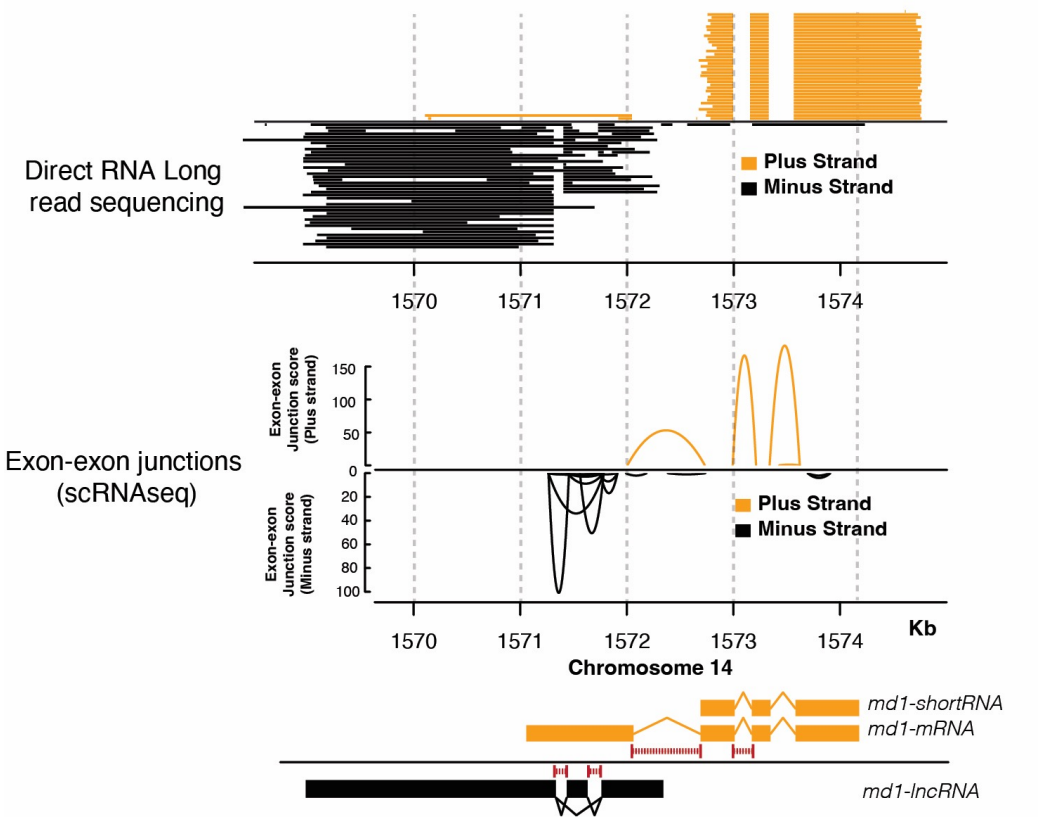
What is the role of *md1* in *P. falciparum* sex determination?



md1 is necessary to determine male fate



Transcription at the *md1* locus

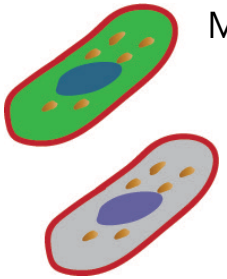


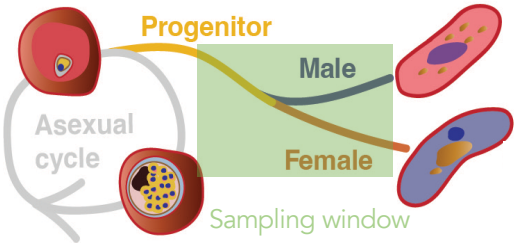
Three different RNA species are produced at the *md1* locus

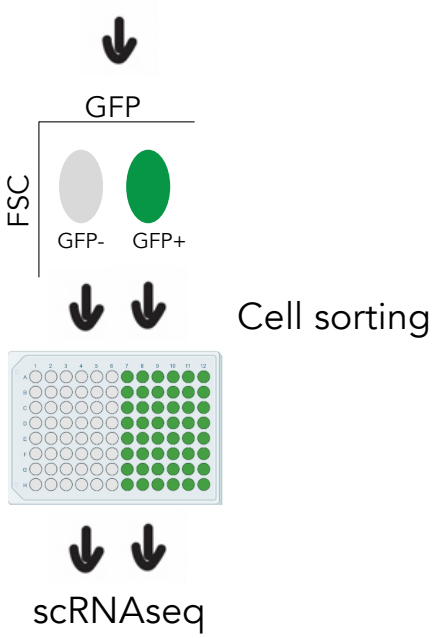
Ana Gomes

Dissecting the pattern of transcription and translation at the *md1* locus

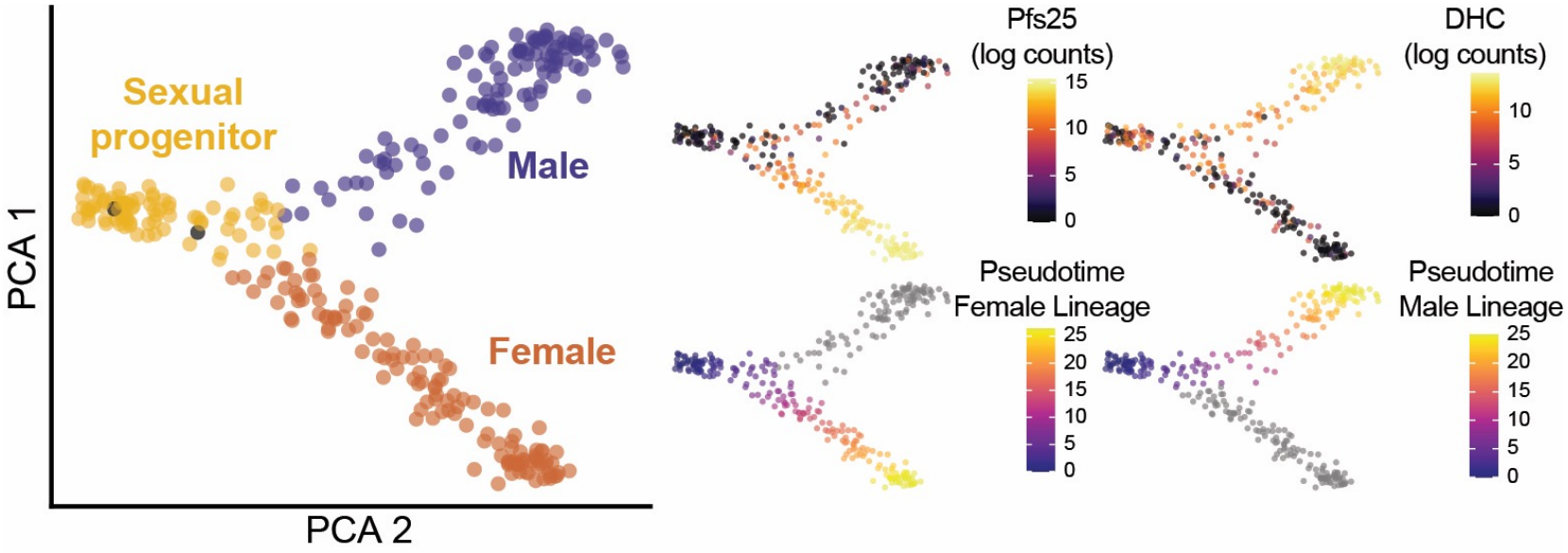
Translational reporter



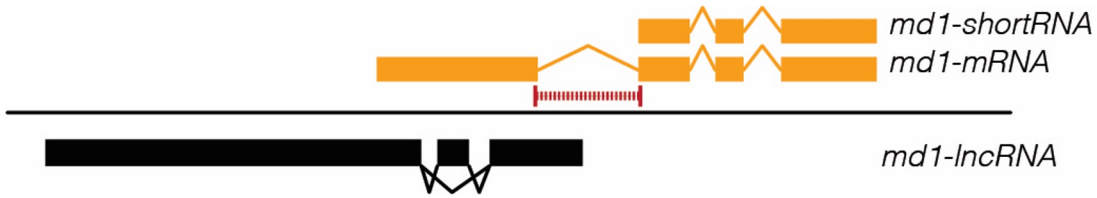




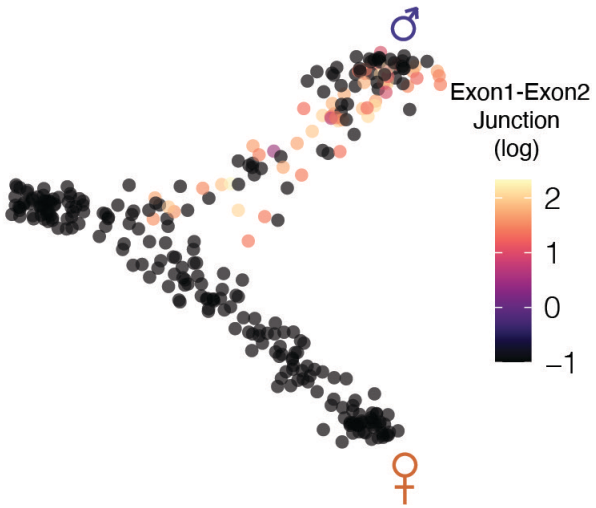
Dissecting the pattern of transcription and translation at the *md1* locus



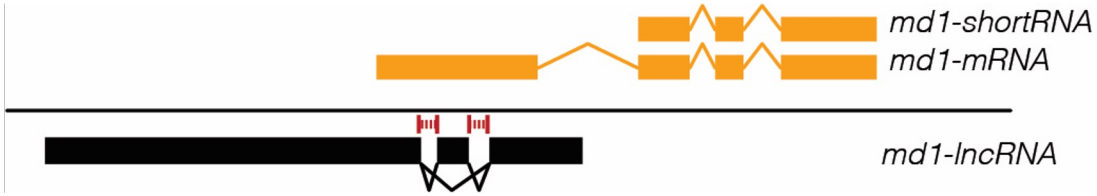
Dissecting the pattern of transcription and translation at the *md1* locus



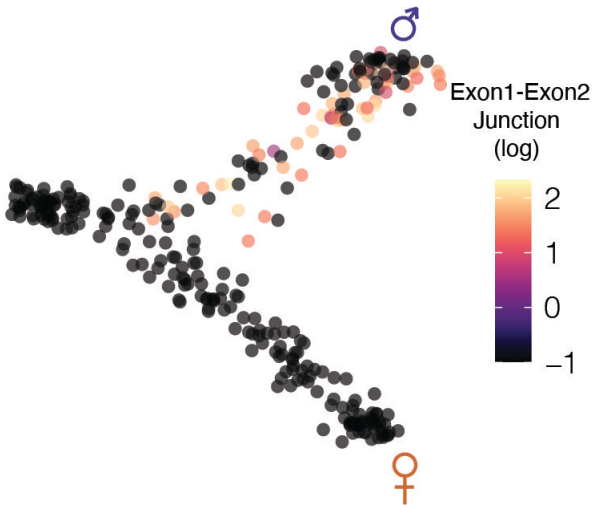
md1 mRNA



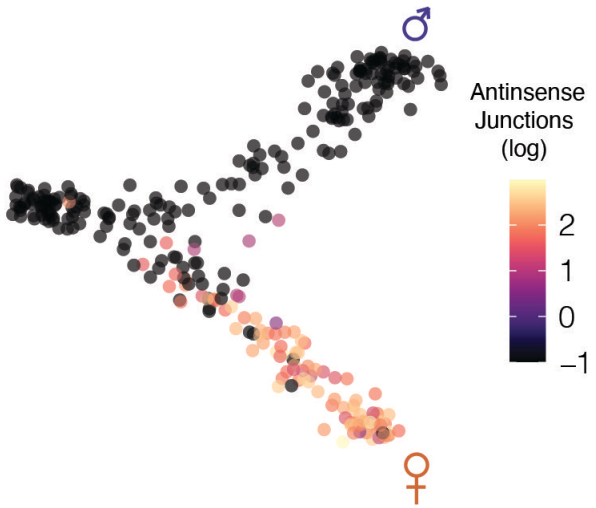
Dissecting the pattern of transcription and translation at the *md1* locus



md1 mRNA

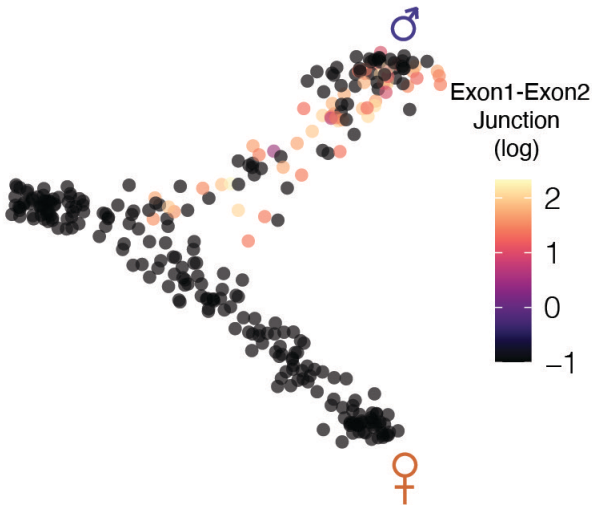


md1 lncRNA

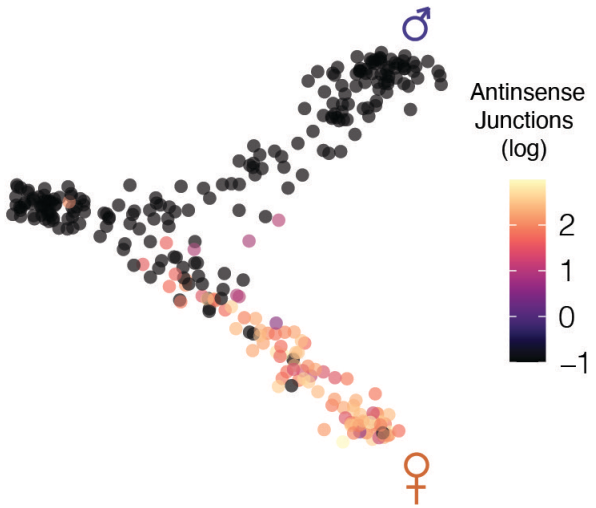


Dissecting the pattern of transcription and translation at the *md1* locus

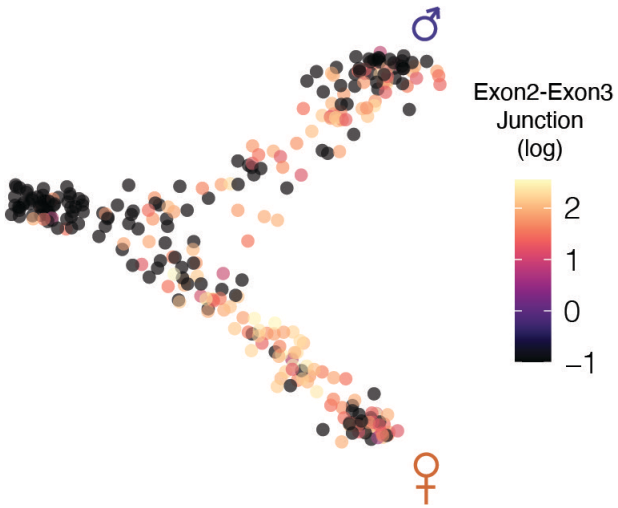
md1 mRNA



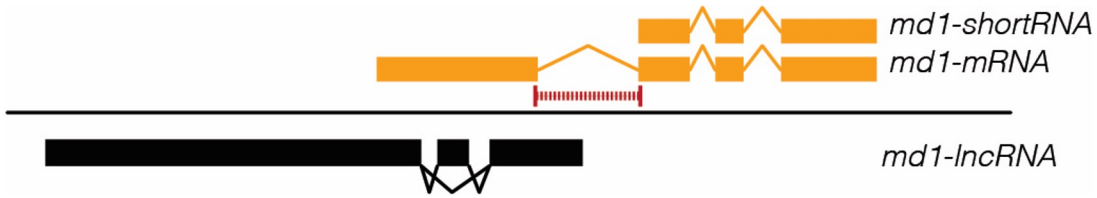
md1 lncRNA



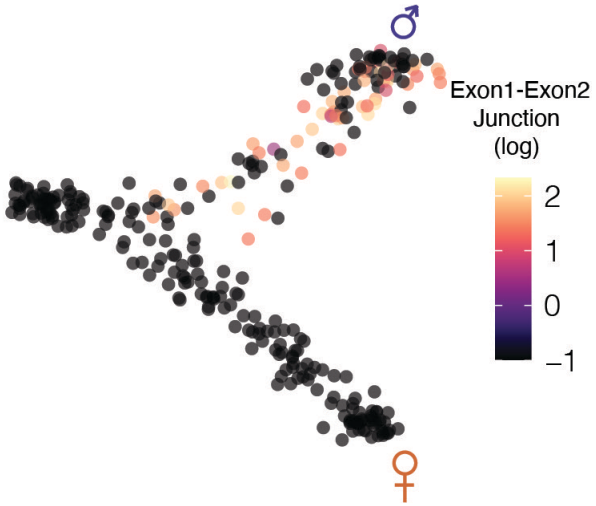
Exon2-Exon3 reads



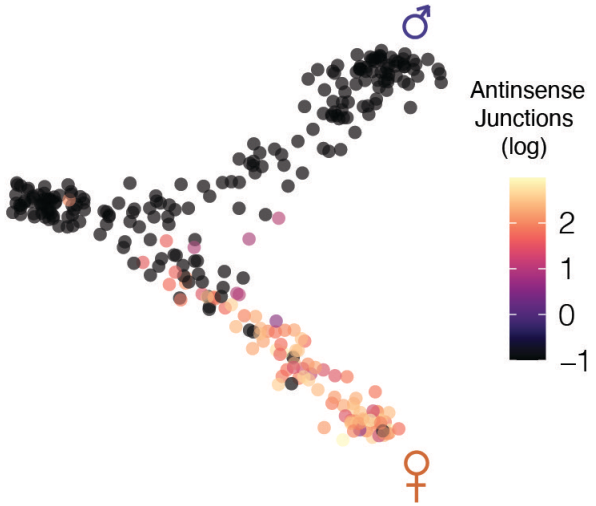
Dissecting the pattern of transcription and translation at the *md1* locus



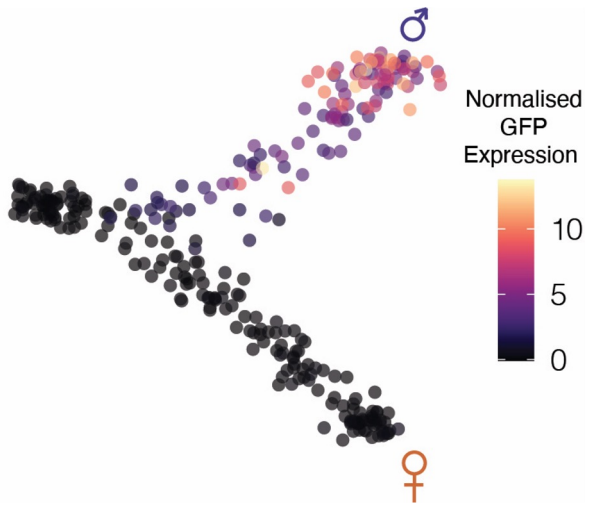
md1 mRNA



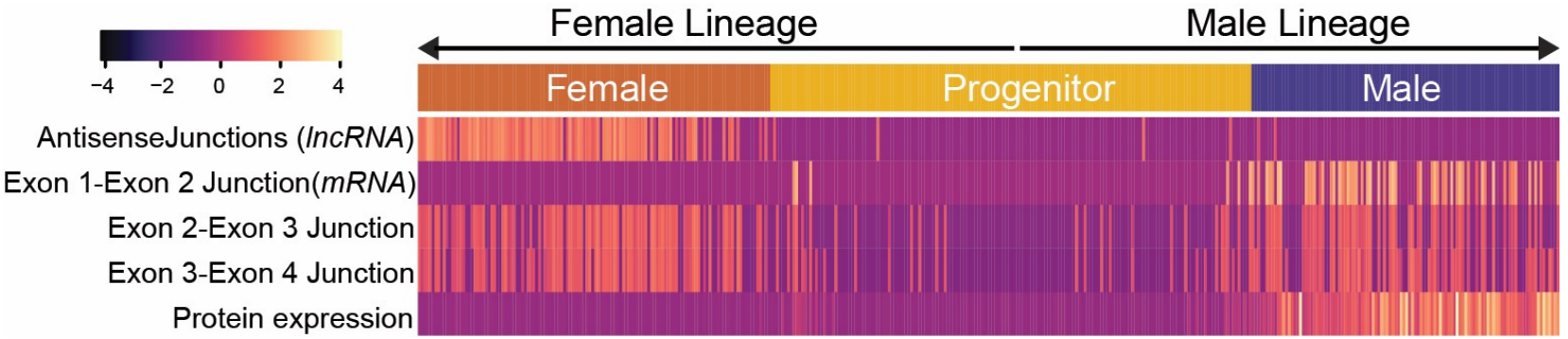
md1 lncRNA

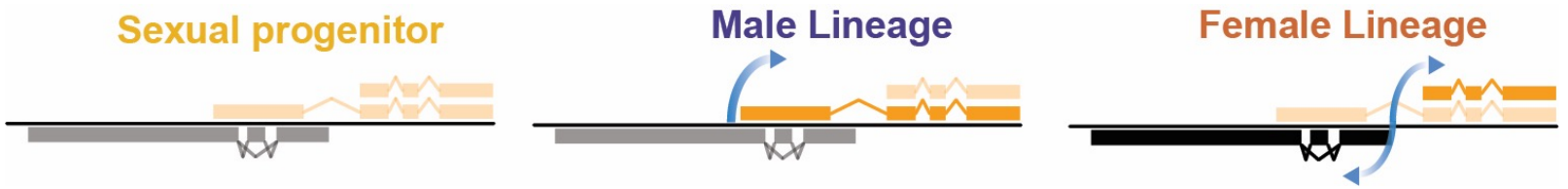


Protein intensity

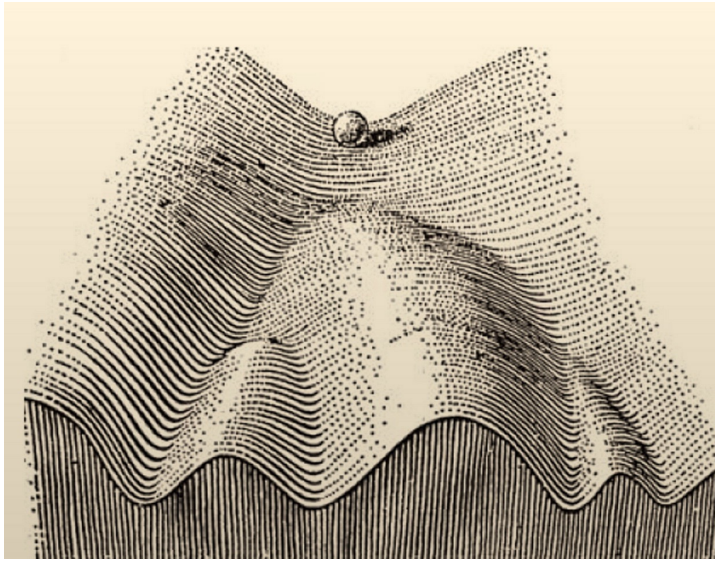


A transcriptional switch at the *md1* locus





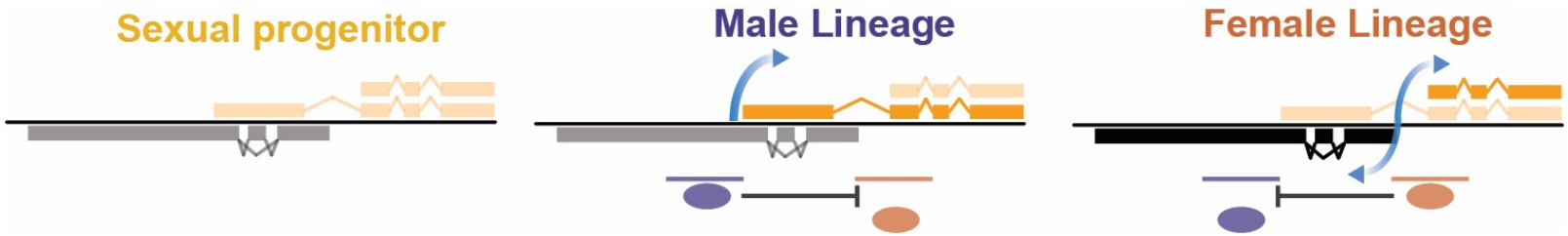
A transcriptional switch at the *md1* locus



_md1_ regulation

Mutually exclusive *md1* transcriptional states are coupled with the sex determining event

Competition/occupancy regulates the locus



Gomes *et al.*, *Nature* 2022

Single Cell technologies – Recent innnovations

Analysis-> new tools released weekly, exciting field of innovation

Multiplexing/Cell hashing-> cheaper but still technically imperfect for 10X solutions

Surface proteomics imputation/index sorting-> Surface proteins measurements coupled to transcriptomes

Long read sequencing -> Better isoforms, important for multigene families

Sc genomes-> genetic variation with single cell resolution

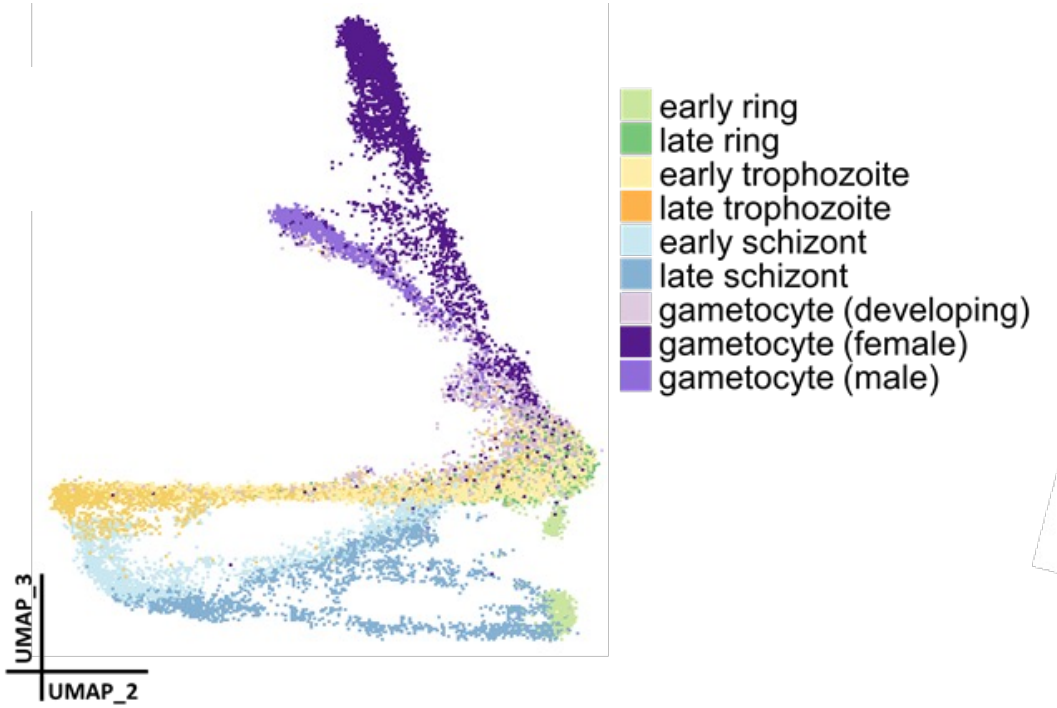
Epigenome-> Atac, chip, methylome, cut and run, HiC…

Multi-omics-> Multiple measurements in the same cell…

Single cell proteomics -> Still a bit far off for small parasites…

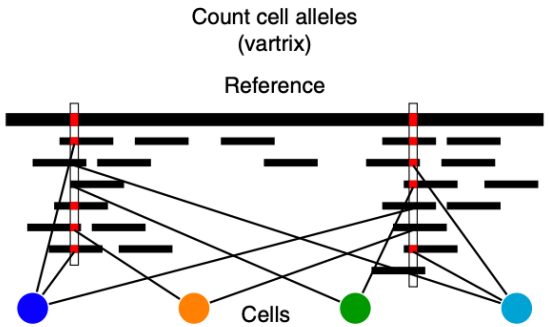
Single Cell technologies – Long reads

A new iteration of the MCA
with long reads
(10x + PacBio Isoseq)

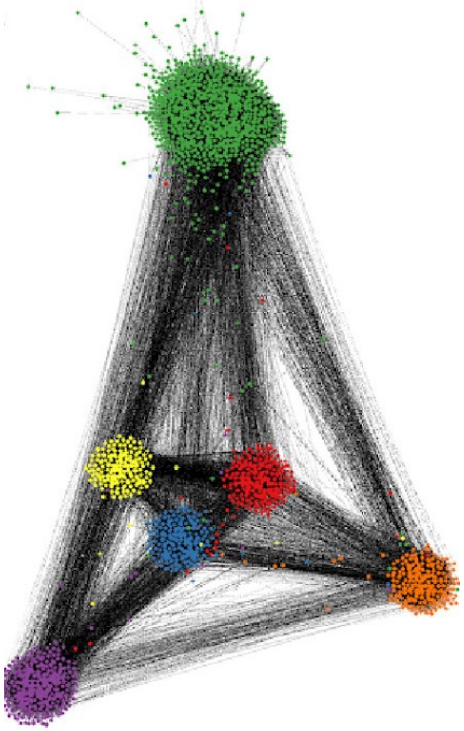


Dogga, Rop, Cudini *et al.,* bioRXiv

Single Cell technologies – Genomics meets transcriptomics



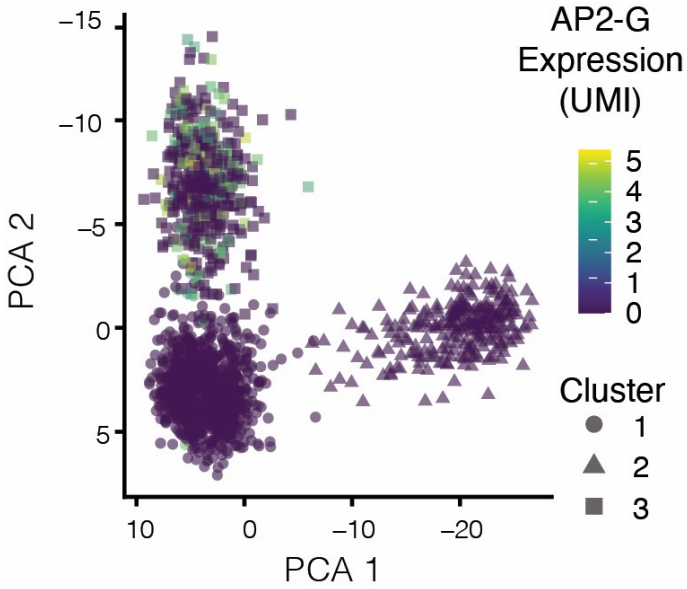
For each cell we get the **stage**, **genotype** and **transcriptome**

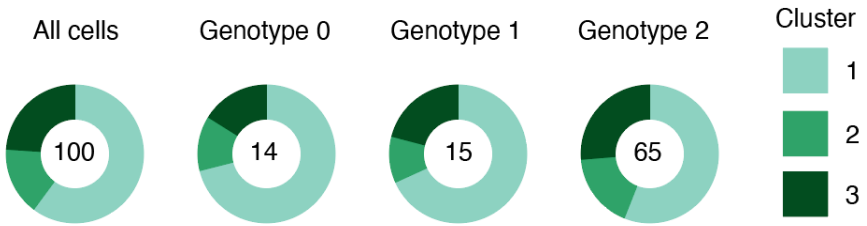


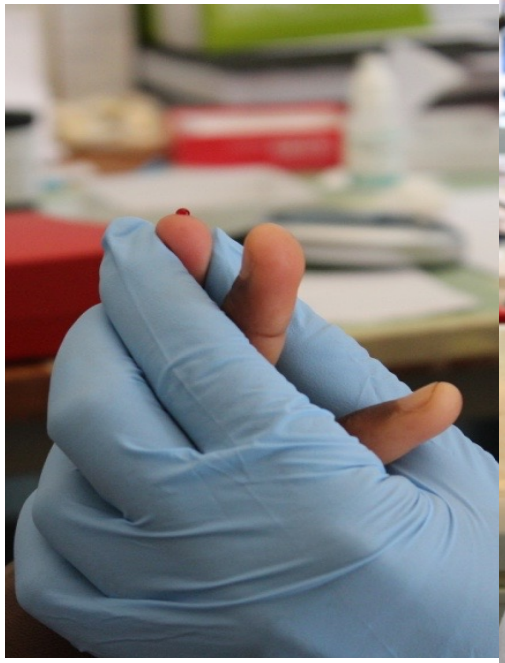
Heaton *et al., Nat methods* (2020)

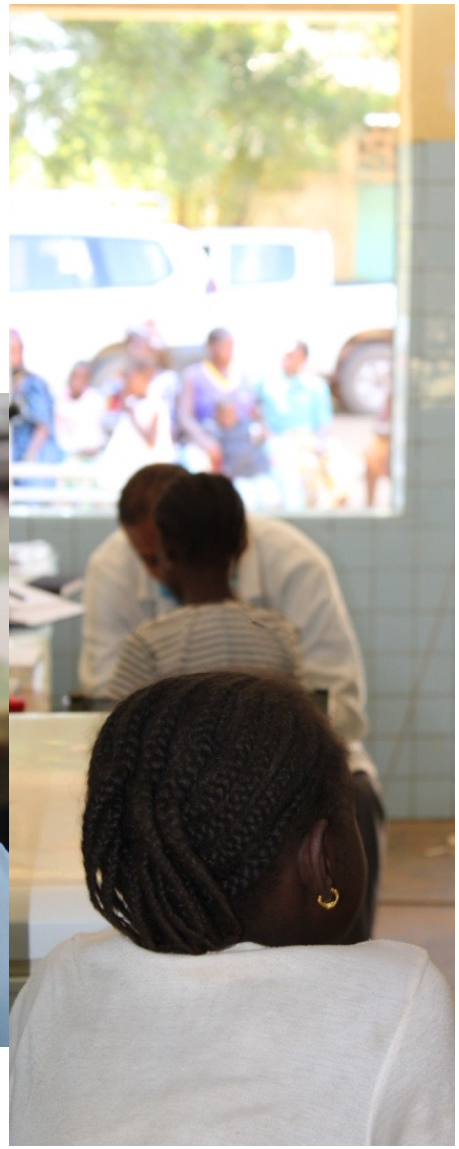
Haynes Heaton

Single Cell technologies with real parasites



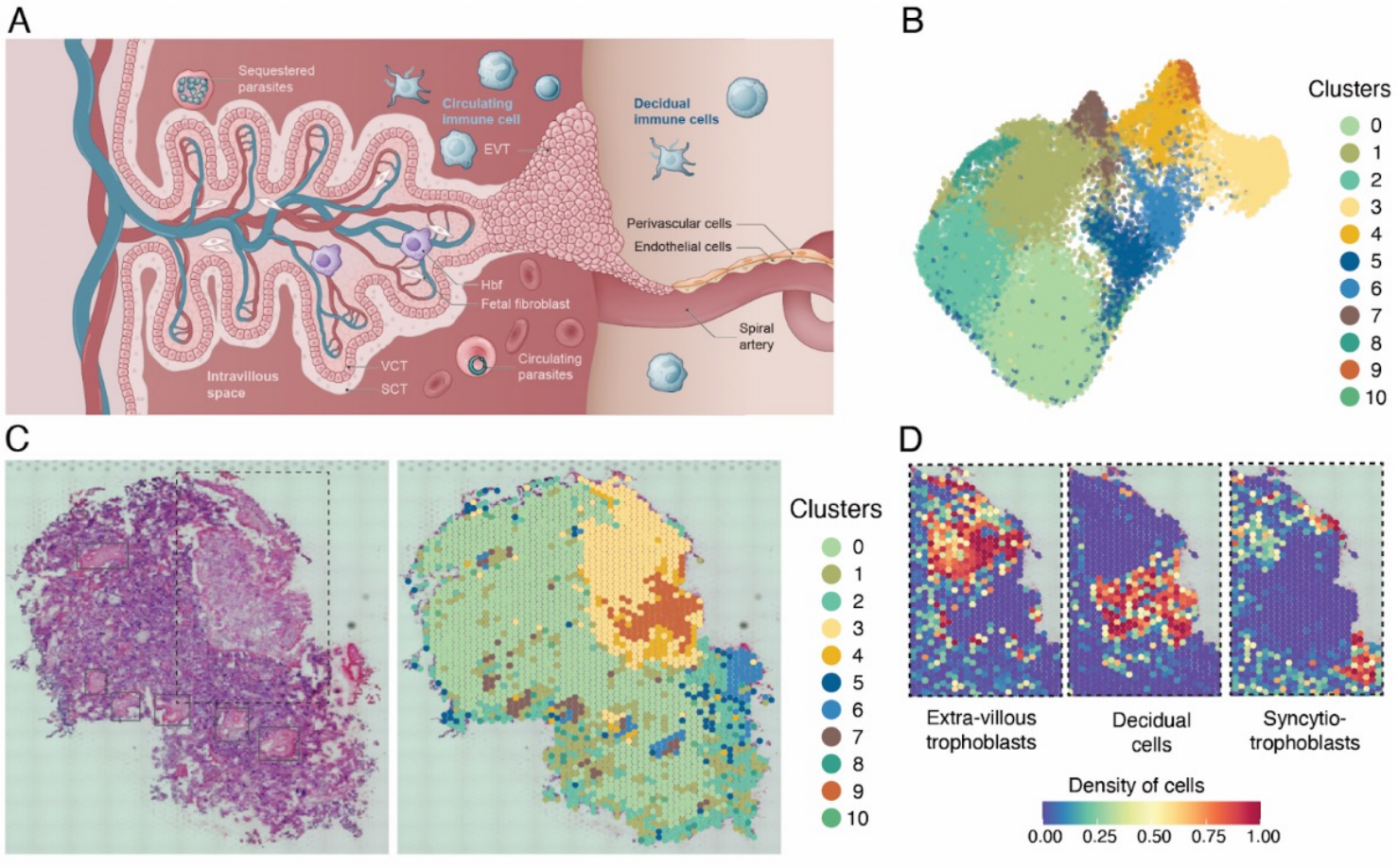






Ngou, Marin Menendez *et al.*, soon

Beyond single cell approaches



Delorme, Cassan, Dechavanne *et al.,* soon

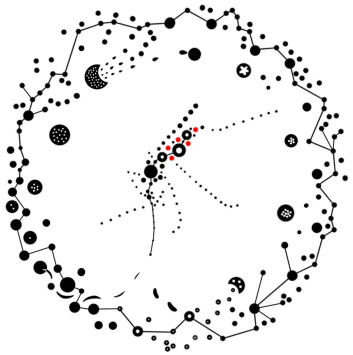
Plasmodium berghei work

Theo Sanderson
Andrew Russell
Tom Metcalf
Frank Schwach
Colin Herd
Burcu Anar
Gareth Girling
Julian Rayner
Mara Lawniczak

Core cytometry and DNA Pipelines
Bee Ng
Jennifer Graham
Chris Hall
Sam Thompson

Ellen Bushell
Vikash Pandey
Claire Sayers
Mirjam Hunziker
Oliver Billker

Katarzyna Modrzynska
Robyn S Kent
Andy Waters



MALARIA
CELL
ATLAS

Plasmodium falciparum work

Celia Bardy
Cecile Cassan
Alejandro Marin Menendez
Christelle Ngou
Emilie Mathis
Ange Tchakounte
François Dao
Quentin Delorme
Irene Rossi
Silvain Pinaud

Laboratory of Pathogen Host Interactions

Ana Gomes
Maryse Lebrun

MRI Montpellier Ressources Imagerie

Christophe Duperray
Elodie Jublanc

AGENCE NATIONALE DE LA RECHERCHE ANR

MGX Montpellier GenomiX

Hugues Parrinello
Dany Severac

wellcome sanger institute

Sophie Adjalley
Marcus Lee

MARIE CURIE ACTIONS

Jessica Bryant (dCas9 plasmids)
Pietro Alano (Pfg377 Ab)

MiVEGEC
Maladies Infectieuses et Vecteurs Écologie, Génétique, Évolution et Contrôle

Institut de Recherche pour le Développement FRANCE

VEuPathDB
Eukaryotic Pathogen, Vector & Host Informatics Resources

UNIVERSITE MONTPELLIER