New genetic approaches to identify antimalarial targets and measure parasite fitness

Marcus Lee

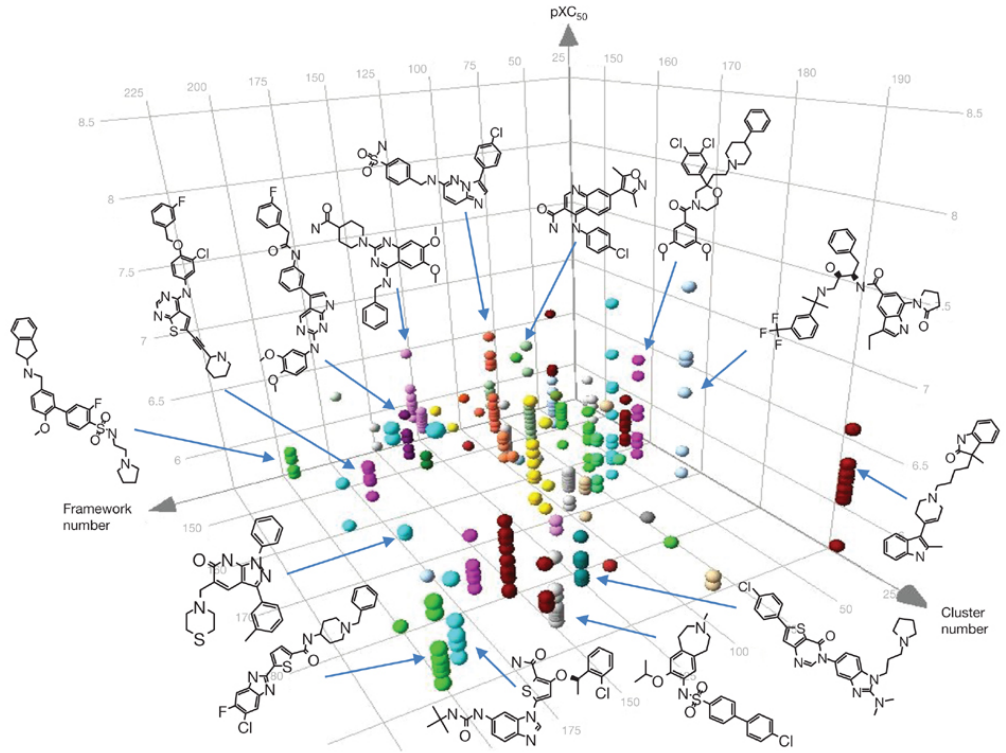
Malaria Programme

A need for new drugs / targets that are not subject to existing resistance mechanisms

Can we exploit chemical diversity to identify new targets and map resistance mechanisms?

How do we translate hits from phenotypic screens into targets?

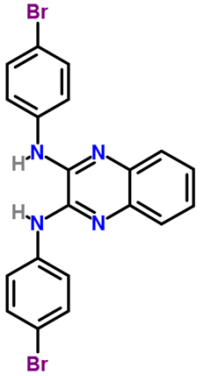
How can we utilise resistant parasites to profile new compounds?



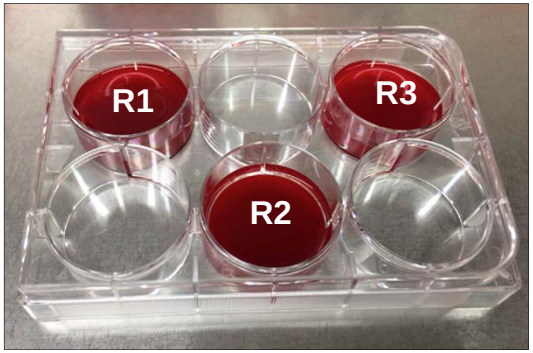
Gamo et al., (2010) Nature

Develop an experimental framework for evaluating new compounds

Unknown MOA

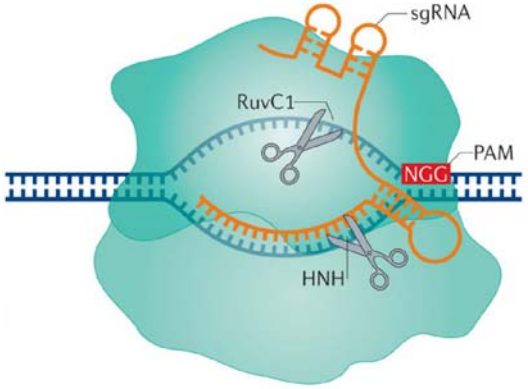


Resistance selections



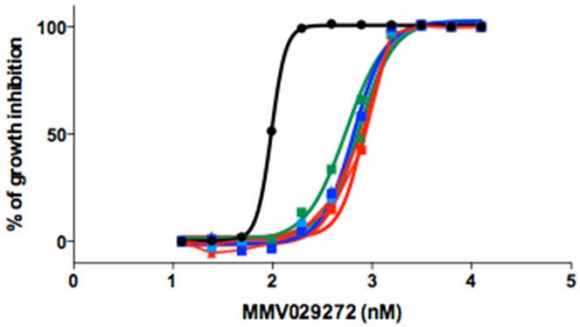
Whole genome sequencing

CRISPR/Cas9 validation

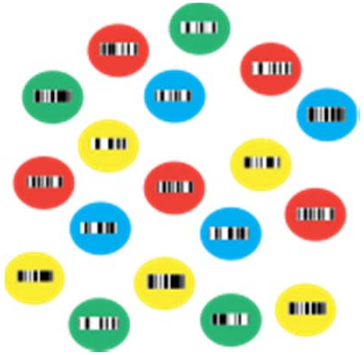


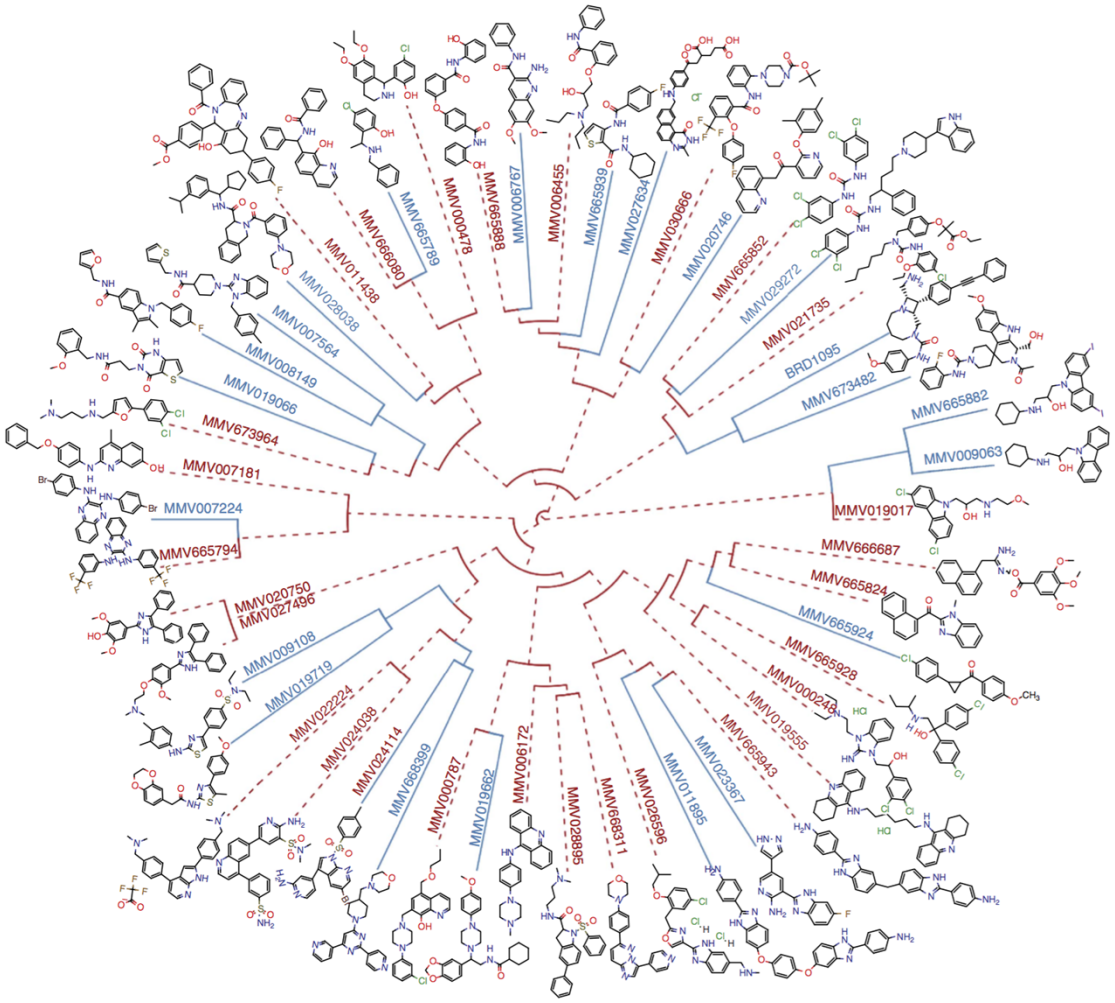
Dominguez *et al.*, (2015)
Nature Rev Mol Cell Biol.

Phenotyping



Barcoding





Corey *et al*.,(2016) *Nat Comm*

Cowell *et al*., BioRxiv

Malaria Drug Accelerator Consortium

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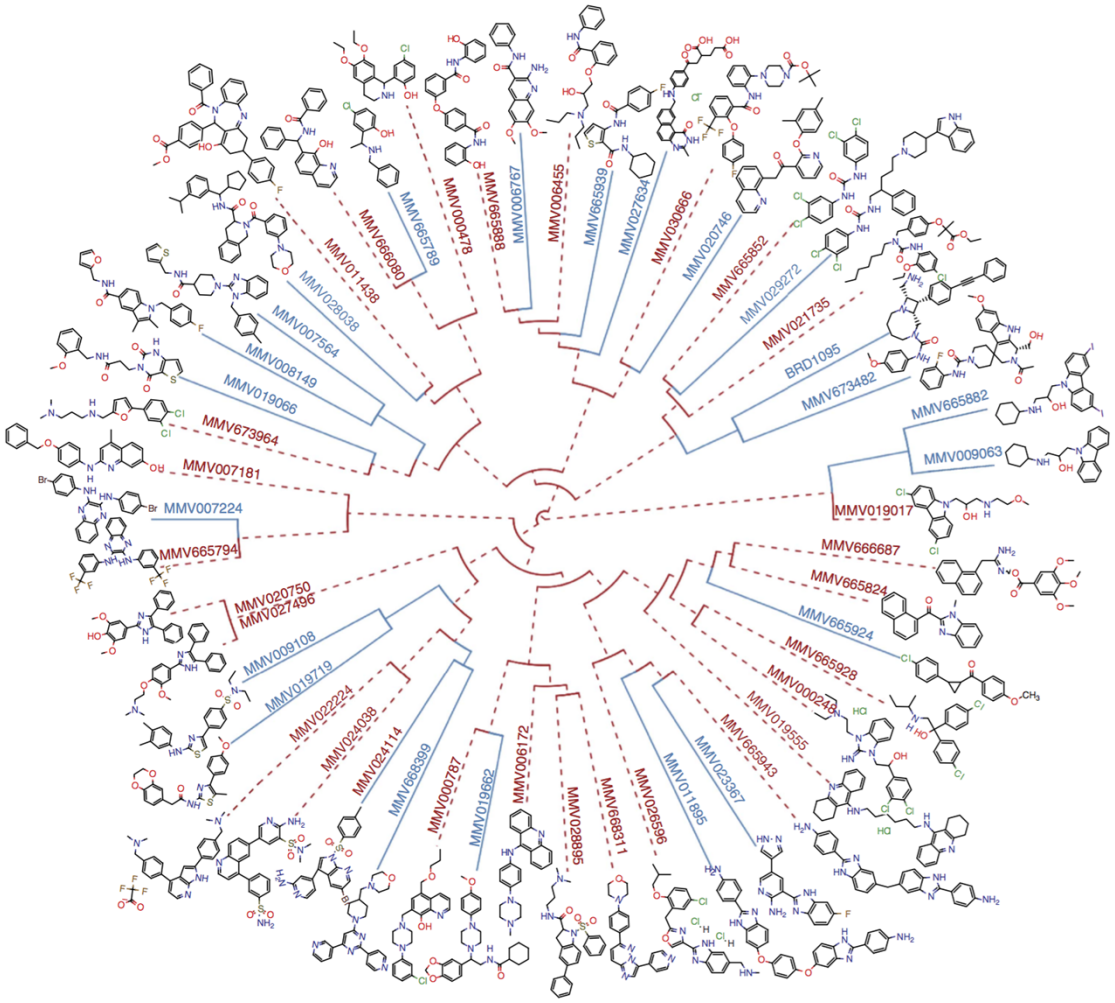
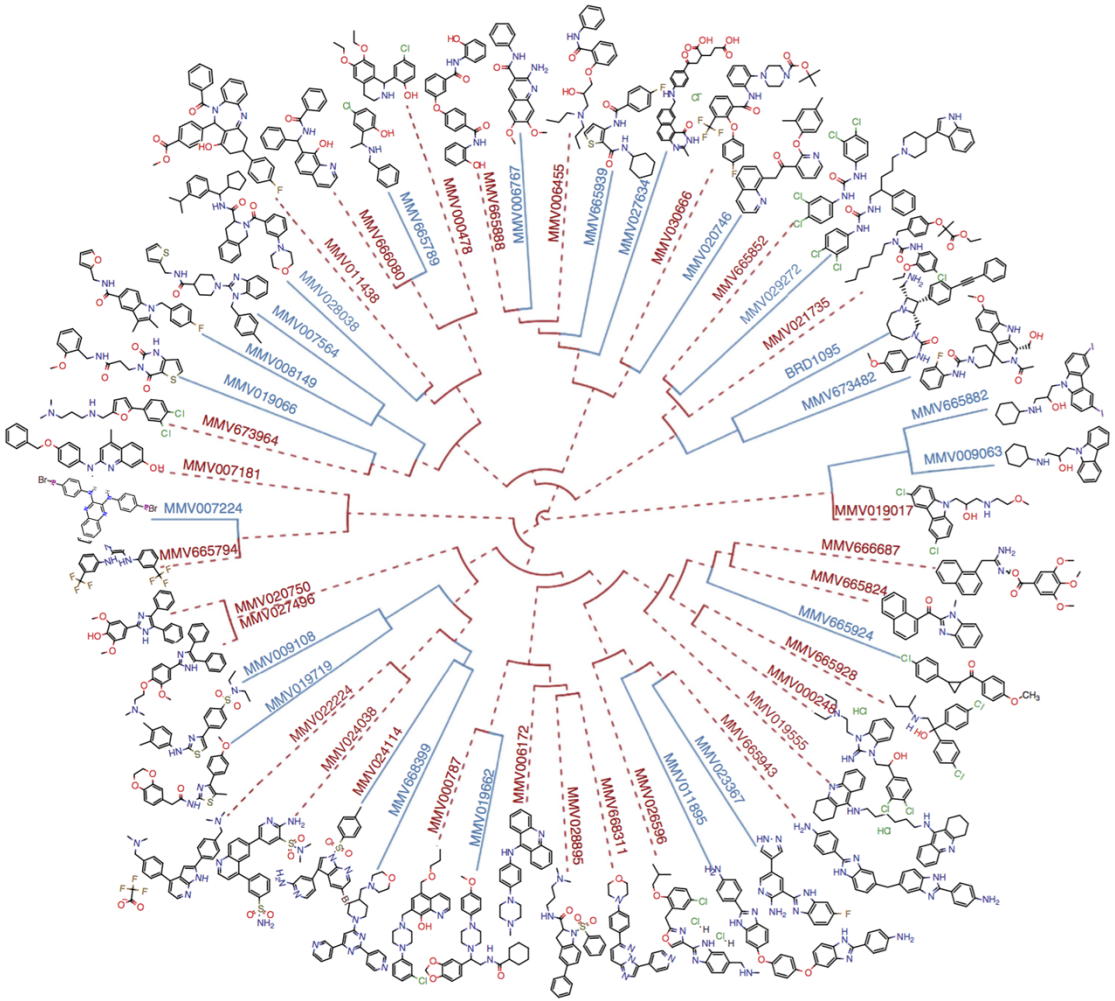
BILL & MELINDA GATES *foundation*



Corey *et al*.,(2016) *Nat Comm*

Cowell *et al*., BioRxiv

Chemically diverse starting points. Drawn from open source collections including the MMV Malaria Box.



Corey *et al*.,(2016) *Nat Comm*

Cowell *et al*., BioRxiv

Challenge parasites with long-term selections.

Whole-genome sequencing of 262 evolved clones.

resistance selected

no resistance

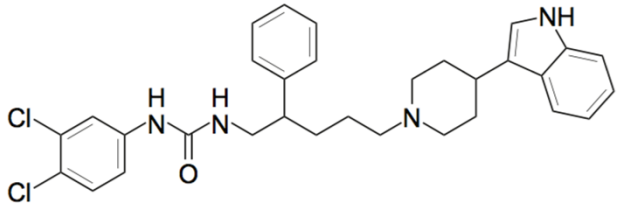
Vacuolar protease DPAP1 is a likely target of MMV029272

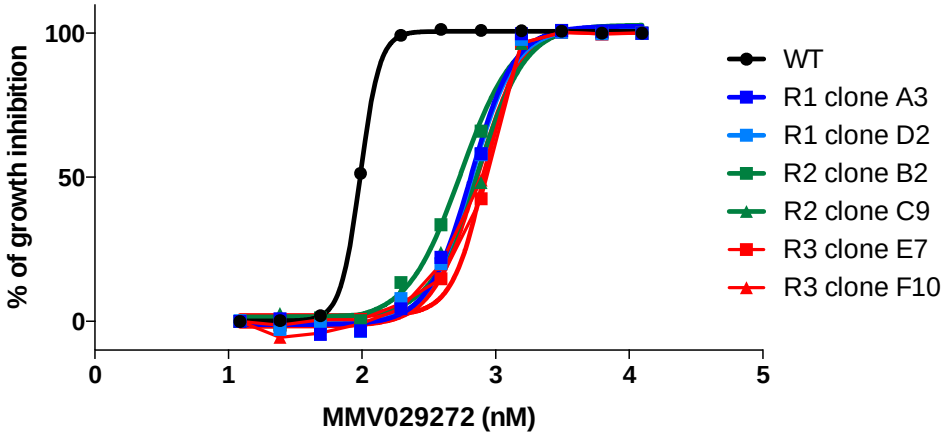
Single-step selections not successful.

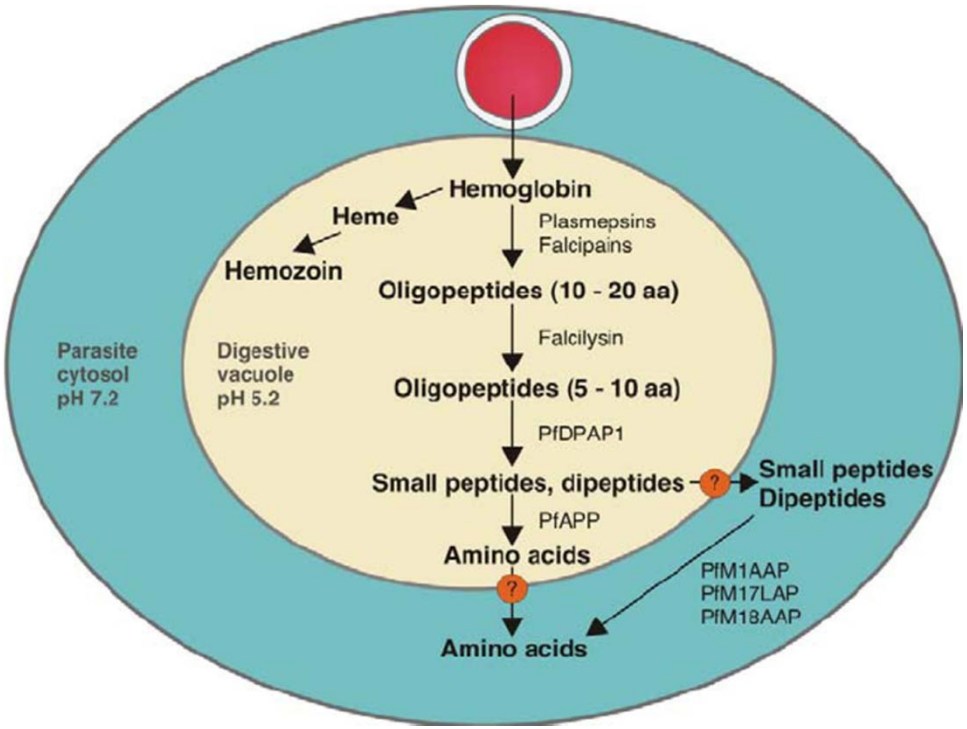
Intermittent pulse of compound yielded resistant parasites.

Resistant clones had a 6 - 8 fold shift in EC50

- sequencing identified mutations in dipeptidyl aminopeptidase 1 (DPAP1)
- N62H, L415P, and L437S







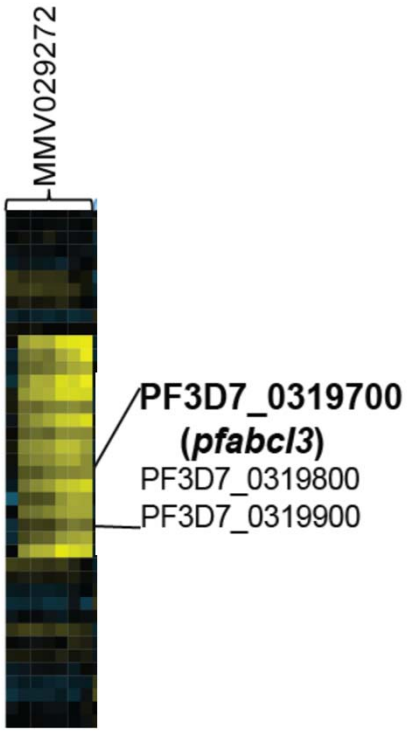
Skinner-Adams *et al.*, (2010) TIBS

ABC transporters modulate resistance to MMV029272

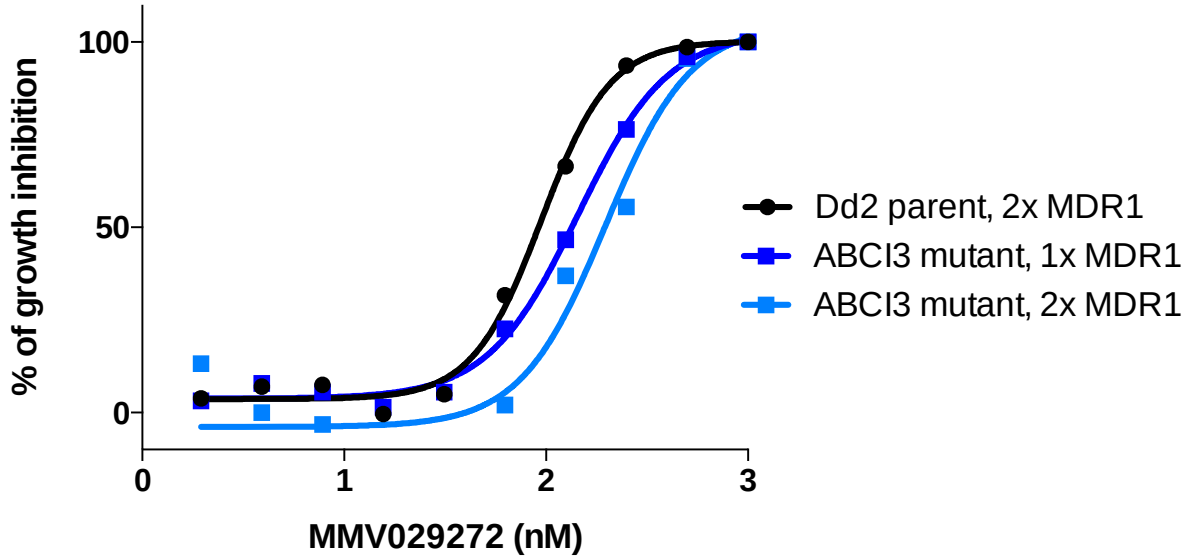
All resistant clones also had a CNV in an ABC transporter, ABCI3.

ABCI3-S678F mutant confers a 2-fold shift in EC50.

Loss of *mdr1* copy number decreases resistance.



Cowell *et al.*, BioRxiv

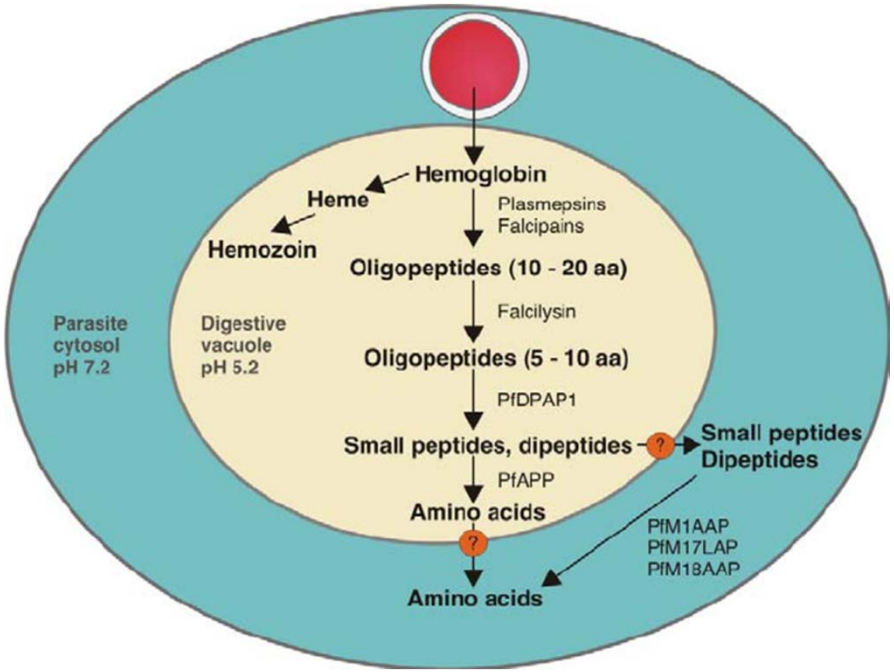


Multiple factors contribute to MMV029272 resistance

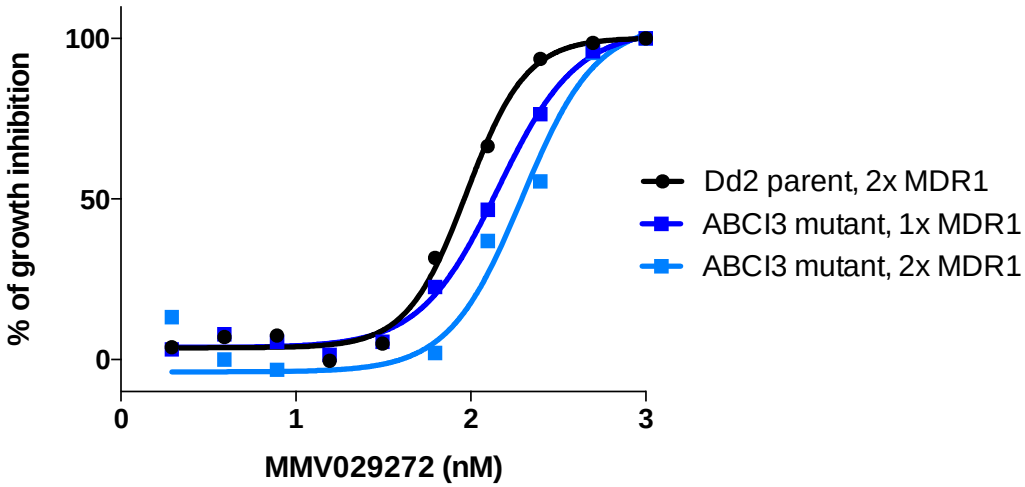
MMV029272 likely acts by perturbing hemoglobin degradation.

ABC transporters contribute to the resistance phenotype.

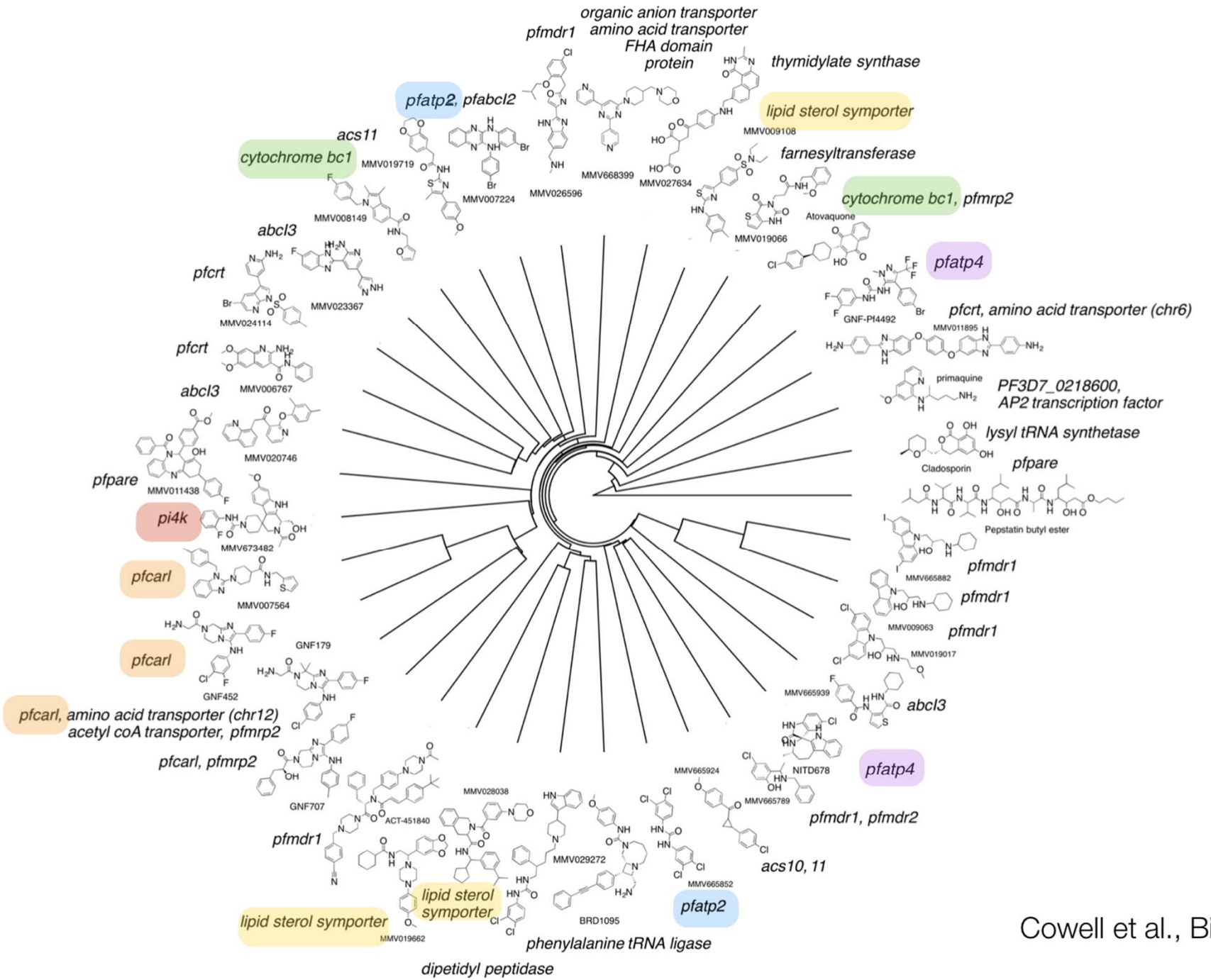
CRISPR dissection of these contributions ongoing.



Skinner-Adams *et al.*, (2010) TIBS



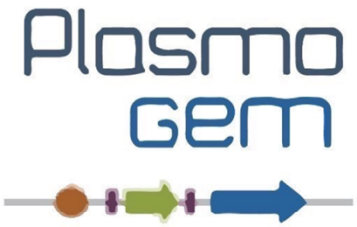
Profiling compound mode-of-action using resistant parasites

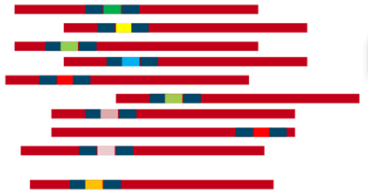


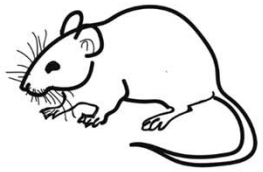
Cowell et al., BioRxiv

Barcode tagging parasites using CRISPR/Cas9

Adapting PlasmoGEM *P.berghei* approach to *P.falciparum*

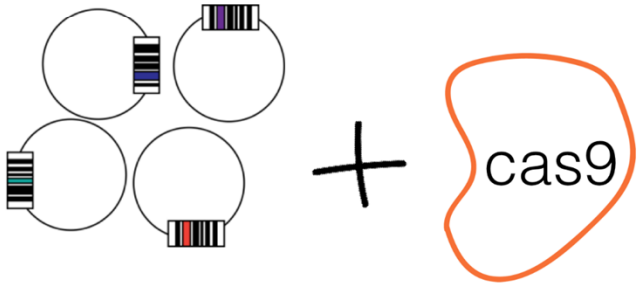




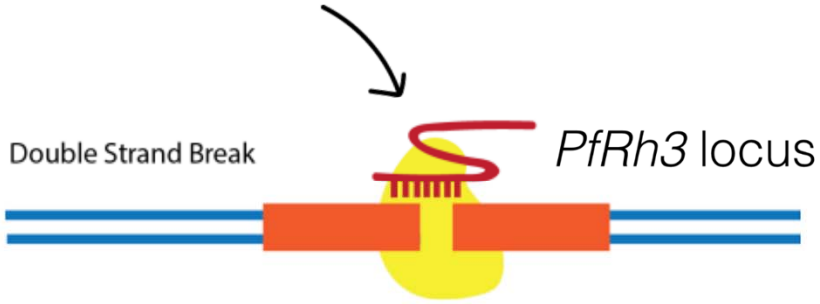


Bushell, E. Gomes, A.R. Sanderson, T. *et al.* (2017) *Cell*

barcode donor plasmid



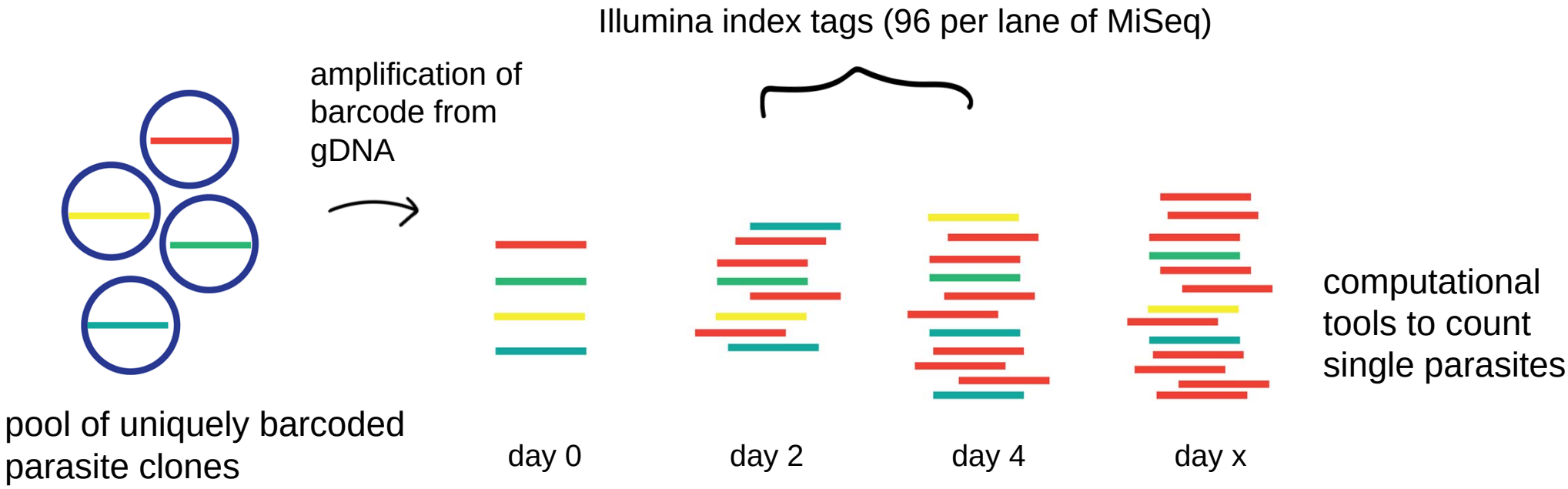
co-transfect donor and Cas9 plasmids



11bp barcode
(+ adapter sites)

Using Next Generation Sequencing as a parasite counter

Up to 96 samples can be multiplexed in one lane of MiSeq
(e.g. timepoints, replicates, conditions)

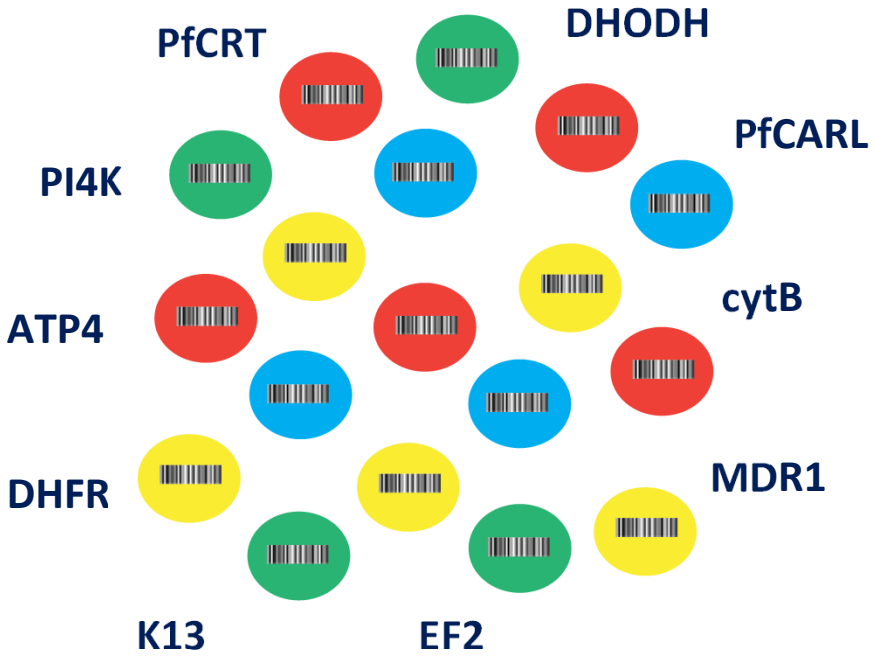


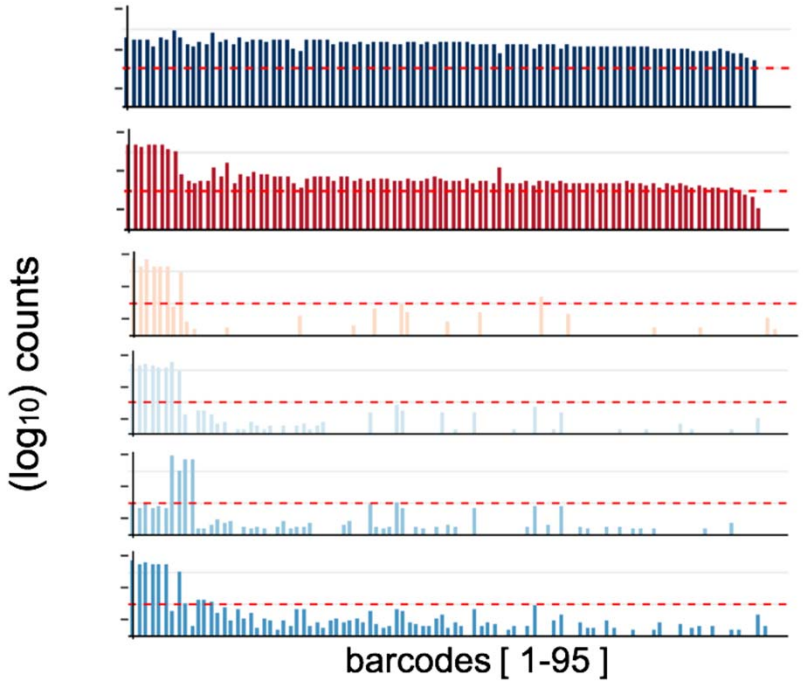
Parasite counts measured as the relative proportion of each barcode over time

Developing a barcoded resistance library for compound prioritization

Goal: to encompass the known parasite resistome in a single well

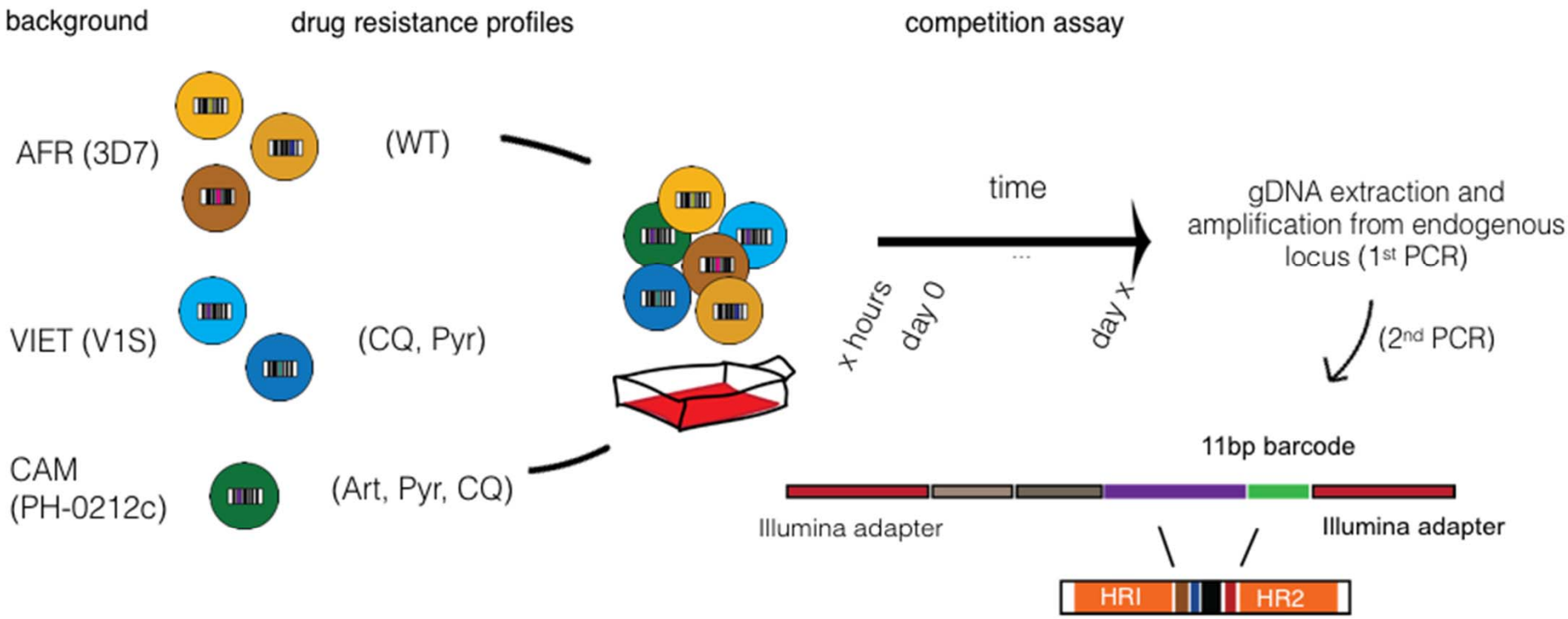
Use CRISPR-Cas9 to generate a comprehensive library of drug-resistant parasites, each individually barcoded.





Manuela Carrasquilla,
Hannah Jagoe, Aslı Akidil,
Julian Rayner

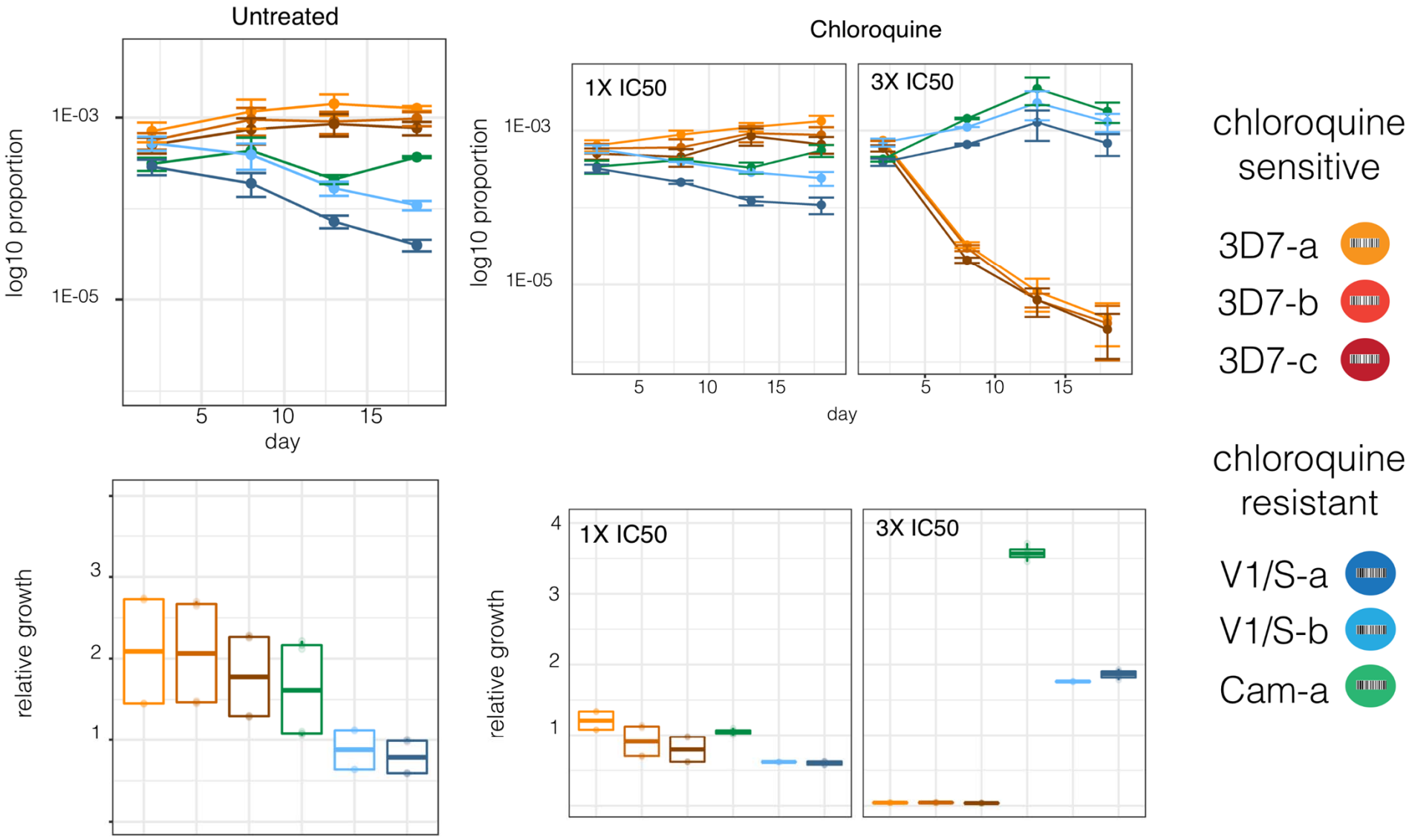
Barcode sequencing (BarSeq) to measure fitness and drug response



Manuela Carrasquilla

Barcode sequencing can reveal fitness and drug response phenotypes

Opposing fitness and chloroquine-resistance phenotypes observed.



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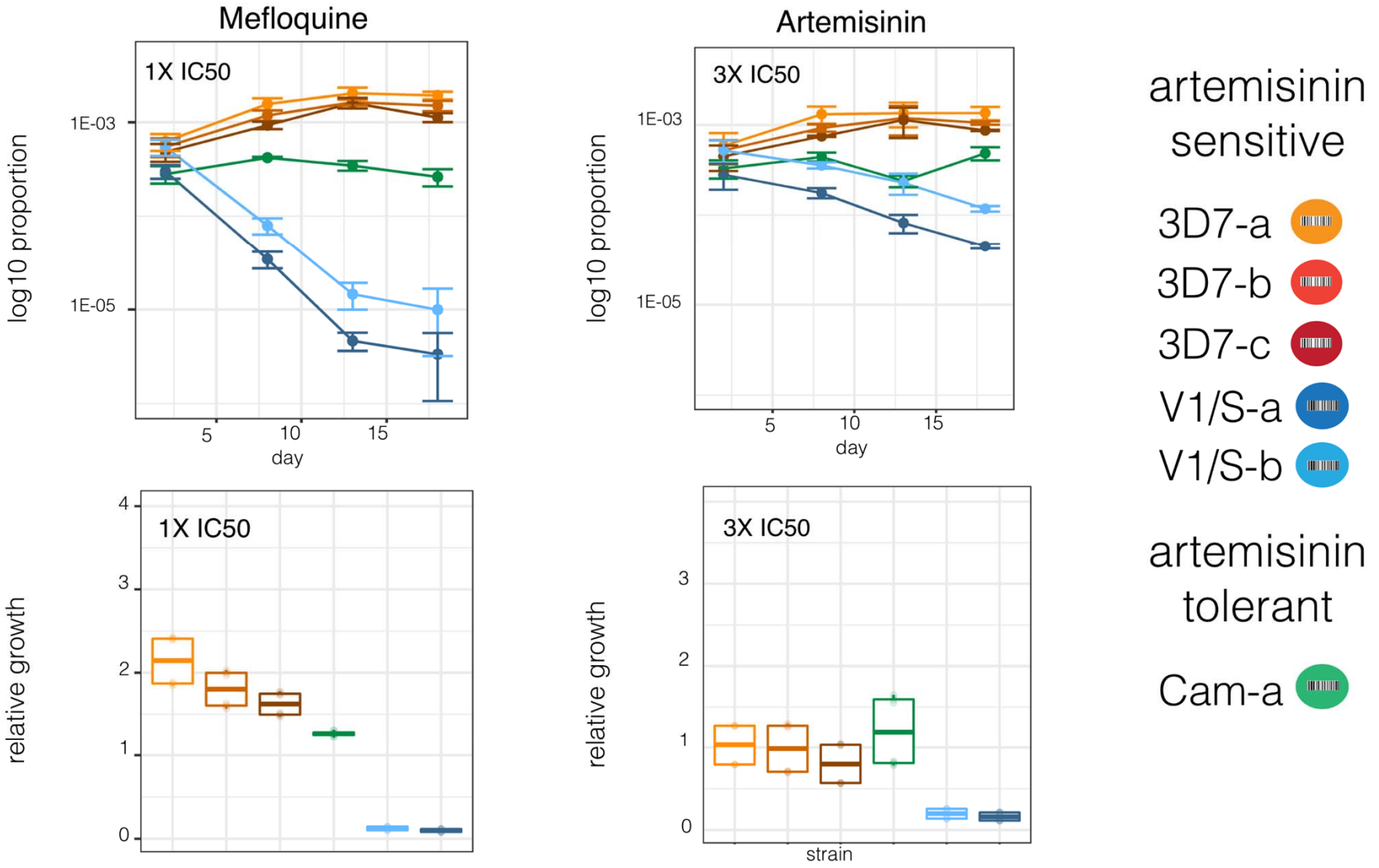
Barcode sequencing can reveal fitness and drug response phenotypes

‘Conventional’ exposure to artemisinin does not clearly identify the K13-mutant parasite



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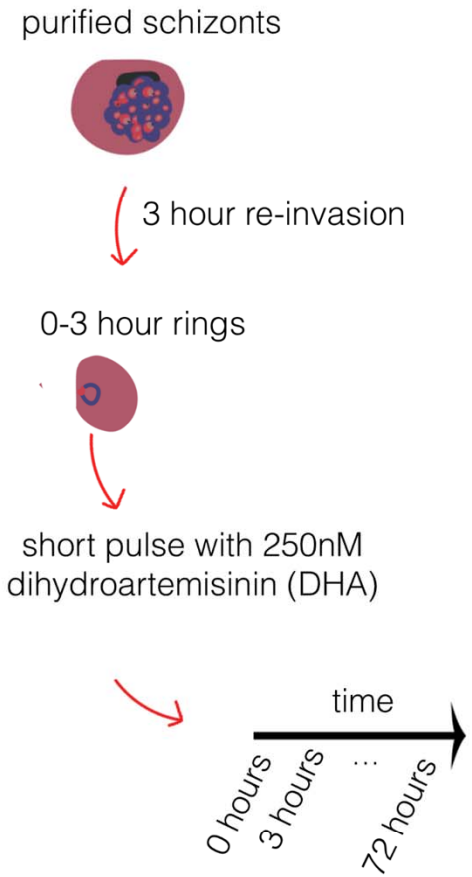
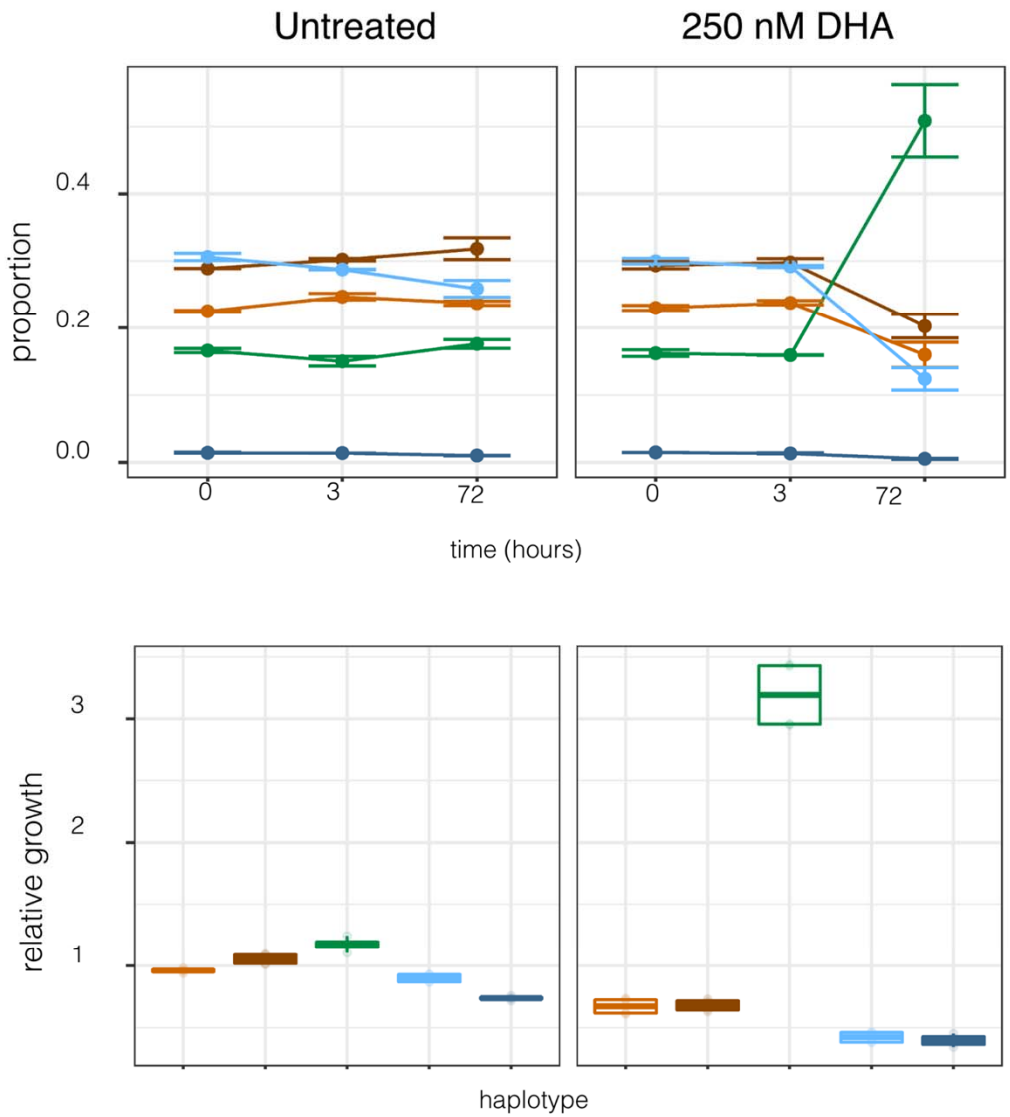
Barcode sequencing can reveal fitness and drug response phenotypes

Artemisinin phenotype of K13-C580Y parasite revealed by early ring-stage pulse





artemisinin sensitive

3D7-a

3D7-b

3D7-c

V1/S-a

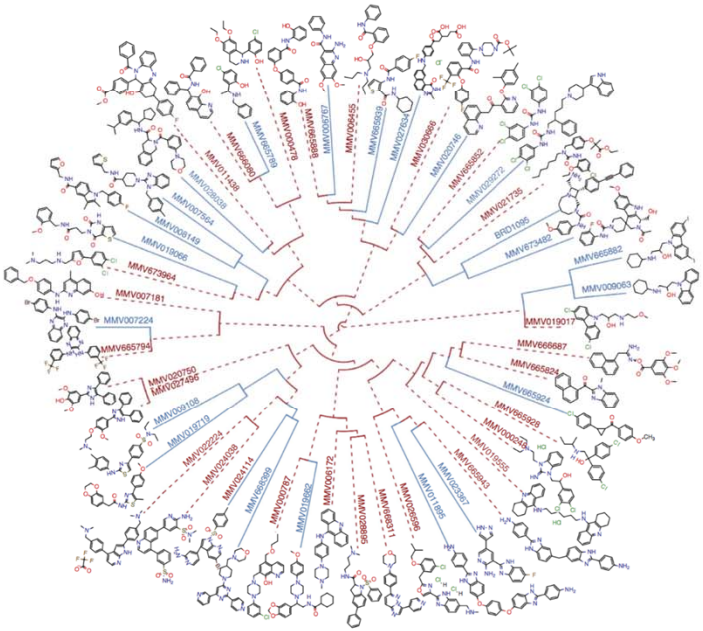
V1/S-b

artemisinin tolerant

Cam-a

Manuela Carrasquilla

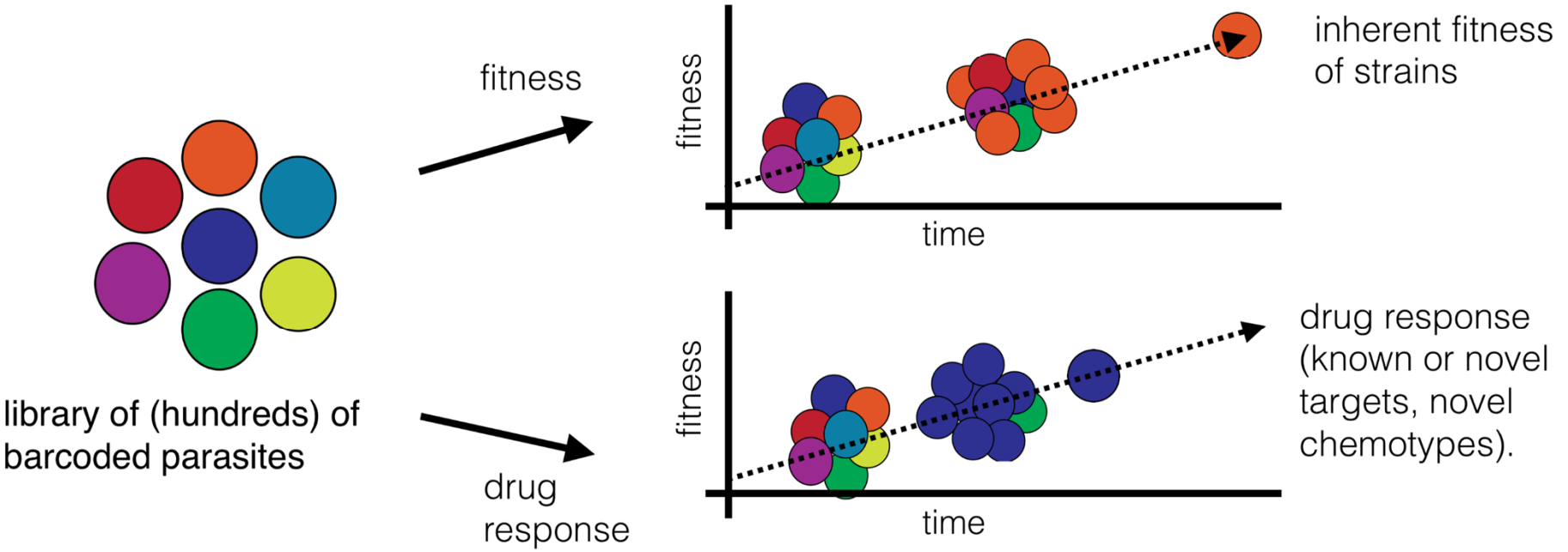
Complementary chemical genetic approaches for target identification



Target validation by CRISPR editing and overexpression.

Building barcoded parasite panel for comprehensive compound profiling.

Insights into how parasites respond to test compounds may identify mechanisms at use in the field.



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Malaria Drug Accelerator Consortium
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