How we halved transfection times, broke up with WR99210 and developed a novel PKG$_{T618Q}$ selection cassette

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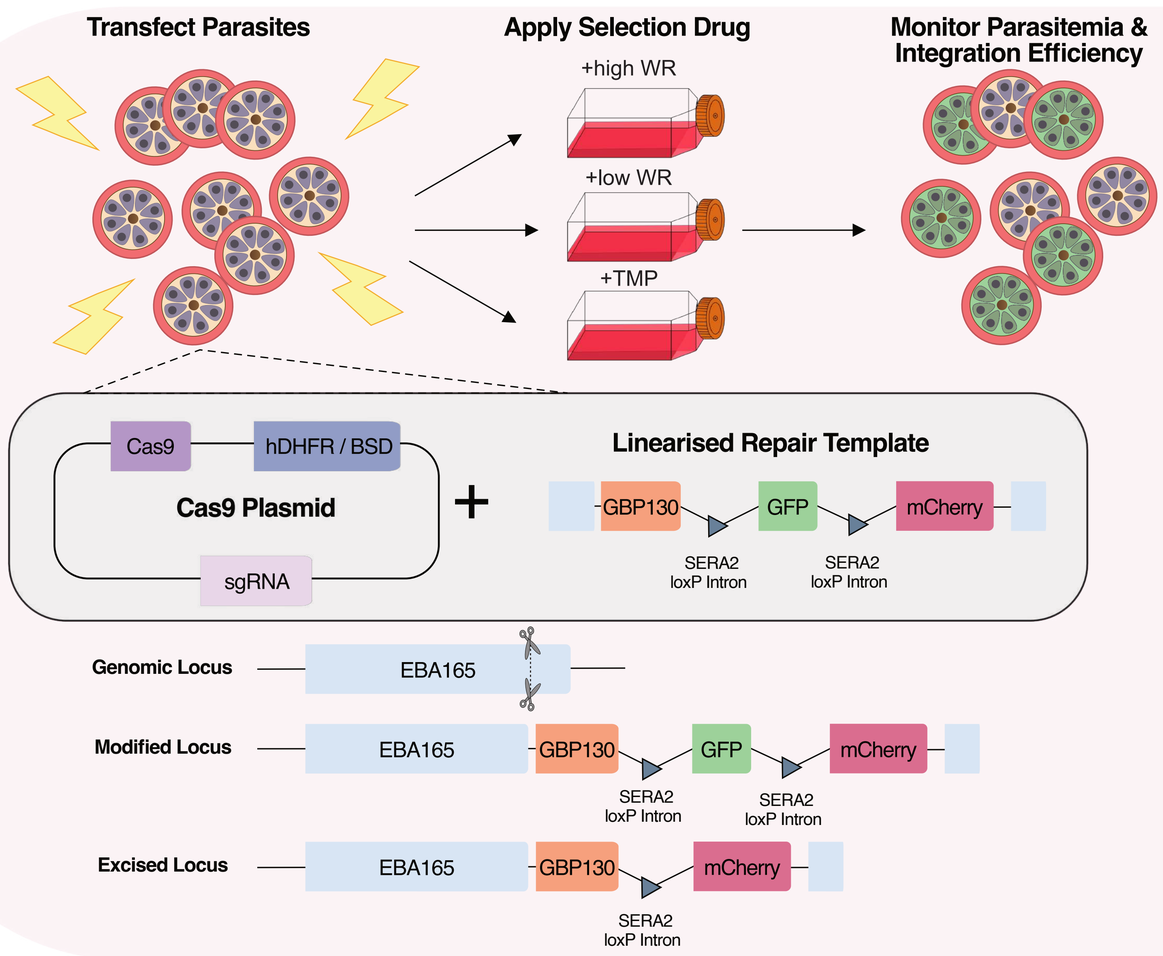
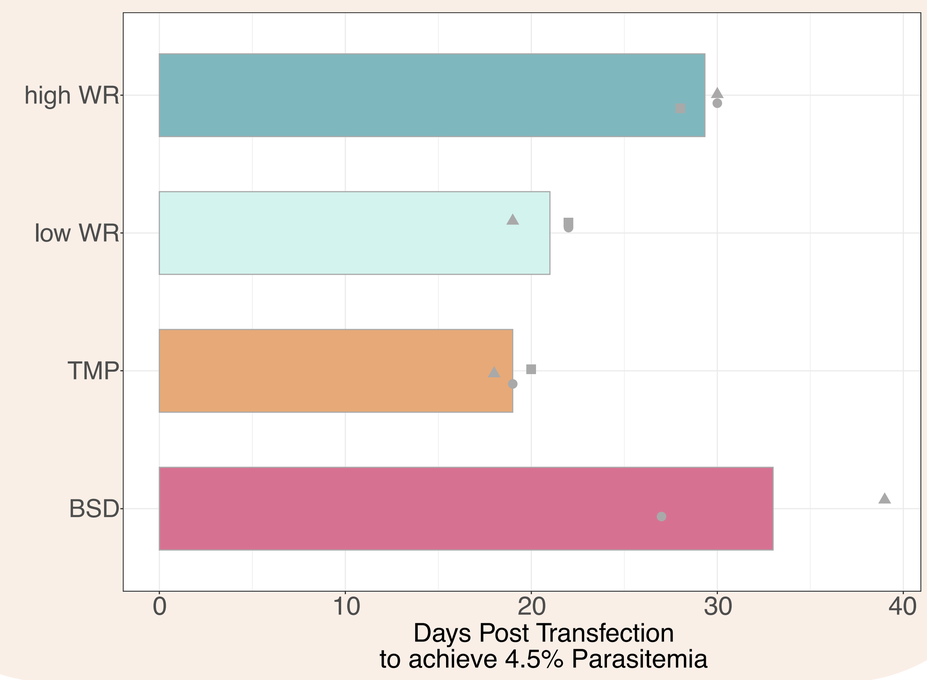
1 Introduction

- WR99210 is widely used to select for transgenic *Plasmodium falciparum* parasites.
- However, WR99210 is becoming harder to source and commercial alternatives have limited efficacy[1].
- Given that WR99210 targets parasite dihydrofolate reductase (DHFR), we reasoned that alternative DHFR inhibitors such as trimethoprim (TMP) could provide a robust and accessible replacement.



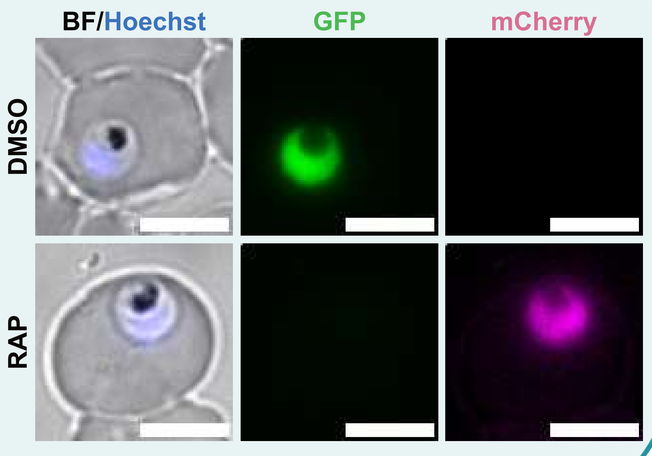


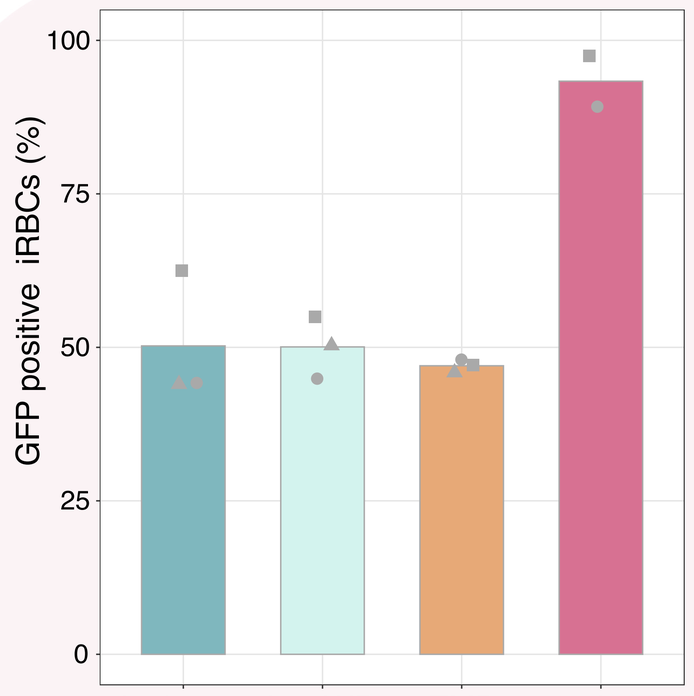
2 TMP works as a WR alternative

- WR99210 is usually used at 80x IC50 (5nM) and TMP at 8x IC50 (10 µM). Therefore, we included a control where we selected with 8x IC50 WR99210 (0.59 nM).
- TMP selection led to faster post-transfection recovery than standard WR99210 selection.
- Reducing WR99210 pressure improved recovery, suggesting that high drug concentrations slow post-transfection growth.

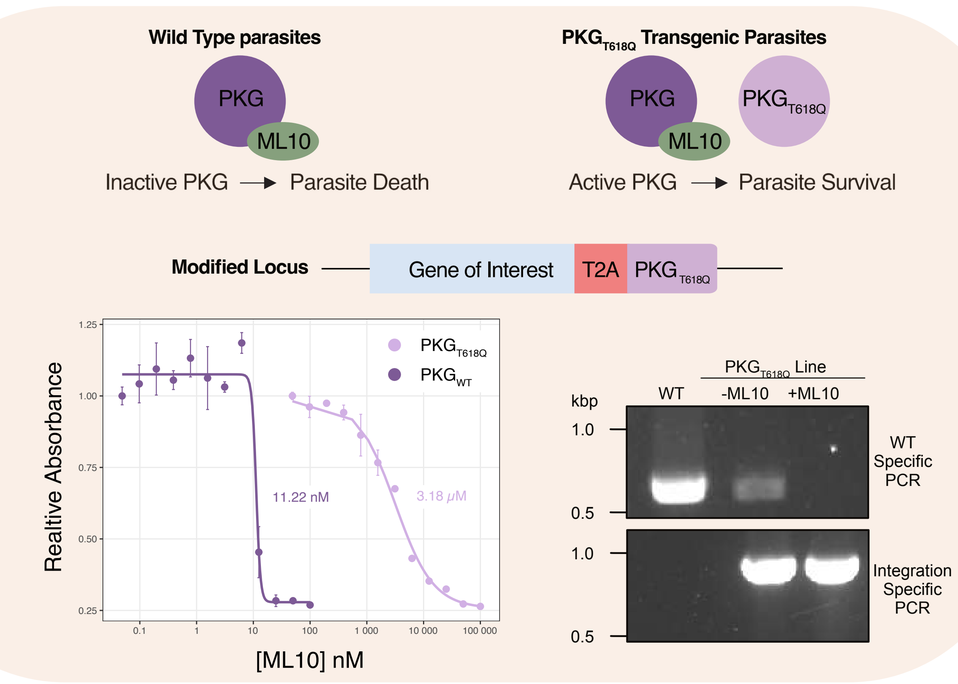
3 Integration efficiency is unchanged

- Using TMP or lower WR99210 concentrations, which both led to faster recovery, did not compromise integration efficiency.
- We also developed a novel BSD-Cas9 plasmid to expand the transgenic parasite selection tool kit.
- Surprisingly, BSD led to a higher integration efficiency compared to transfections using the hDHFR-Cas9 plasmids, but takes significantly longer than TMP or low WR selection for parasites to recover.





4 PKG as a novel selection cassette



- A common challenge to generating transgenic parasite lines is the limited number of drug selection tools available.
- We developed a novel selection cassette based on a T618Q mutation in the cGMP-dependent protein kinase (PKG), which confers resistance to PKG inhibitors such as compound 2 or ML10[2,3,4].
- This cassette proved highly effective using Selection-Linked Integration (SLI).
- Parasites can still be blocked from egress with C2/ML10 if higher concentrations are used

References

1. **Remcho** et al., 2020 Antimicrobial Agents and Chemotherapy; **2. Taylor** et al., 2010 Ekaryotic Cell; **3. McRobert** et al., 2008 PLoS Biology; **4. Ressurreição** et al., 2020 PLoS One

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