



## **Protocol for drug testing in *P. berghei* blood stage infected mice**

### **Experimental animals**

Female Swiss Webster mice, weighing 25 to 30 g. Groups of five mice are divided in control (vehicle treated) and the different groups of drug treatments.

### **Transgenic parasite line**

The transgenic *P. berghei* line 676m1cl1 line (PbGFP-Luccon) is used (Franke-Fayard et al., 2005. PNAS 102(32):11468-73. PMID:[16051702](#)). It expresses a fusion GFP (mutant 3) and firefly luciferase (LucI<sub>AV</sub>) and has been generated in the reference clone of ANKA strain cl15cy1. Parasites of line 676m1cl1 contain the PbGFP-Luc gene fusion stably integrated as a single copy gene by double cross over recombination into the 230p locus and the reporter gene is under control of the constitutive eef1a $\alpha$  promoter. This line has been selected by FACS-sorting of GFP-expressing parasites and therefore does not contain a drug-selectable marker.

### **In vivo development of blood stages + Drug treatments**

Groups of 5 mice are infected via i.p. injection with  $10^3$  ( $\sim 5 \times 10^{-5}\%$  infection) *P. berghei*-Luccon infected erythrocytes obtained from another infected mouse.

Two days after infection treatment starts for 5 days. The control group is treated with the vehicle for 5 days (route of administration either i.p., i.v. or oral gavage). The drug-treated groups are treated with the test compounds at the determined mg/kg body weight once a day for 5 days. As control, a group of mice will be treated with chloroquine at 20 mg/kg/day for i.p. and 40 mg/kg/day orally.

One day after 5 days of treatment (day 7 after infection), the mice were anesthetized by inhalation of isofluorane (controlled flow of 2.5% isofluorane in air was administered through a nose cone via a gas anesthesia system). Mice are injected i.p. with 150 mg/kg of D-Luciferin Potassium-salt (Goldbio) dissolved in PBS. Mice are imaged 5 to 10 min after injection of luciferin with an IVIS 100 (Xenogen, Alameda, CA) and the data acquisition and analysis are performed with the software LivingImage (Xenogen). This provides the level of infection.