Materials List for:

Protocol for Production of a Genetic Cross of the Rodent Malaria Parasites

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Materials

Name	Company	Catalog Number	Comments
Glyerolyte 57 solution	Cenmed	4A7833	
Mouse *Mus musculus*	Charles River Laboratories		Female, inbred, strain Balb/C
Heat-inactivated calf serum	Invitrogen	26010-066	
Phosphate buffered saline (PBS) solution	Invitrogen	10010-072	pH 7.4; Cell Culture grade
Malaria parasite *Plasmodium y–lii y–lii* 17XNL(1.1)	MR4	MRA-593	deposited by DJ Carucci
Malaria parasite *Plasmodium y–lii nigeriensis* N67	MR4	MRA-427	deposited by W Peters, BL Robinson, R Killick Kendrick
Mosquito *Anopheles stephensi*	MR4	MRA-128	deposited by MQ Benedict
Cellometer automatic cell counter	Nexcelom Bioscience	Cellometer Auto T4	
Cellometer CP2 disposable hemacytometer	Nexcelom Bioscience	Cellometer CP2	
High Pure PCR template preparation kit	Roche Group	11 796 828 001	
Calcium chloride	Sigma-Aldrich	C5670	Cell culture tested; insect cell culture tested
Giemsa stain, modified	Sigma-Aldrich	GS500	
Ketamine hydrochloride	Fort Dodge Animal Health	NDC 0856-2013-01	Pharmaceutical grade; concentration to 100 mg/mL
Potassium chloride	Sigma-Aldrich	P5405	Cell culture tested; insect cell culture tested
Sodium chloride	Sigma-Aldrich	S5886	Cell culture tested; insect cell culture tested
Trisodium citrate dihydrate	Sigma-Aldrich	S4641	
Xylazine	Akorn Inc	4811-20ml	Pharmaceutical grade; concentration to 20 mg/mL
Glass wool	VWR international	32848-003	
Glass capillary (1 µL)	VWR international	53440-001	
Hemocytometer	VWR international	15170-168	Complete chamber set
Homogenizer	VWR international	KT749520-0090	Pestle with matching tube, 1.5 mL

SUPPLEMENTARY MATERIALS:

- Maintenance of laboratory mice
- Maintenance of laboratory mosquit–s
- Microscopic examination of thin blood smears stained with Giemsa stain
- Measurement of red blood cell density

Maintenance of laboratory mice

Females of inbred laboratory mouse strain BALB/c, aged 5 to 8 weeks old, are used in the study. Mice are housed in a standard solid-bottom polycarbonate cage with wire-bar lid, equipped with feeder and a water bottle. Mice are maintained at a constant temperature (25 ± 1°C) on 12:12 hour light:dark cycle. Mice are allowed to feed on 2018S Harlan Teklad Global 19% protein extruded rodent diet (sterilizable; from Harlan-Teklad) and supplied with acidified drinking water ad libitum. Experiments on animals are performed in accordance with the guidelines and regulations set forth by the Animal Care and Use Committee at the National Institute of Allergy and Infectious Disease under protocol LMVR11E (National Institutes of Health, Bethesda, Maryland).

Maintenance of laboratory mosquit–s

Mosquit–s are from a laboratory-bred colony of Anopheles stephensi. The adults are maintained in nylon cages kept in a temperature- and humidity-controlled room (23 to 25°C for *Plasmodium y–lii* and *Plasmodium chabaudi*, and 19 to 21°C for *Plasmodium berghei*; 80 to 95% humidity; on 12:12 hours light:dark cycle). Adult mosquit–s are fed with 10% glucose and 2.00% para-aminobenzoic acid (PABA) supplemented water solution. To obtain high-quality adults, 500 larvae are grown in a low-density condition in 1 L of distilled water in a 1,000-cm^3 open dish supplied with approximately 1 mg of sodium bicarbonate. After hatching, the larvae are given tetramin powder (PETCO) until they develop into the pupa stage and are transferred to the adult mosquito cages for emerging.

Microscopic examination of thin blood smears stained with Giemsa stain

Using clean scissors snip off the tip (1.0 mm) of the infected mouse's tail. Place one drop (0.5-1.0 µL) of tail blood onto a clean specimen slide. Mouse will stop bleeding in 1-2 min. Place a clean spreader slide on top of the blood drop, maintaining it at a 45° angle relative to the specimen slide, and allow the blood to adsorb to the entire width of the spreader. Hold the specimen slide and push forward the spreader slide rapidly and smoothly to produce a thin smear. Let the blood film dry, and then immerse the slides in absolute methanol. Allow the slide to air dry once more before covering it with Giemsa stain (10% Giemsa dye in distilled water). After incubating the thin blood films for 10-15 min at room temperature, carefully rinse the slides with tap water and let it air dry. Examine the number of infected red blood cells (iRBC; see Figure 3 for morphology of infected RBC) under a light microscope with immersion oil at 1000x magnification (with 100x objective lens) and calculate parasitemia (the number of iRBC per 100 RBC counted). Different strains of malaria parasites vary in growth rate and pathogenicity. Monitoring of blood stage parasitaemias can be performed 24hrs after injections, depending on the dose of the blood stage malaria parasites. For example, mice will be microscopically positive 24 hrs when injected with 10^7 infected RBC intraperitoneally or 10^6 infected RBC intravenously.

Measurement of red blood cell density

Like the levels of parasitaemias, red blood cell (RBC) density in infected mice varies throughout the course of infection. RBC density should be measured within 1-2 hrs before the start of the single- and mixed-clone infection and the cloning experiments. There are two methods for measurement of RBC density: a manual counting using Neubauer hemocytometer and an automatic counting using a Cellometer (Nexcelom Bioscience). In both methods, withdraw 1 µL of mouse tail blood using a glass capillary (VWR) and dilute in 10 mL of PBS and mix well. To use a Neubauer hemocytometer, load 20 µL of the suspension onto the hemacytometer. Place the hemacytometer on a light microscope with 10x objective lens. The hemacytometer contains a grid divided into 9 large squares, and 4 large squares at the corner are further divided into 16 small squares. Count the total number of cells in each of the 16 small squares in the four corner squares. To avoid counting bias or counting cells that overlap a grid line, count a cell as "in" if it overlaps the top or right lines and "out" if it overlaps the bottom or left lines. Estimate the number of cells per one small square and divide by 0.00625 (the volume of one small square is 6.25 nL). This yields the number of cells per microliter (µL). From this data, calculate the final red blood cell density by multiplying with 10,000 (a dilution factor). Rinse the cover slip and counting chamber with distilled water and 70% ethanol; air dry. Alternatively, load 20 µL of the suspension onto a Cellometer counting chamber slide. Insert the slide into a Cellometer slide chamber (the reader). Start the Cellometer software, select the "red blood cell" option, and enter a dilution factor of 10,000. Record the RBC density.

