APPENDIX I: CRYOPRESERVATION

1. Prepare a 40% (v/v) sterile glycerol solution in Trypanosome dilution buffer (see below).
2. Dispense 0.5 mL of anticoagulant solution (see below) into a 15 mL test tube. Add to the anticoagulant tube blood collected by a facility-approved method from mice that had reached or are near peak parasitemia. Invert the tube several times to mix the blood with the anticoagulant.
3. In a separate test tube, add the heparinized blood dropwise to the 40% glycerol solution. Note that blood should be mixed with glycerol solution in a 1:1 ratio to obtain a final concentration of cryoprotectant of 20% (v/v). Mix slowly by inversion and place the tube on ice. The freezing process should start 15 to 30 minutes following the addition of the heparinized blood to the cryoprotectant solution.
4. Dispense 0.5 mL aliquots of blood suspension into 1 to 2 mL sterile plastic screw-capped cryovials. Place the vials in a controlled rate freezing unit. From room temperature, cool the vials at -1°C/min to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through this phase. At -40°C, plunge vials into liquid nitrogen. Alternatively, place the vials in a Nalgene 1°C freezing container. Place the container at -80°C for 1.5 to 2 hours and then plunge vials into liquid nitrogen.
5. To thaw a frozen ampule, place in a 35°C to 37°C water bath, until thawed (2 to 3 min). Immerse the ampule just sufficient to cover the frozen material. Do not agitate the ampule.
6. Immediately after thawing, remove the contents of the ampule aseptically with a syringe and inoculate an immunosuppressed mouse. Follow the protocol for *in vivo* propagation above.

Trypanosome Dilution Buffer
20 mM Na_2HPO_4
2 mM NaH_2PO_4
80 mM NaCl
5 mM KCl
1 mM $MgSO_4$
20 mM Glucose

Adjust the pH of the solution to 7.7 and filter-sterilize.

Yaeger's Anticoagulant Solution

Sodium citrate	1.33 g
Citric acid	0.47 g
Dextrose	3 g
Sodium heparin	0.2 g
Distilled H_2O to	100 mL

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898