Protocol macrophage isolation and stimulation (modified from Crystal L. Loving USDA)	PI	Dr. Chris Tuggle
	Submitter	Dr. Juber Herrera Uribe
2255 Kildee Hall Department of Animal Science, ISU	Date	Feb. 12 2019

Macrophage collection/culture

1. Lavage lungs with ~300ml of PBS/gentamicin recovering as much volume as possible (about 200ml).
2. Pellet cells by centrifugation at 1500 rpm for 10 minutes at 4°C.
3. Resuspend cells in 10ml of media A and transfer to a large petri dish. Add 20ml of media A to bring final volume to 30ml. (may need 2 plates per pig if large pellet)
4. Incubate at 37°C 5% C02 for 2 hours or overnight.
5. Remove and discard non-adherent cells using cold PBS.
6. Add 15ml cold media B and scrape up cells. Continue to add media B and scrape until all cells are collected. Collect into 50 mL conical.
7. Centrifuge at 1200 rpm for 10minutes at 4°C
8. Resuspend cells in 1-3 mL of media B.
9. Count and transfer the appropriate number of cells for the assays (non-stimulated, LPS and Poly (I:C) treatment) to a petri dish (100 mm). Bring to 5 mL with media B.
10. Incubate at least 1 hour at 37°C 5% C02 before adding stimulant.

Macrophage stimulation

1. Add the LPS (O55:B5 *E. coli* at 1µg/mL final concentration) and Poly (I:C) (0.5 µg/mL final concentration) to each plate and incubate the cells for 2 and 6 hours at 37°C 5% C02.
2. After each timepoint scrape until all cell are collected. Transfer the cells into 50 mL canonical tube, count and take the cell number needed for each assay (ATAC-seq, RNA and DNA isolation).

Reagents

Media A

Reagent	100 ml	500ml
RPMI	90 ml	452ml
Swine Sera (5%)	5 ml	25 ml
HEPES (25mM) 1M Stock	2.5 ml	12.5 ml
L-glutamine (2mM)	1 ml	5 ml
Pen-Strep (1%)	1 ml	5 ml
Gentamicin (100ug/ml) 50µg/ml Stock	50µl	500ul

**if plating cells longer than a few hours, bump up sera to final 8-10% concentration.

Media B

Reagent	100 ml	500ml
RPMI	87.5 ml	439.5ml
FCS (10%)	10 ml	50 ml
L-glutamine (2mM)	1 ml	5 ml
Pen-Strep (1%)	1 ml	5 ml
Gentamicin (100ug/ml) 50µg/ml Stock	50µl	500ul