How many individuals? Pull out immune gene (Jak-STAT expression)

Ribo-depleted

Lark:

Kasen: high pathogen load in infected bugs if it is a pathogen

Bacterial ribodepletion kit in addition

Read viral discovery papers

Lark: even in a pool of 100 mosquitos you might only get one or two viruses

Maybe do big pools to maximize your chances of finding stuff

Pools of pools 10 pools of a 100, so 1000 bugs

Most discovers would argue that if you see even part of the genome that that is compelling evidence of presence.

*Make sure you have that positive control plasmid*

Pooled 30 mosquitos, 8 pools in a high-seq run, so 240 bugs in one run.

Do small pools of bugs or individual bugs

96 samples can be

Barcode

Power analysis, what prevalence would you deem as significantly different

Kasen library prep: If your virus isn’t at a high titr doing ribodepletion, its ineffiecnet you only get about 1-5% of the rna that comes out the back side

Its good to be thinking about what you consider positive

Assememble, put in reference viruses and see if that will help you pull out related sequences.

Myseq vs. high-seq

Mixer mill for extracting rna.

NEB next ultra rna seq kit for library prep

Percent coverage and depth correlates with viral load, up to a certain limit.

Risk of high number of bugs is you might miss viruses

Viral discovery in mosquitos. .2 percent were viral reads.

Do back of the envelope calc for reads.

Per pool 10-50

50-100 million reads per pool

7- 12 pools per lane

Do one sample first,

There are viral discovery pipelines for bioinformatics

Have the storage ready for the data. You get about 100 gigs of data out of a run (compressed) end is terabyte of data

The process is very prone to cross contamination. Separate out high and low cannibalism bugs in time.

Run a bioanalyzer

Turn around is week and a half for myseq

Three weeks for highseq or week and half. Run on Friday

Definitely do paired end 150

Pool after rna extraction

Def do a practice library prep.

Buy part of lane for practice library.