Basic workflow:

Wet-lab work (Borna Lab)
Immune cells (macrophages, dendritic cells, neutrophils, alveolar macrophages) and epithelial cells will be treated with aspergillus, and samples (RNA, protein and supernatant – suspension on which cells live) will be collected. Expression for select proteins will be measured.

Sequencing (The Jackson Lab)
RNA will be sequenced, and data will be provided to Reinhard Lab.

Model (Reinhard Lab)
Differential gene expression analysis across different conditions will be performed. Based on differential expression analysis and literature review, a mathematical model will be built for each cell type. Once individual models are built, they will be integrated into a multi-scale model, which will also include other modules – iron diffusion, molecule transport, macrophage movement etc. Simulations will be performed for each individual model and the multi-scale model. The simulations for the completed individual models will be generated in 2019 and for the multi-scale model once we have multiple modules to integrate together.

Experimental validation (Borna Lab)
All predictions will be tested in-vitro in cells in Borna Lab for individual models.
Multi-scale model predictions will be validated in mouse model.

Experiment I

BORNA LAB
Macrophages/Dendritic cell and Aspergillus Co-incubation: Macrophages or Dendritic cells will be treated with aspergillus fungus and samples collected at different time points.

Cell Type	Condition	0 hrs	2 hrs	4 hrs	6 hrs	8 hrs	10 hrs	Total	# of Patients	
Macrophages	No aspergillus	1	1	1	1	1	1	9	6	54
	Aspergillus		1	1	1	1	1			
Dendritic Cells	No aspergillus	1	1	1	1	1	1	9	6	54
	Aspergillus		1	1	1	1	1			
TOTAL SAMPLES										108

For all Conditions and time points (total 108): supernatant, proteins, RNA will be collected. Data will be recorded.

- Experiment design and protocol **(.pdf, .doc)**
- RNA quantity/quality data **(.xlsx)**

- Protein Expression **(.jpeg/.png, .xlsx)**

THE JAX LAB

The Jackson Laboratory will sequence 108 RNA samples received from Borna Lab. They will provide the Laubenbacher Lab with sequencing data.

- Protocol, design **(.pdf)**
- Sequencing Data **(Text files(2-3Gb/each file))**
    - **.fastq** (Example from Wiki Page).

A FASTQ file normally uses four lines per sequence)

- Line 1 begins with a '@' character and is followed by a sequence identifier and an *optional* description
- Line 2 is the raw sequence letters.
- Line 3 begins with a '+' character and is *optionally* followed by the same sequence identifier (and any description) again.
- Line 4 encodes the quality values for the sequence in Line 2, and must contain the same number of symbols as letters in the sequence.

A FASTQ file containing a single sequence might look like this:

```
@SEQ_ID
GATTTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTTT
+
!''*((((***+))%%%++)(%%%%).1***-+*''))**55CCF>>>>>>CCCCCCC65
```

A .fastq file can be really big. For our experimental datasets, I expect ~ 2-3 Gb.

LAUBENBACHER LAB

Bioinformatics Analysis

Raw sequencing data will be cleaned up using tools like FASTQC, Cutadapt, Trimmomatic etc. The cleaned sequencing data will be aligned with reference genome using alignment algorithms eg. STAR.

Differential gene expression analysis will be performed using bioinformatics packages in R such as EdgeR or DEseq2

- Scripts for running various kinds of analysis **(Text files(kb): .sh, .py)**
- Quality report of the raw .fastq data **(Text files (kb-mb) - .html, text files))**
- Cleaned raw data **(Text files (2-3gb): .fastq)**
- Aligned data **(Text files (2-5gb), .sam- alignment file, .bam – binary version of .sam)**
- Differential Expression Analysis results **(Heatmaps, .csv, .xlsx)**
- Pathway Analysis Results **(.pdf, heatmaps/networks - .jpeg/.png)**

Mathematical Modeling

- Logical networks **(Text files(kb): .txt)**

- Simulations **(Images, text files, .pdf)**

We will perform the same workflow for more cell types. So far, we have macrophages and dendritic cells. We will have neutrophils (in the first half of 2019), followed by epithelial cells and alveolar macrophages.

Experiment II – Neutrophils (Wet lab experiment being done in Borna Lab) Same workflow as above.