



Introduction to Differential Gene Expression Analysis

Harvard Chan Bioinformatics Core

<http://tinyurl.com/hbc-intro-to-dge>



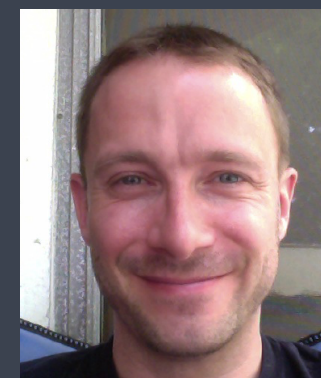
Shannan Ho Sui



John Hutchinson



Brad Chapman



Rory Kirchner



Meeta Mistry



Radhika Khetani



Mary Piper



Victor Barrera



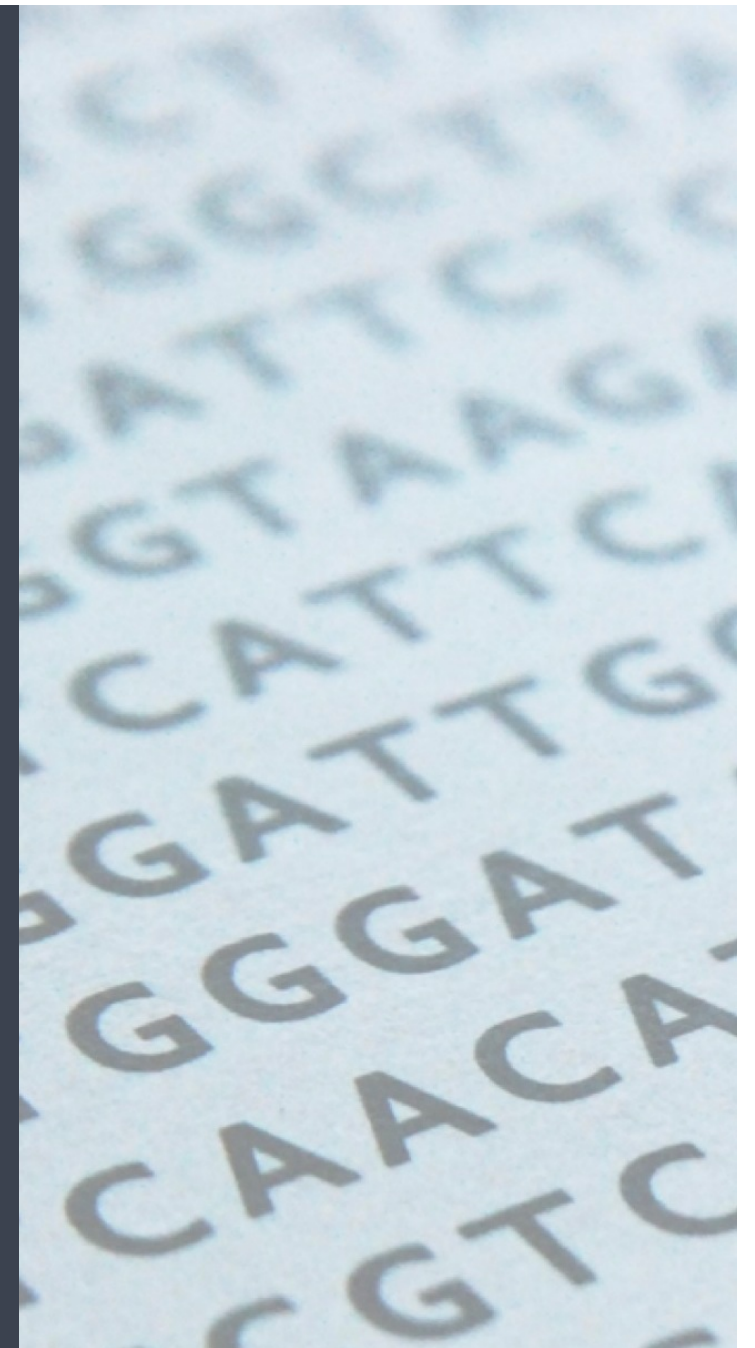
Lorena Pantano



Peter Kraft

Consulting

- RNA-seq, small RNA-seq and ChIP-seq analysis
- Genome-wide methylation
- WGS, resequencing, exome-seq and CNV studies
- Quality assurance and analysis of gene expression arrays
- Functional enrichment analysis
- Grant support





HARVARD
T.H. CHAN
SCHOOL OF PUBLIC HEALTH

HSCI
HARVARD STEM CELL
INSTITUTE



HARVARD
CATALYST
THE HARVARD CLINICAL
AND TRANSLATIONAL
SCIENCE CENTER



HARVARD
MEDICAL SCHOOL

NIEHS / CFAR
Bioinformatics
Core

Center for Stem
Cell
Bioinformatics

Harvard
Catalyst
Bioinformatics
Consulting

HMS
Tools &
Technology



HARVARD
T.H. CHAN

SCHOOL OF PUBLIC HEALTH

HSCI
HARVARD STEM CELL
INSTITUTE



HARVARD
CATALYST

THE HARVARD CLINICAL
AND TRANSLATIONAL
SCIENCE CENTER



HARVARD
MEDICAL SCHOOL

NIEHS / CFAR
Bioinformatics
Core

Center for Stem
Cell
Bioinformatics

Harvard
Catalyst
Bioinformatics
Consulting

HMS
Tools &
Technology

Training

We have divided our short workshops into 2 categories:

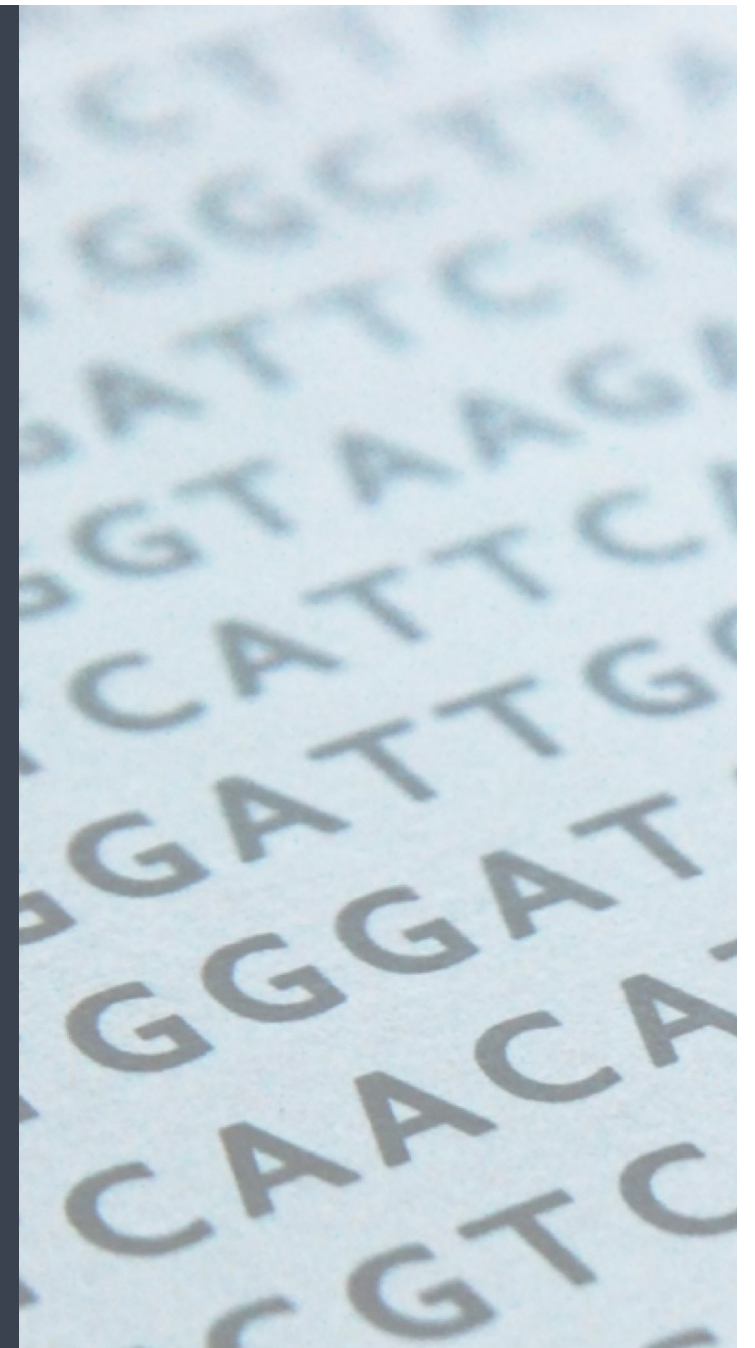
1. Basic Data Skills - No prior programming knowledge needed (no prerequisites)
2. Advanced Topics: Analysis of high-throughput sequencing (NGS) data - Certain “Basic” workshops required as prerequisites.

Any participants wanting to take an advanced workshop will have to have taken the appropriate basic workshop(s) within the past 6 months.

https://hbctraining.github.io/main/training_spring2019.html

<http://bioinformatics.sph.harvard.edu/training/>

<https://hbctraining.github.io/main/>



Introductions!



Shannan Ho Sui



John Hutchinson



Brad Chapman



Rory Kirchner



Meeta Mistry



Radhika Khetani



Mary Piper



Victor Barrera



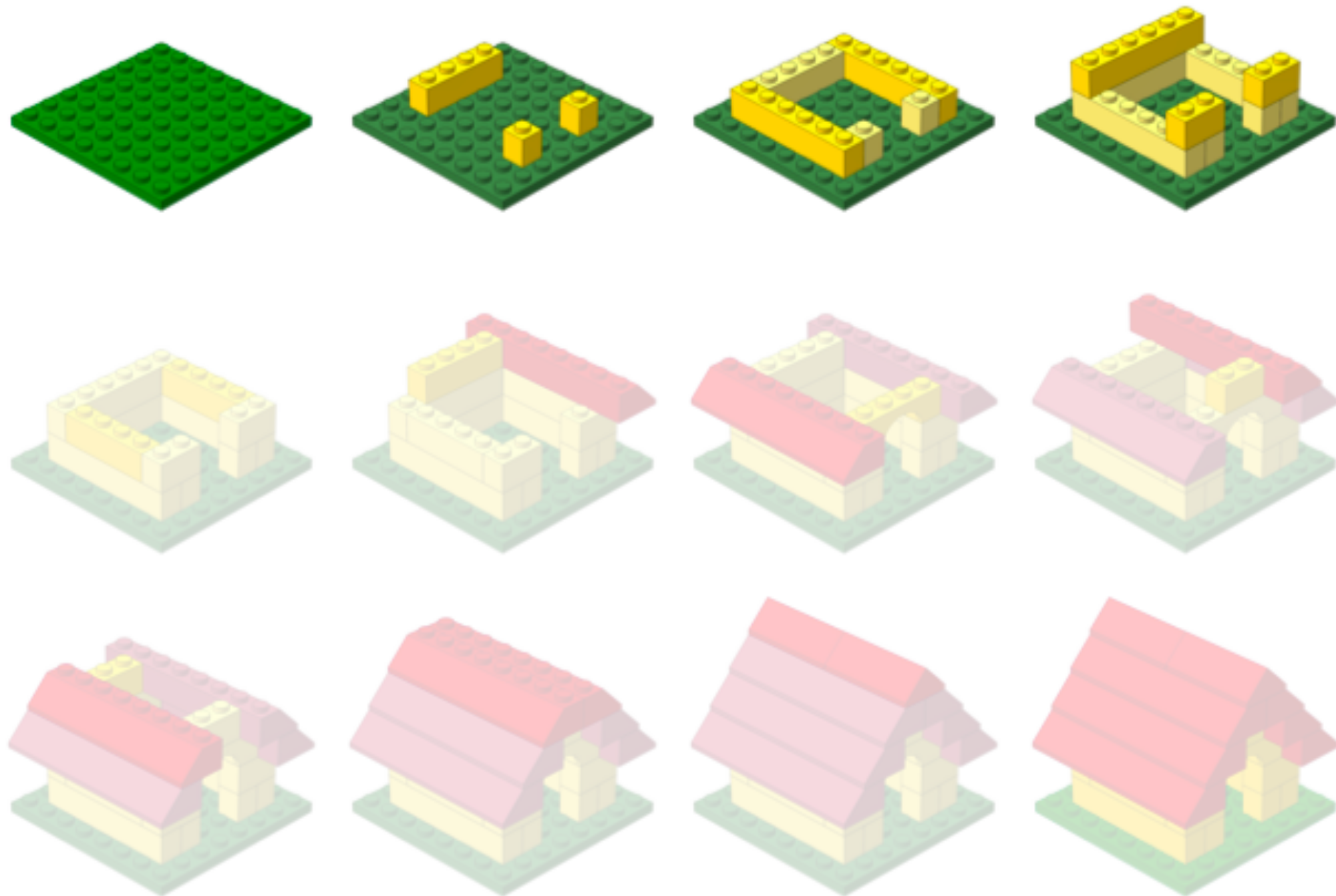
Lorena Pantano



Peter Kraft

Class Introductions!

Workshop Scope...



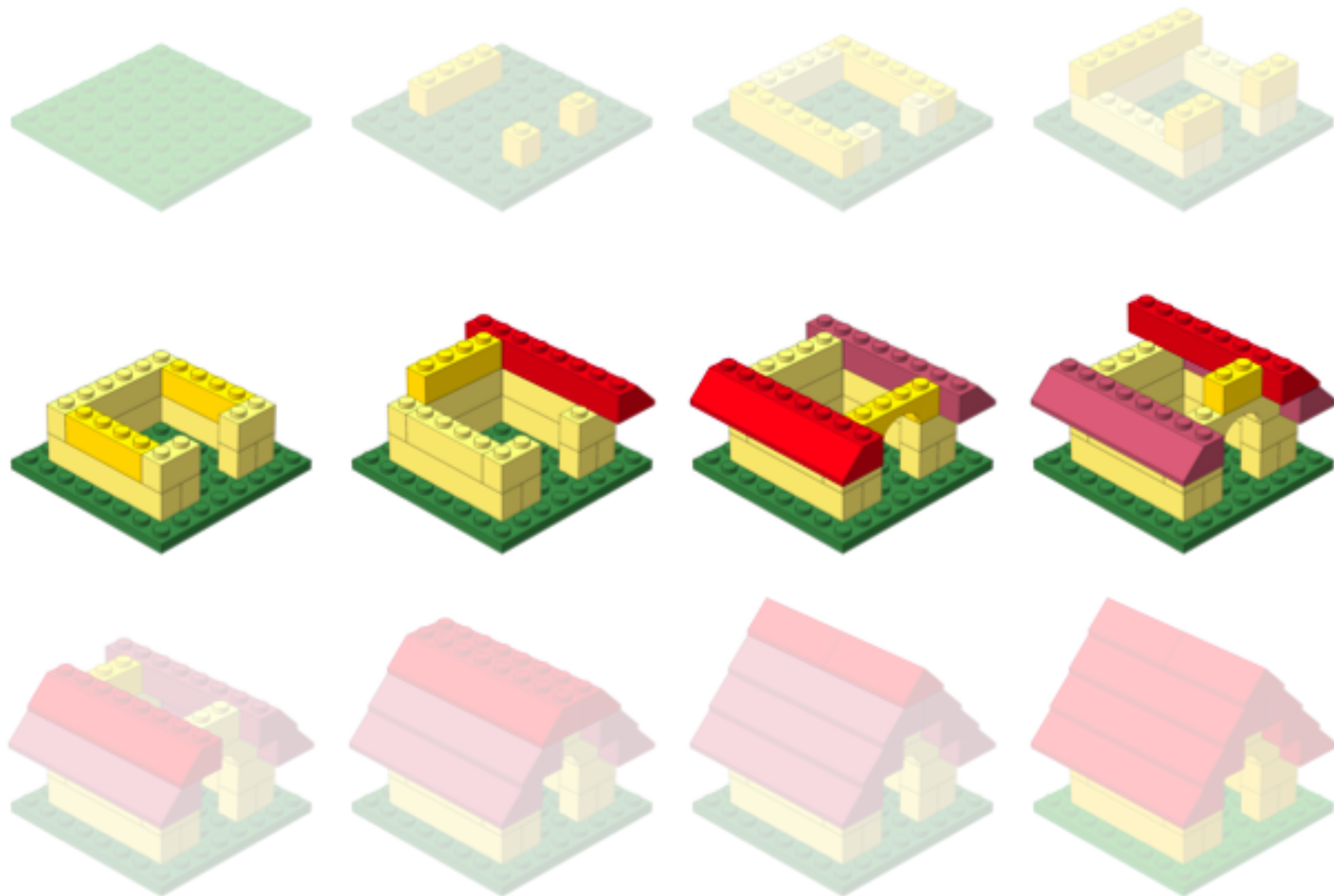
<http://anoved.net/tag/lego/page/3/>

Setting up to perform Bioinformatics analysis

Setting up...



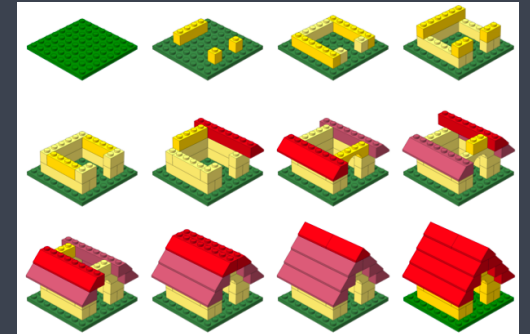
- ✓ Introduction to the command-line interface (shell, Unix, Linux)
 - Dealing with large data files
 - Performing bioinformatics analysis
 - Using tools
 - Accessing and using compute clusters
- ✓ R
 - Parsing and working with smaller results text files
 - Statistical analysis, e.g. differential expression analysis
 - Generating figures from complex data



<http://anoved.net/tag/lego/page/3/>

Bioinformatics data analysis

Workshop Scope



Differential Gene Expression analysis

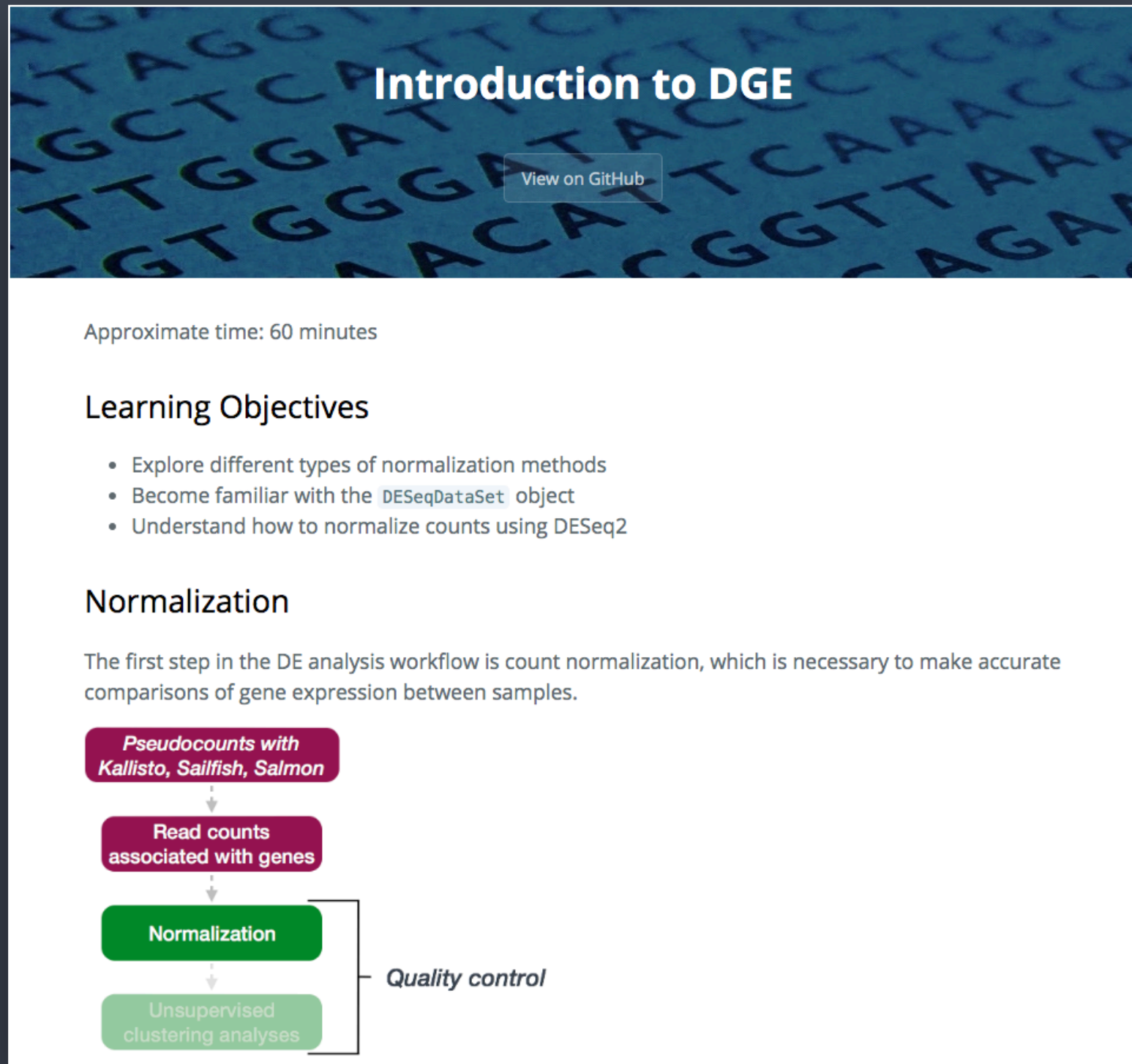
- ✓ Understand the considerations for performing statistical analysis on RNA-seq data
- ✓ Start with gene counts (after alignment and counting)
- ✓ Perform QC on count data
- ✓ Use DESeq2 to perform differential expression analysis on the count data and obtain a list of significantly different genes
- ✓ Visualize results of the analysis
- ✓ Perform functional analysis on the lists of differentially expressed genes

Logistics

Course webpage (wiki)

<http://tinyurl.com/hbc-intro-to-dge>

Course materials online



Introduction to DGE

[View on GitHub](#)

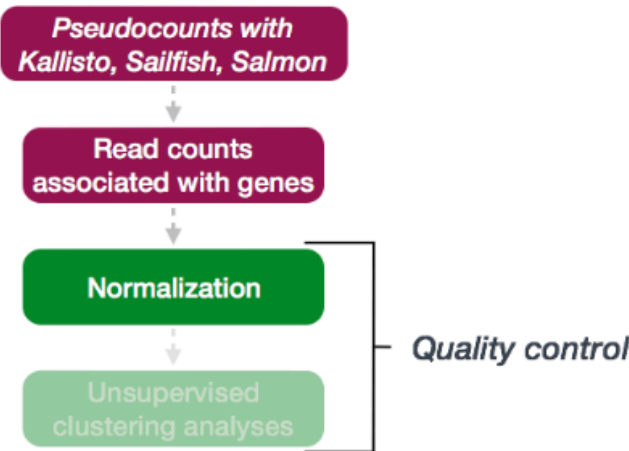
Approximate time: 60 minutes

Learning Objectives

- Explore different types of normalization methods
- Become familiar with the `DESeqDataSet` object
- Understand how to normalize counts using DESeq2

Normalization

The first step in the DE analysis workflow is count normalization, which is necessary to make accurate comparisons of gene expression between samples.



```
graph TD; A["Pseudocounts with Kallisto, Sailfish, Salmon"] --> B["Read counts associated with genes"]; B --> C["Normalization"]; C --> D["Unsupervised clustering analyses"]; C -.-> E["Quality control"]; D -.-> E;
```

The flowchart illustrates the DE analysis workflow. It starts with 'Pseudocounts with Kallisto, Sailfish, Salmon' (dark red box), followed by 'Read counts associated with genes' (dark red box), then 'Normalization' (green box), and finally 'Unsupervised clustering analyses' (light green box). A bracket on the right side of the 'Normalization' and 'Unsupervised clustering analyses' steps is labeled 'Quality control'.

Odds and Ends

- ❖ Name tags: Tent Cards
- ❖ Post-its
- ❖ Wi-Fi: **HMS Public** or **HMS Secure**
- ❖ Lunch locations
- ❖ Bathrooms
- ❖ Water Fountain
- ❖ Phones on vibrate/silent!

Contact us!

HBC training team: hbctraining@hsph.harvard.edu

HBC consulting: bioinformatics@hsph.harvard.edu

Twitter

[@bioinfocore](https://twitter.com/bioinfocore)