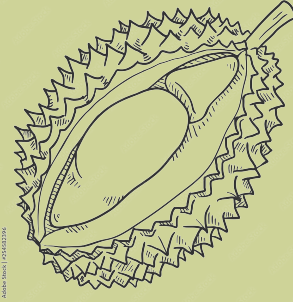
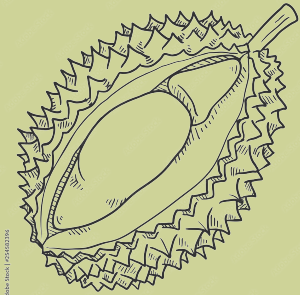


# The draft genome of tropical fruit durian (*Durio zibethinus*)



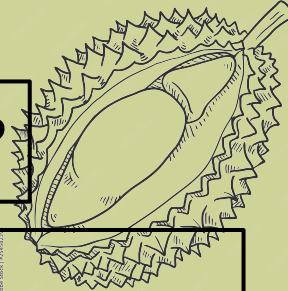
Andreas Bergfeldt | CC-BY 4.0

Genome Analysis 2023  
Paper IV  
Andreas Bergfeldt



Andreas Bergfeldt | CC-BY 4.0

# What is a Durian and why do this assembly?



- **What?**

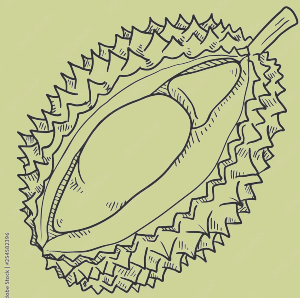
- Fruit popular in southeast asia
- Pungent odor, not allowed in some public spaces

- **Why?**

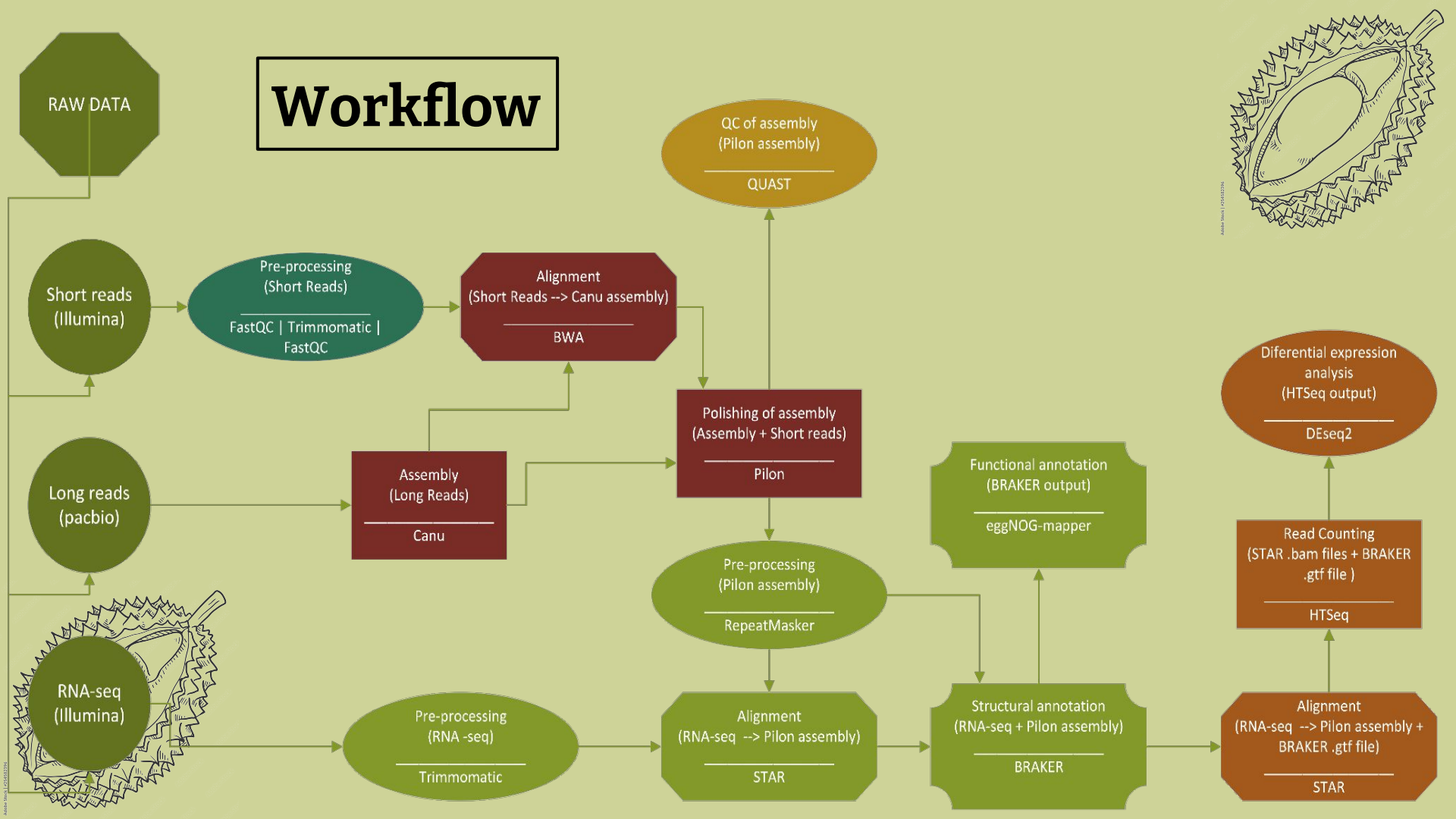
- 2016 China imported 600 mUSD
- Almost non existent genetic research

## My assembly

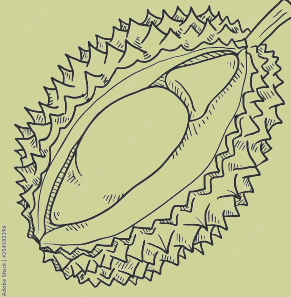
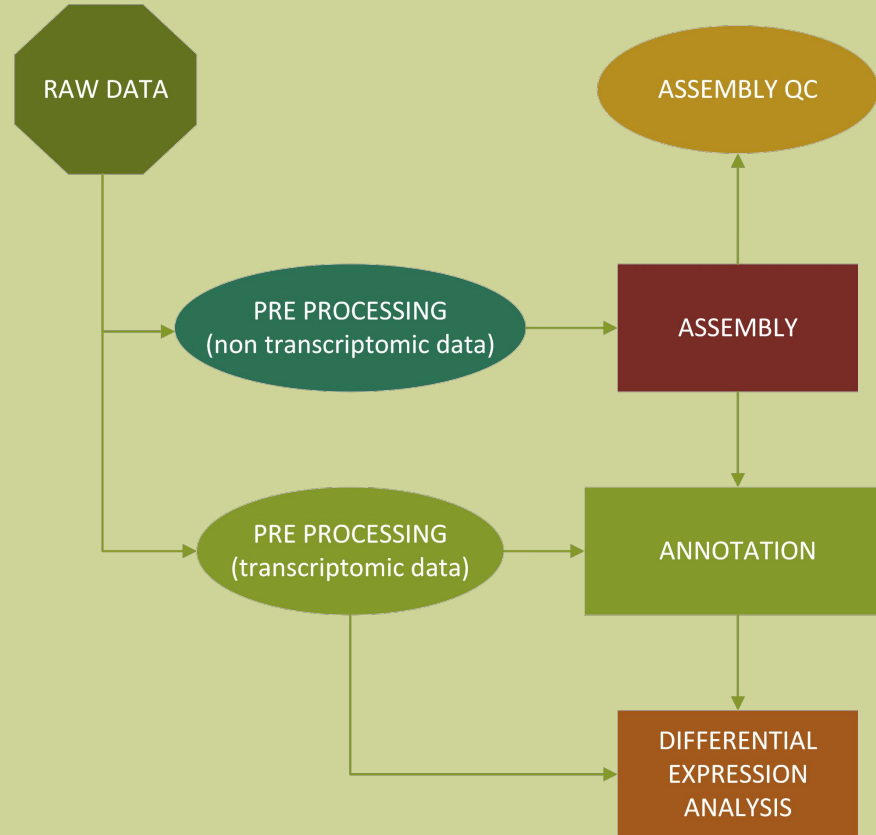
- One scaffold
  - Scaffold 10
- Too much to do whole genome
  - Computing power
  - Time



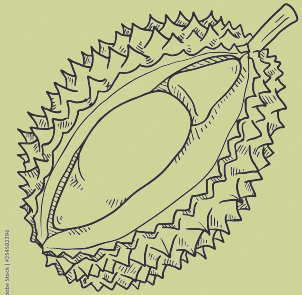
# Workflow



# Workflow - simplified



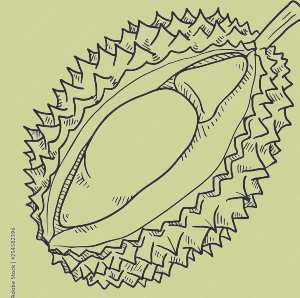
Adrian Jones | C0561276



Adrian Jones | C0561276

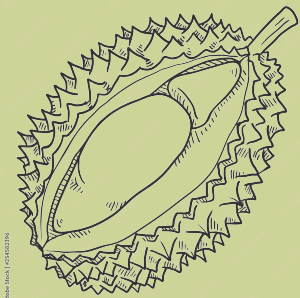
# Pre-processing - Trimmomatic

- Pre-trimmed data
  - Trimmed again to make sure it is good
- Trimming to remove adapters and low quality bases
- Quality control was done before and after



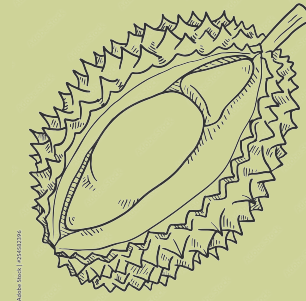
## Parameters - Trimmomatic

- ILLUMINACLIP = TruSeq3-SE:2:30:10
- LEADING = 3
- TRAILING = 3
- SLIDINGWINDOW = 4:15
- MINLEN = 36



# Pre-processing - FastQC

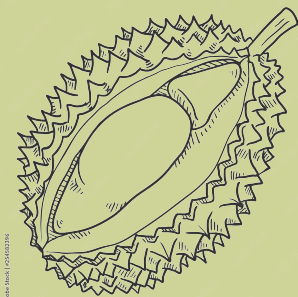
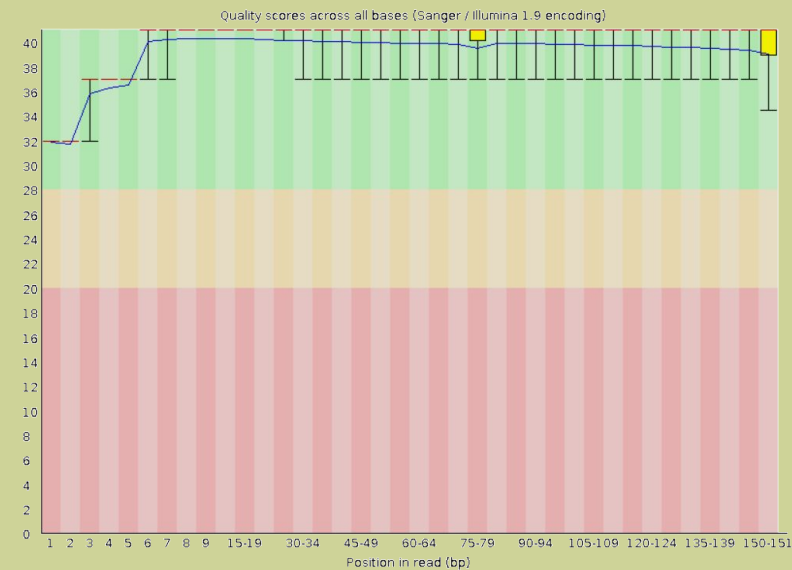
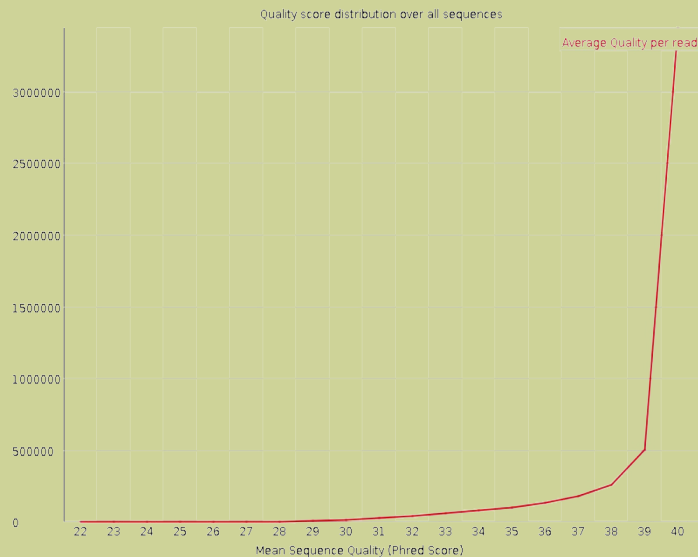
- Important to check data before proceeding!



- Quality deemed good enough

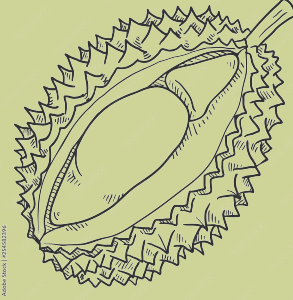
Pictures are from after trimming

## Per base sequence quality



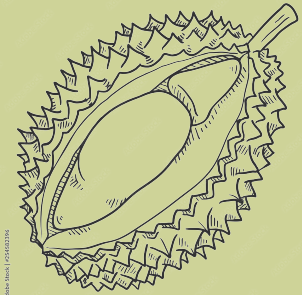
# Assembly - Canu

- Assembly of the long reads
- Plant genome with many repeats:
  - `corrMaxEvidenceErate` parameter
- `useGrid` for running in UPPMAX



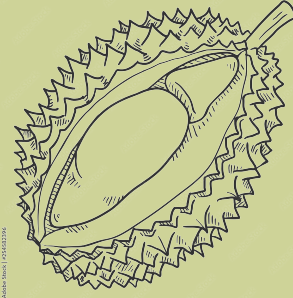
## Parameters - Canu

- `useGrid = false`
- `genomeSize = 30m`
- `corrMaxEvidenceErate = 0.15`

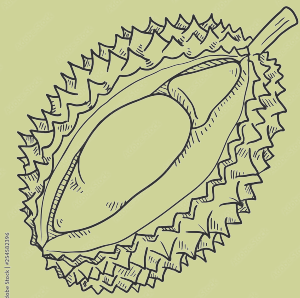


# Assembly - BWA (+ samtools)

- **BWA**
- Mapping short reads to long reads
  - Necessary for polishing later
- Manipulating BWA output with samtools to get .bam file
  - .fasta → .sai → .sam → .bam



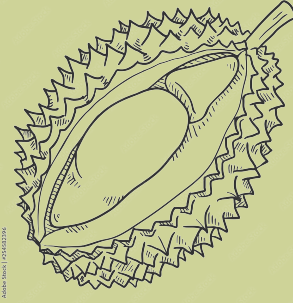
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Adrian Jones | CC BY-SA 4.0

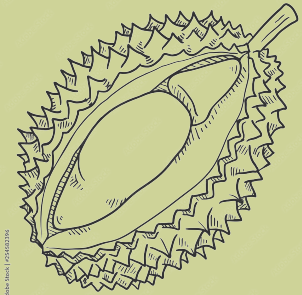


# Assembly - Pilon



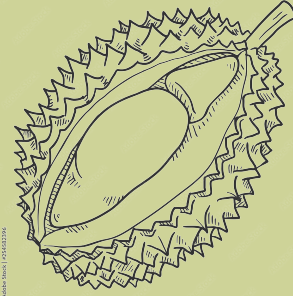
- **Pilon**
- Polishing assembly with short read data
  - Higher accuracy to long scaffolds

- **Results:** *(No good summary logfile so taken random reads)*
  - Total Reads: 18201
  - Confirmed 177014 of 192342 bases (92.03%)
  - Corrected 11 snps; 0 ambiguous bases  
corrected 96 small insertions totaling 102 bases  
41 small deletions totaling 42 bases

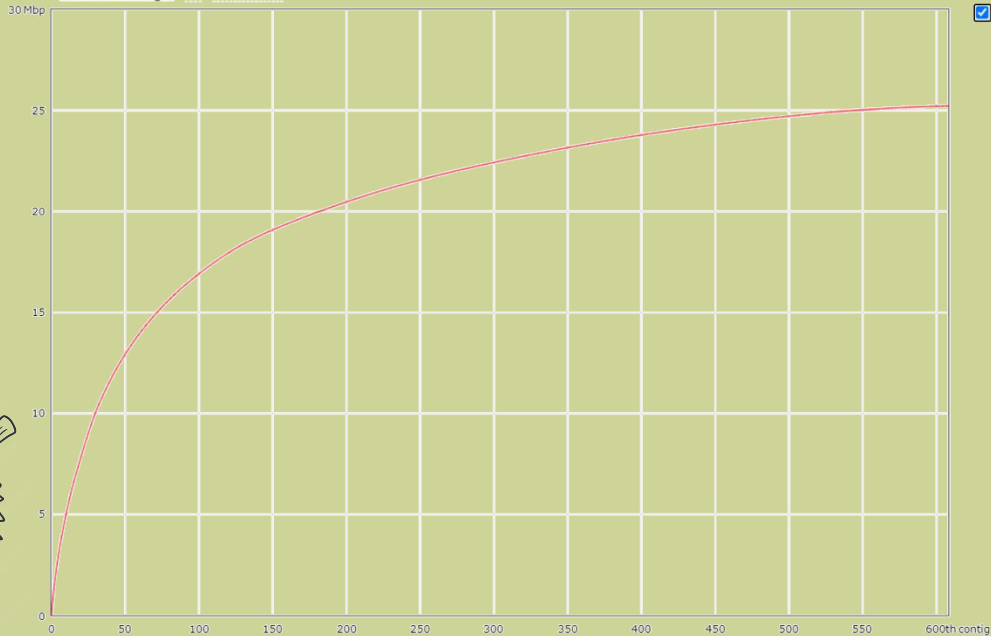


# Assembly QC - QUAST

- Was the assembly any good?



Plots: Cumulative length Nx GC content



Contigs are ordered from largest (contig #1) to smallest.

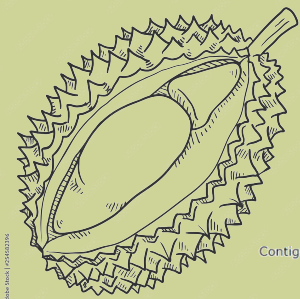
☒ durian\_pilon

## Statistics without reference ☒ durian\_pilon

# contigs	608
# contigs ( $\geq 0$ bp)	608
# contigs ( $\geq 1000$ bp)	608
# contigs ( $\geq 5000$ bp)	551
# contigs ( $\geq 10000$ bp)	433
# contigs ( $\geq 25000$ bp)	199
# contigs ( $\geq 50000$ bp)	114
Largest contig	826 217
Total length	25 217 558
Total length ( $\geq 0$ bp)	25 217 558
Total length ( $\geq 1000$ bp)	25 217 558
Total length ( $\geq 5000$ bp)	25 033 298
Total length ( $\geq 10000$ bp)	24 133 282
Total length ( $\geq 25000$ bp)	20 443 885
Total length ( $\geq 50000$ bp)	17 656 612
N50	120 097
N90	14 884
auN	198 308
L50	48
L90	318
GC (%)	31.45

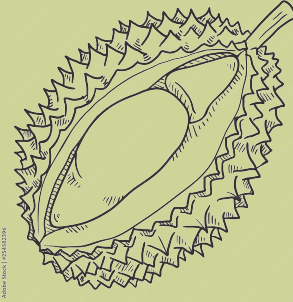
## Mismatches

# N's per 100 kbp	0
# N's	0



# Annotation - Trimmomatic + repeatMasker

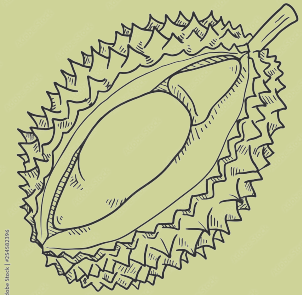
- Pre-processing before annotation
- Trimmomatic → same as before
- RepeatMasker
  - softmasking repeats for better annotation
  - Important to specify softmasking



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## Softmasking

- Identifies and “masks” repeats
- Changes bases in the fasta file from uppercase to lowercase
- Hardmasking removes info, softmasking does not



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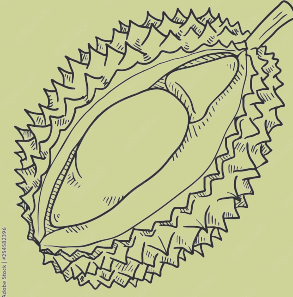
# Structural annotation -

## BRAKER

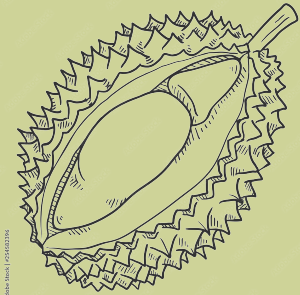
- Pipeline consisting of Augustus and GeneMark
- Annotates based on reference genome (masked assembly), and transcriptomic data
- Gives way to many outputs, still don't know exactly what is what

### **BRAKER results**

- Hardmasked scaffold → 96 genes identified
- Softmasked scaffold → 110 genes identified
- What are the genes?
  - who knows...
  - will come later



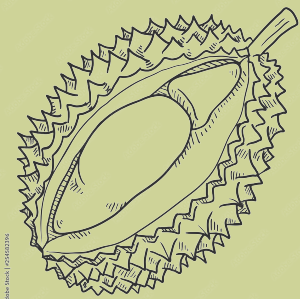
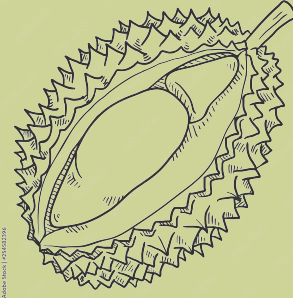
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Adrian Jones | CC-BY-NC-SA

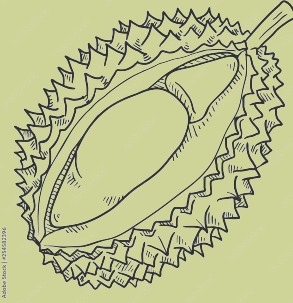
# Functional annotation - eggNOG-mapper

- Web based UI
- Loads of information about found hits
- Does not say a lot at this stage
  - Vital for differential expression analysis
  - Yes more patience is needed..



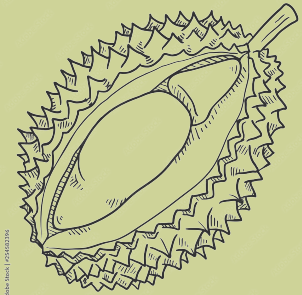
# Counting reads - STAR and HTSeq

- For transcriptomic reads to be counted they first need to be aligned
  - Done with STAR
- HTSeq counts the reads that are aligned to each predicted gene (from BRAKER), using a .gtf file



## HTSeq results

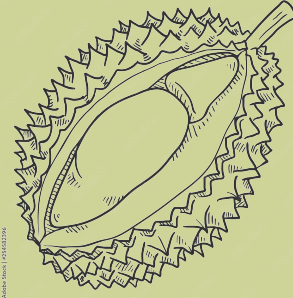
- 8 files with reads of varying length
- Total 29 269 185 record pairs
- 835 record pairs with missing mate record
  - 0.003% of total record pairs



# Differential expression analysis

- R-module for expression analysis

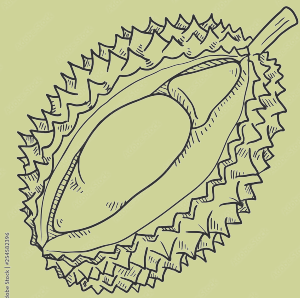
- Finally some tangible results!



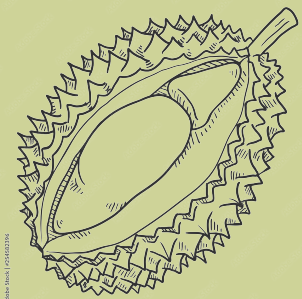
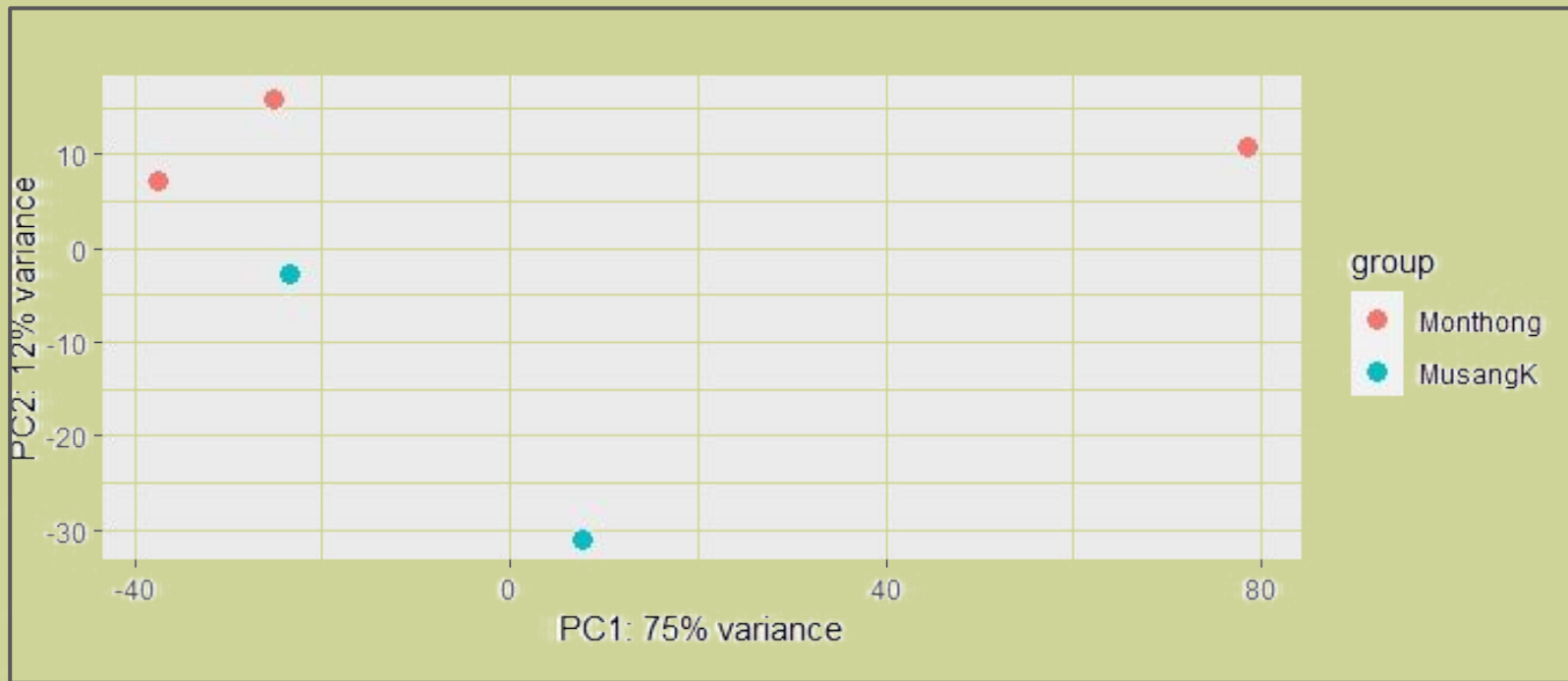
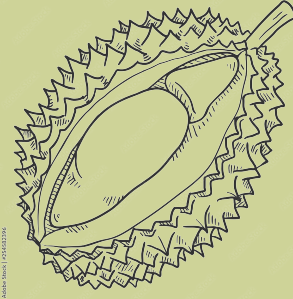
## The analysis in short

(In depth on future slides)

- 2 different species
  - Musang King and Mothong
- 2 different type of analysis
  - Between species (*Musang king, Mothong*)
  - Within species (*Musang King*)
    - Different plant organs (*aril, stem, leaf, root*)
- Types of visualization
  - PCA
  - Heatmap



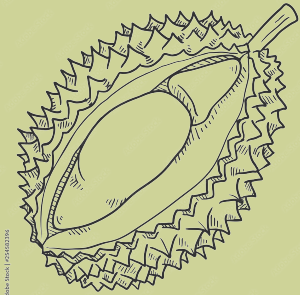
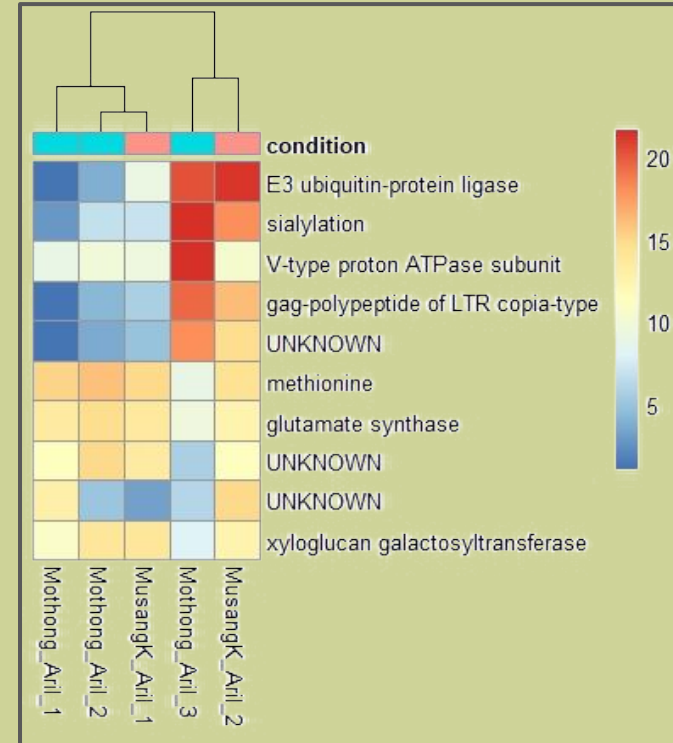
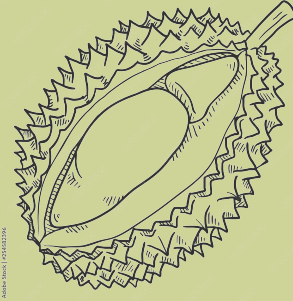
# DE - Between species - PCA



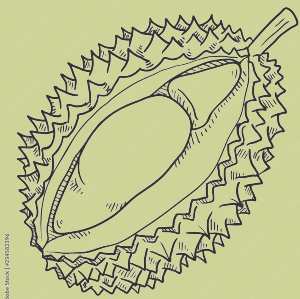
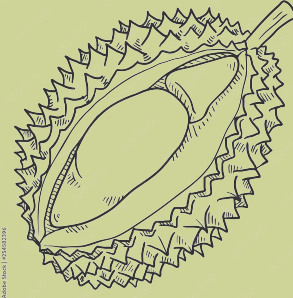


# DE - Between species - Heatmap

- Overall hard to separate the two species
- On PCA the grouping is not good
  - Groups Musang with Mothong
  - Does not group all of same species
- The heatmap does not show any patterns in which the species can be separated

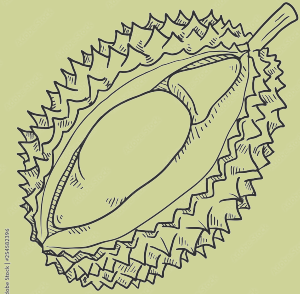
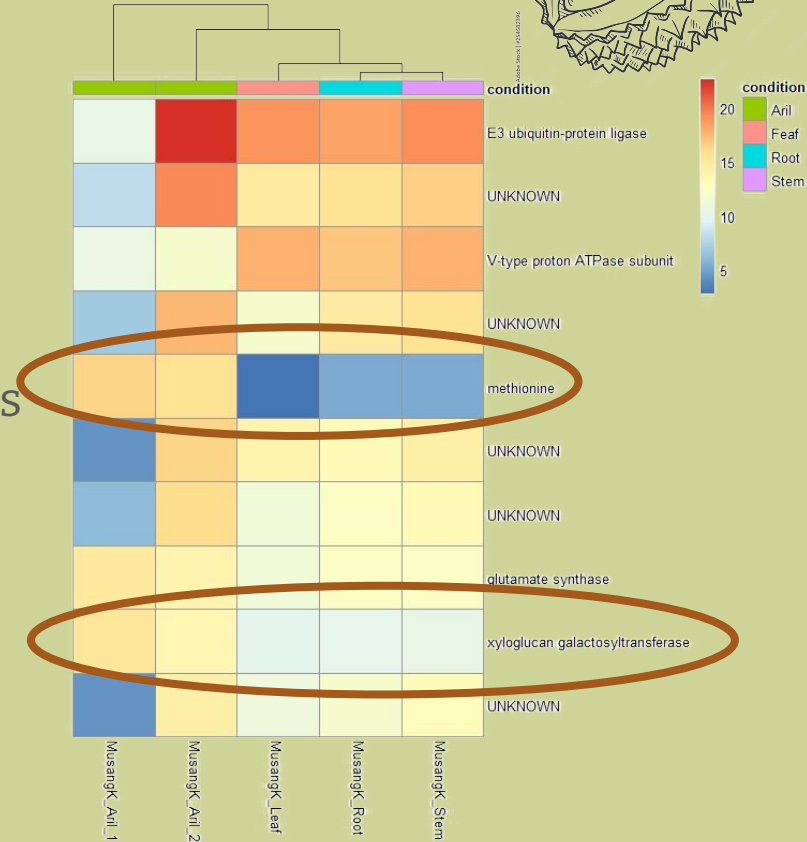
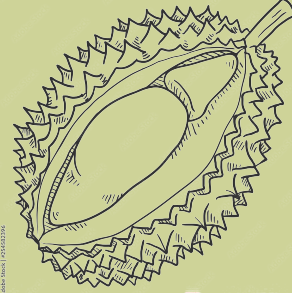


# DE - Within species - PCA



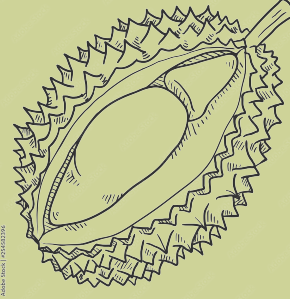
# DE - Within species - Heatmap

- Better separation between plant organs compared to plant species
  - PCA did okay
  - Possible separations at least
- Some patterns can be seen that separates arils from the rest of the plant organs
  - Eg. methionine and xyloglucan

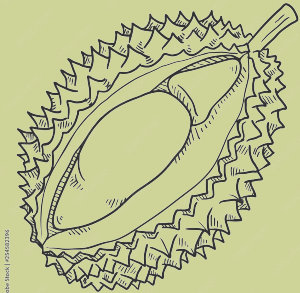
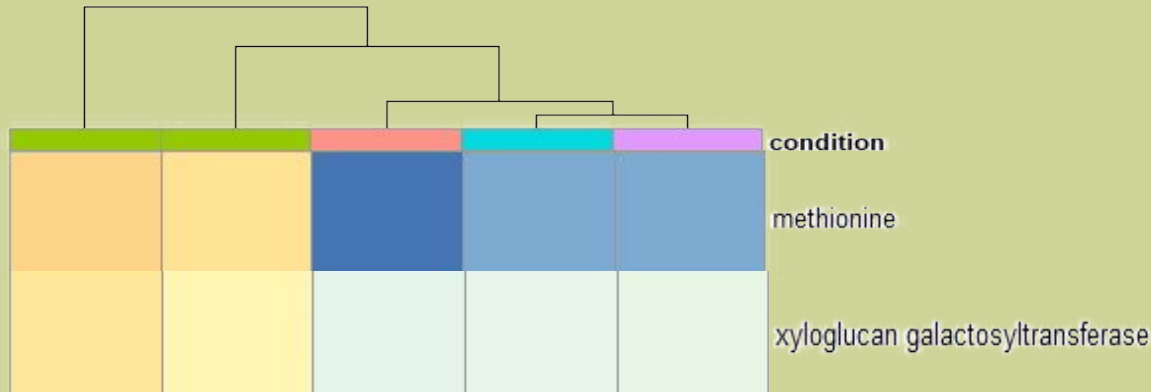
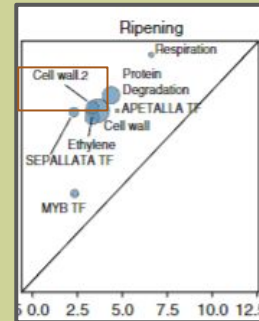
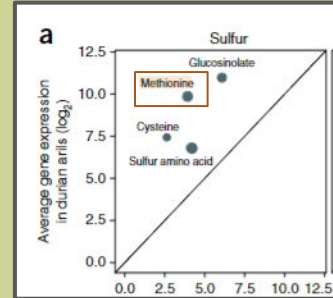


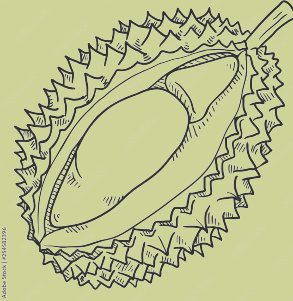
# DE - Biological conclusions

- Methionine converted to methanethiol with the enzyme methionine  $\gamma$ -lyase
  - Methanethiol has key role in odor of durian
- Xyloglucan building block of cell wall 2
  - “interlace cellulose microfibrils in most flowering plants.”
  - Genes with association to cell wall 2 upregulated in arils



Figures from paper IV





# Thank you for your attention!

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For more in depth information about programs etc. please look at my github wiki for this project: <https://github.com/A-Bergfeldt>

