Rapid Transcriptome Characterization for a nonmodel organism using 454 pyrosequencing

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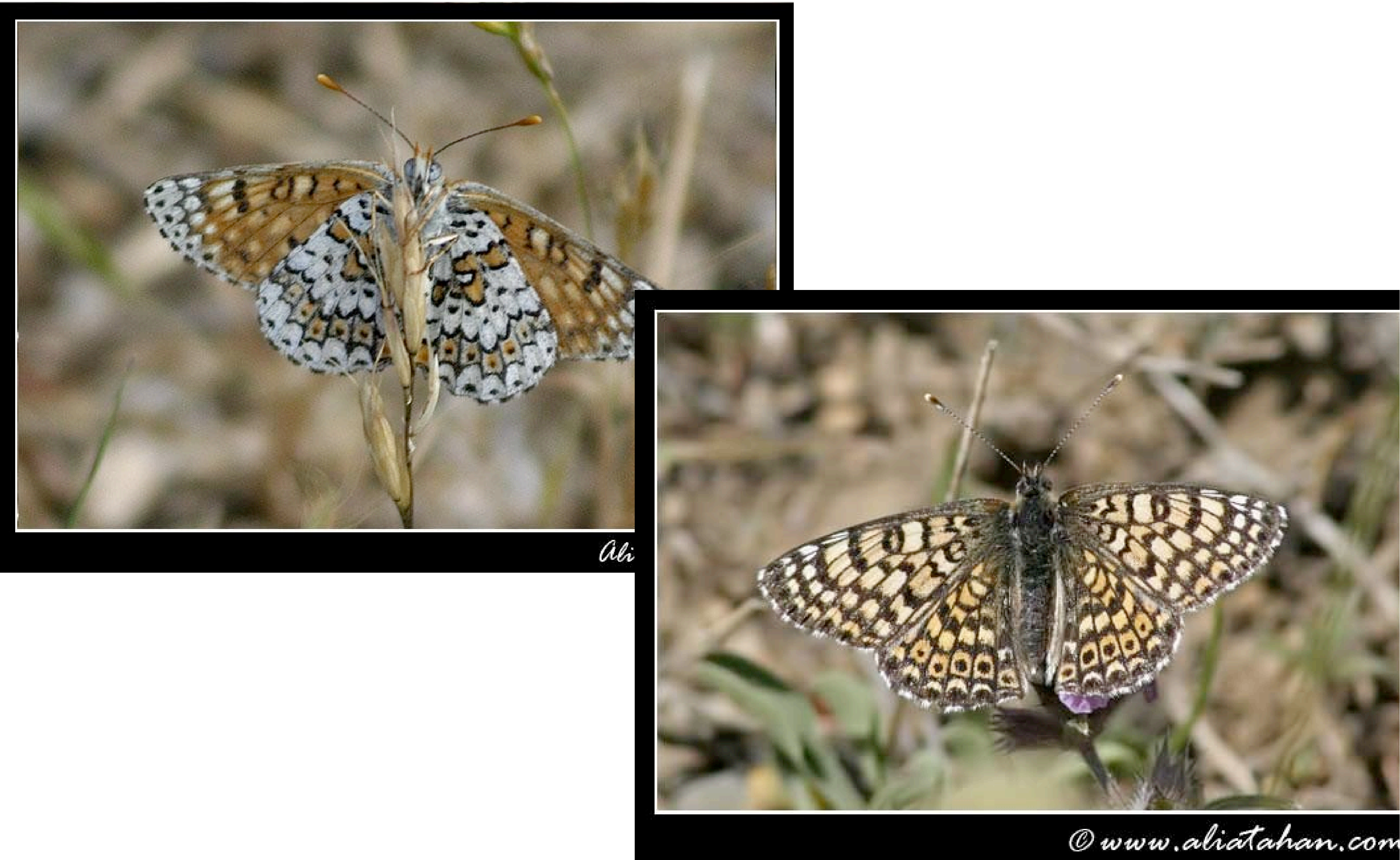
The problem and the Paper

- Goal: Assemble the Transcriptomes/cDNA using NGS
  - Its **cheaper** than using Sanger
- Details:
  - Sequence cDNA with 454 and Sanger
  - Show that the 454 is useful for many tasks, and is no worse than Sanger (but cheaper).

The subject: Glanville Fritiliy butterfly

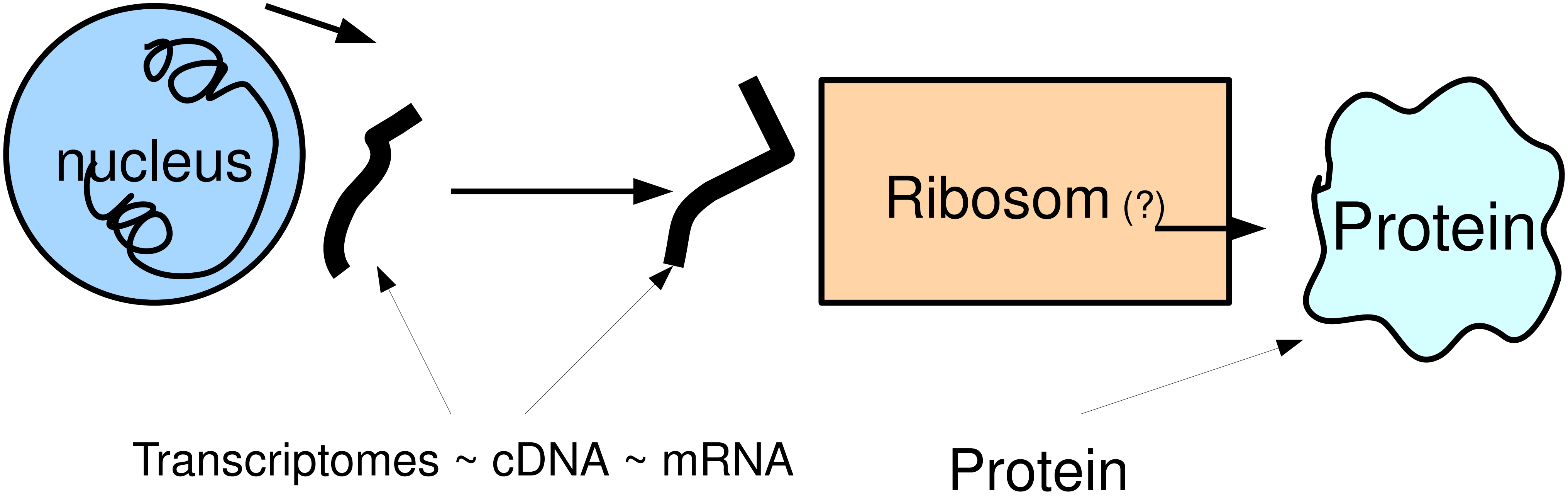


Recap: 454 and Sanger

- 454:
  - 4.5 hours
  - $2K
  - Read length: 110 bp
  - 300,000 reads
  - ~ 30 Mbase
- Sanger: expensive:
  - Read length: 500bp

Transcriptomes and cDNA

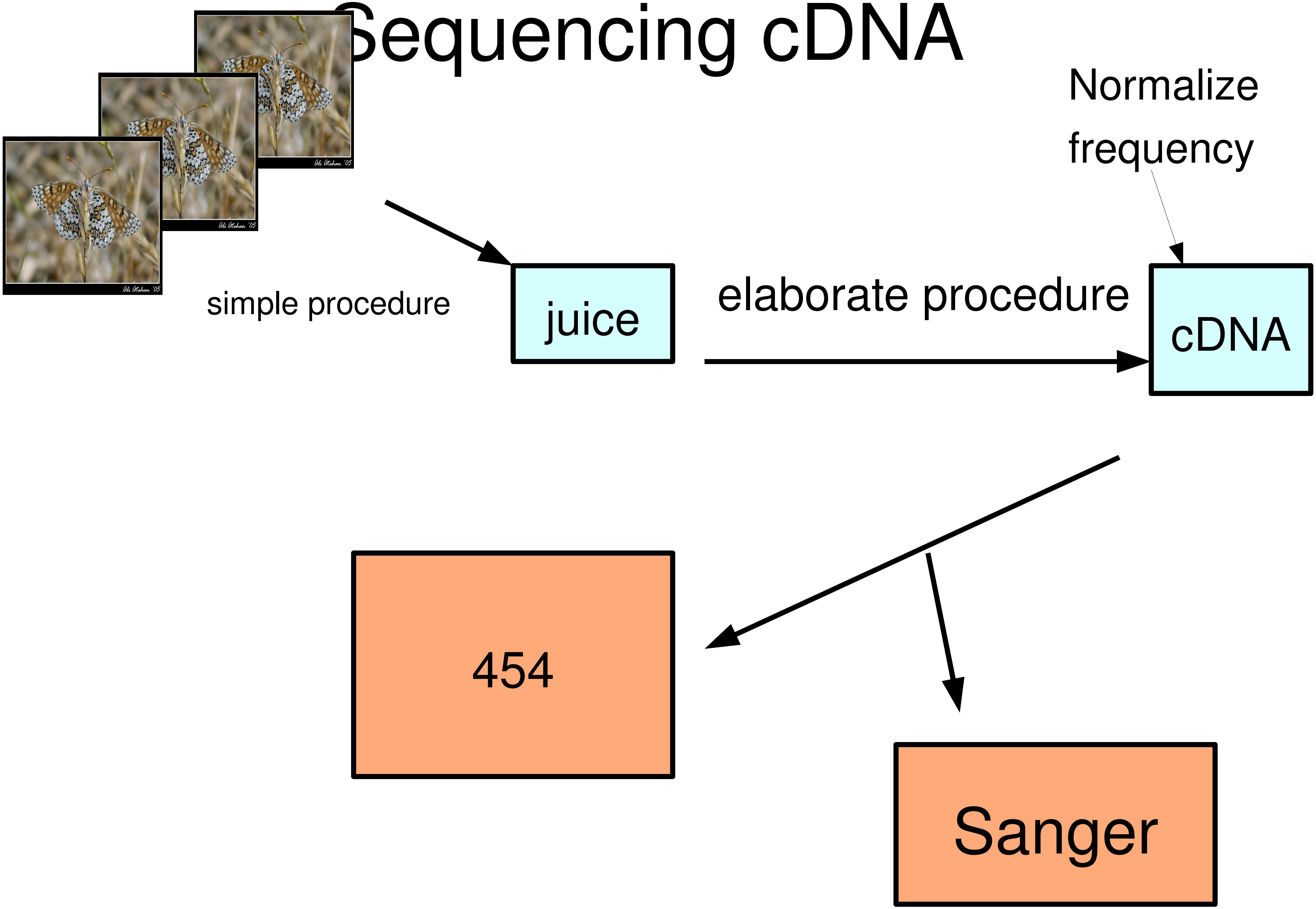
- (I think that) these are the DNA sequences that are currently used to generate proteins.
- They correspond to the expressed proteins.



Comparison to previous work

- 454 was used before for transcriptome sequencing
- But ...
  - Either Sanger was also used or a reference genome was known
  - Or lower coverage was used, so assembly was impossible
-

Sequencing cDNA



Ali Atahan '05

Details of the process

- Get RNA from larvae, pupae, and from adults.
  - From a diverse population
  - The butterfly will have different transcriptomes in different stages of its life
- RNA -> cDNA (magic)

Algorithm

- SEQMAN PRO 7.1
  - Use it to get rid of low quality data
  - Use it to assemble the reads from Sanger and from the 454 – get contigs.
  - That's it.

What to do with the data?

- Take a database of proteins, Uniprot 9.2
- Align the contigs to the proteins, to find which proteins are expressed in the butterfly
- More alignments to proteins of :
  - *Bombyx mori*
  - *Drosophila melanogaster*
  - *M. cinxia*
  - Butterflybase

Microarrays

- Some good contigs (ones that matched good proteins, I think) were used as probes for microarrays
- 200K microarray probes were generated
- Microarrays tell us what genes are expressed

Results of sequencing

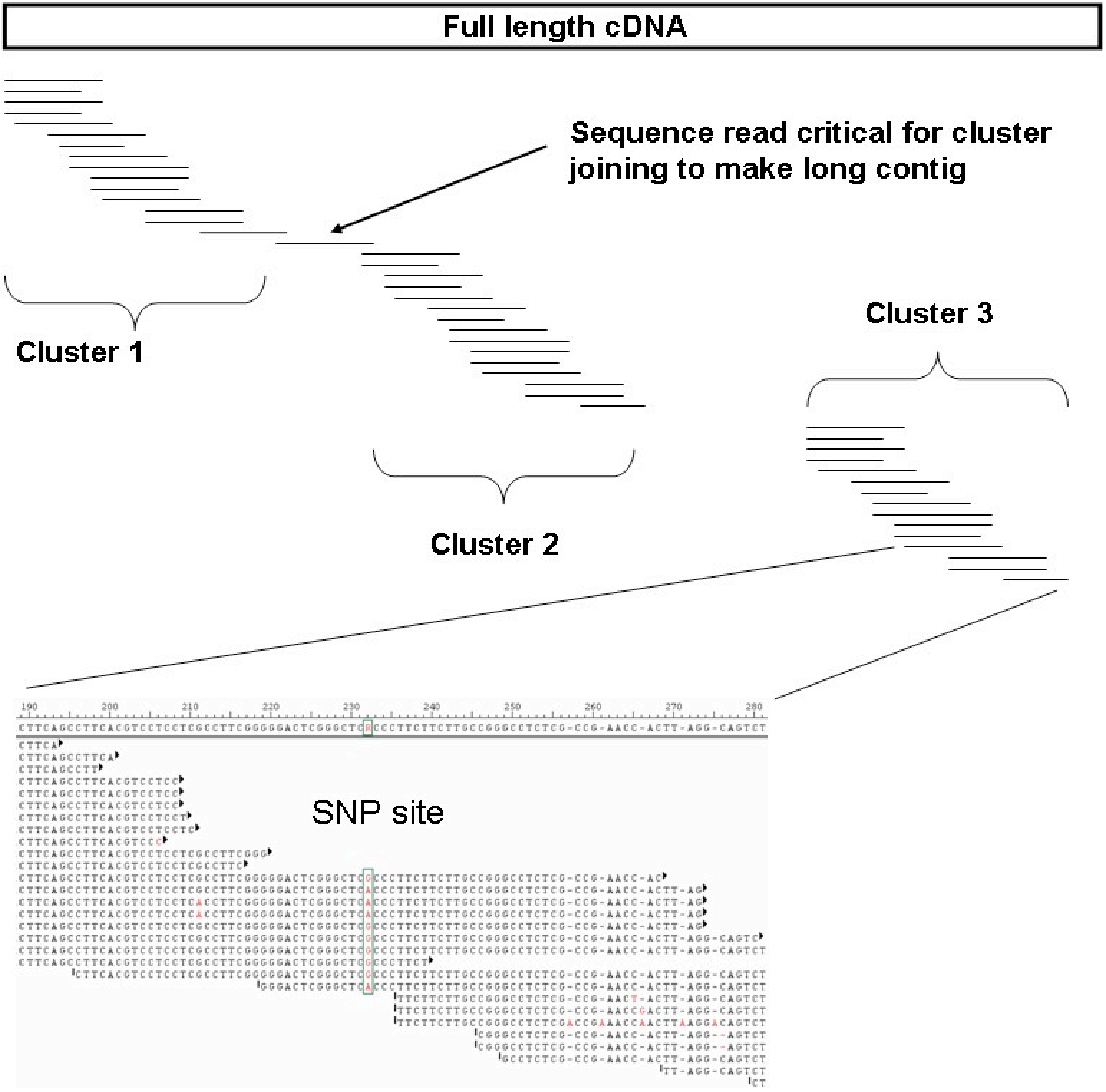
- 50K contigs, mean length 200 bp (it seems short to me)
- They tried to look for exact matches between contigs. But most of these matches matched to different proteins (except 2%)
- So these must be motifs in different proteins

Sanger vs 454

- 92% of Sanger reads had strong alignments to 454 contigs
- Contigs had very few gaps when aligned to Sanger

Coverage is important for assembly

- They have evidence for that.



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CTTCAGCCTT
CTTCAGCCTTCACGTCCTCC
CTTCAGCCTTCACGTCCTCC
CTTCAGCCTTCACGTCCTCC
CTTCAGCCTTCACGTCCTCCT
CTTCAGCCTTCACGTCCTCCTC
CTTCAGCCTTCACGTCCC
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                                                         CGGGCCTCTCG-CCG-AACC-ACTT-AGG--AGTCT
                                                         CGGGCCTCTCG-CCG-AACC-ACTT-AGG--AGTCT
                                                            GCCTCTCG-CCG-AACC-ACTT-AGG-CAGTCT
                                                                                TT-AGG-CAGTCT
                                                                                           CT
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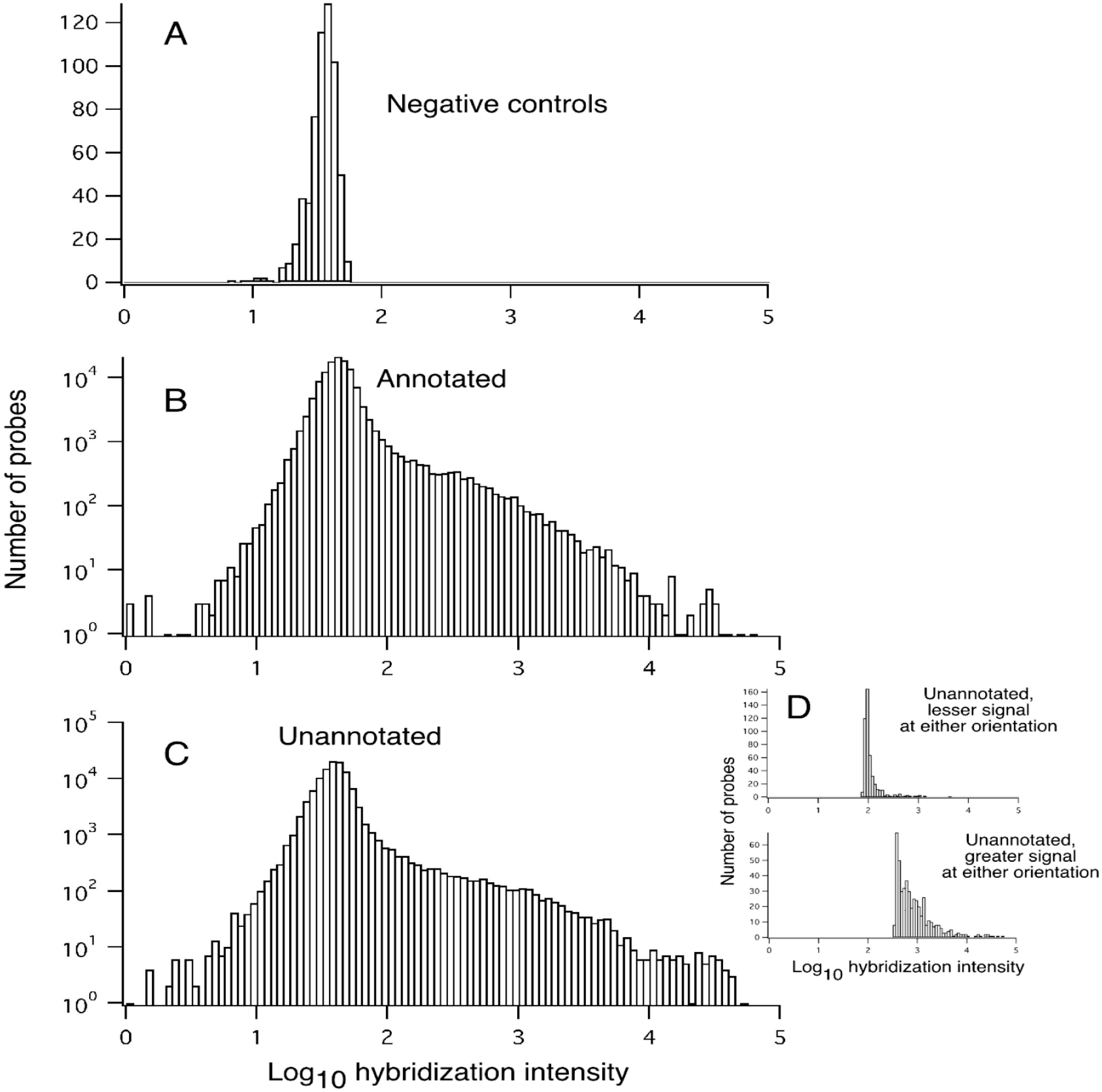
Transcriptome coverage Breadth

- 20% of the contigs were well aligned to proteins in the different databases
- 9000 unique proteins were detected this way
  - with 73% amino acid identity
- If we microarray some of the unmatched reads, the responsiveness of the microarray is the same for annotated and unannotated (matched) contigs. So more proteins were found.


A
Negative controls
120
100
80
60
40
20
0
0
1
2
3
4
5
B
Annotated
Number of probes
10^4
10^3
10^2
10^1
10^0
0
1
2
3
4
5
C
Unannotated
10^5
10^4
10^3
10^2
10^1
10^0
0
1
2
3
4
5
Log$_{10}$ hybridization intensity
D
Unannotated,
lesser signal
at either orientation
160
140
120
100
80
60
40
20
0
0
1
2
3
4
5
Unannotated,
greater signal
at either orientation
60
50
40
30
20
10
0
0
1
2
3
4
5
Number of probes
Log$_{10}$ hybridization intensity

Functional annotation

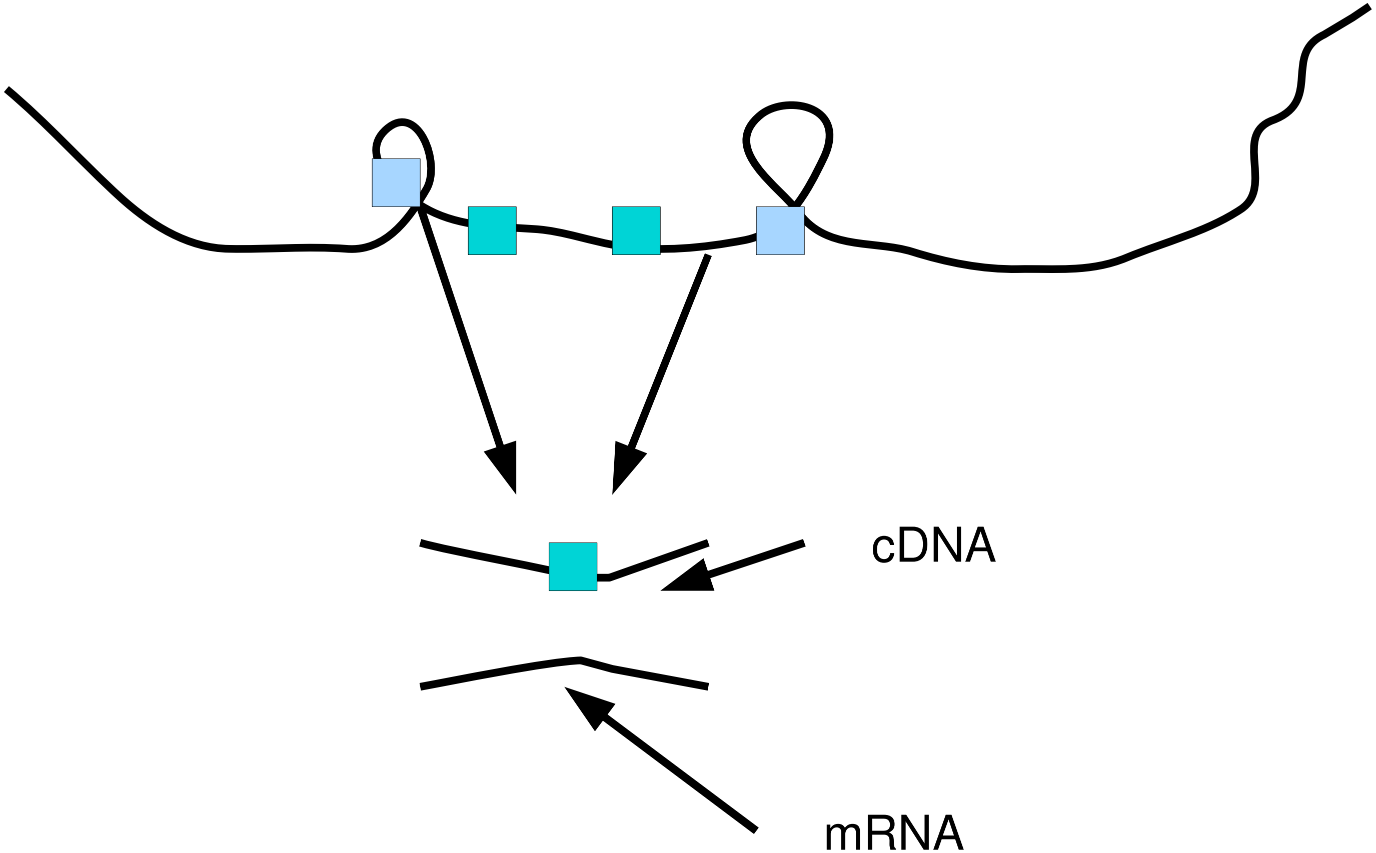
- Not too sure...
- The reads/contigs were matched to known proteins with known function
- This way, the function of the reads was guessed

SNP discovery

- Take the contigs, and discover SNPs
- 6.7 SNPs per 1000 base pairs
- 751 SNPs at 6X covered sites, in 355 contigs

Alternative splicing

- It is when the dna is spliced before turning to cDNA and mRNA



Alternative splicing effects on assembly

- Characterize 2 such genes using PCR, cloning method, amplification of cDNA ends
- The genes have deep coverage
- Somehow, it made things more difficult

Detection of intracellular parasite

- Many reads had alignment to sequences of non-insects
- That's pretty much it!