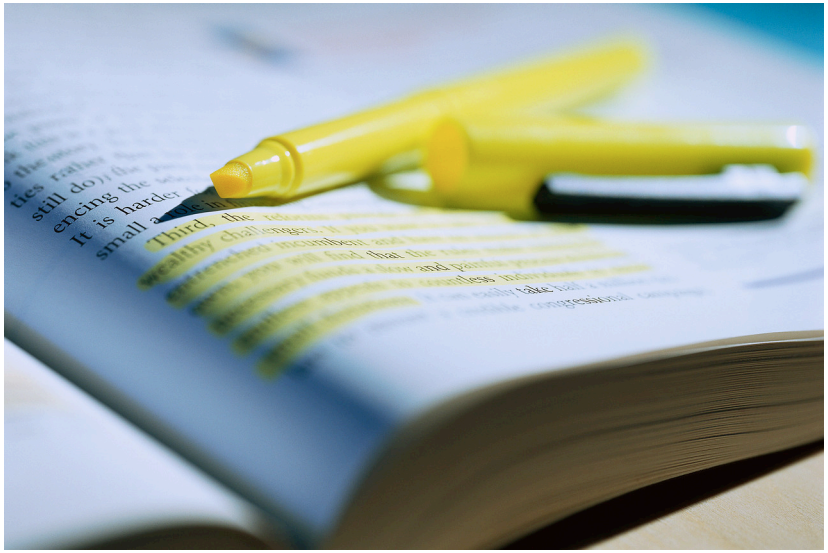
Basics of Genome Annotation

Daniel Standage
Biology Department
Indiana University

An-no-ta-tion \ˌa-nə-ˈtā-shən\

1. A critical or explanatory note or body of notes added to a text
2. The act of annotating



http://dictionary.reference.com/browse/annotation?s=t

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AAAGGGTCG
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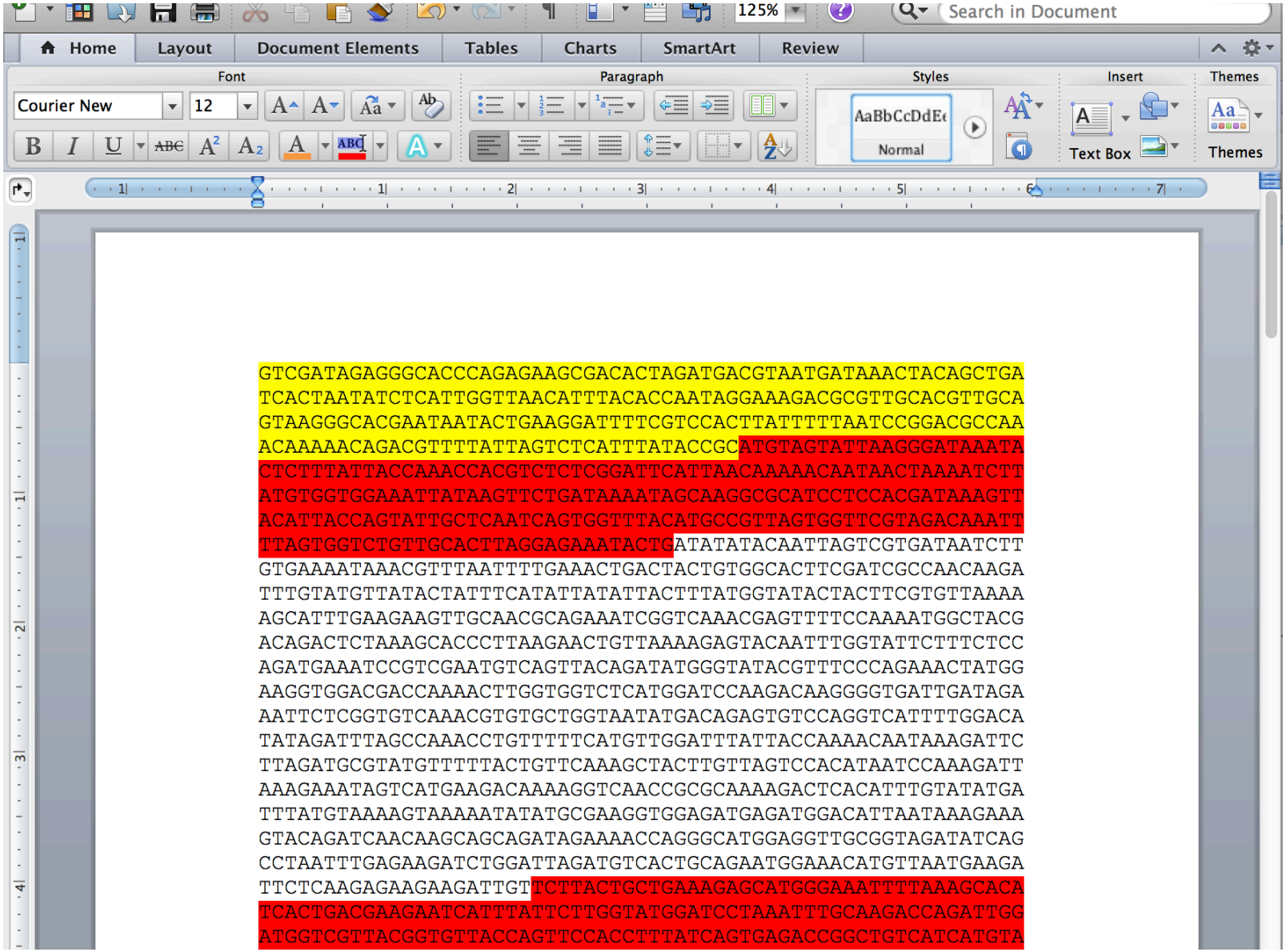
CATTCATTT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Document Elements
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Charts
SmartArt
Review
Font
Courier New
12
Paragraph
Styles
AaBbCcDdEe
Normal
Insert
Text Box
Themes
Themes
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TTCTCAAGAGAAGAAGATTGTTCTTACTGCTGAAAGAGCATGGGAAATTTTAAAGCACA
TCACTGACGAAGAATCATTTATTCTTGGTATGGATCCTAAATTTGCAAGACCAGATTGG
ATGGTCGTTACGGTGTTACCAGTTCCACCTTTATCAGTGAGACCGGCTGTCATCATGTA

Genome annotation

```
##gff-version   3
##sequence-region   chr8 1 100000
chr8    Gaze    gene    10503   11577   7.84    -       .       ID=GSVIVG01033678001;complete=1
chr8    Gaze    mRNA    10503   11577   7.84    -       .       ID=GSVIVT01033678001;Parent=GSVIVG01033678001;complete=1
chr8    Gaze    CDS     10503   10593   -0.4    -       1       ID=GSVIVT01033678001.cds1;Parent=GSVIVT01033678001;complete=1
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chr8    Gaze    exon    22057   22382   .       +       1       ID=GSVIVT01033677001.exon1;Parent=GSVIVT01033677001
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chr8    Gaze    three_prime_UTR 23023   23119   0.878   +       .       ID=GSVIVT01033677001.utr1;Parent=GSVIVT01033677001
###
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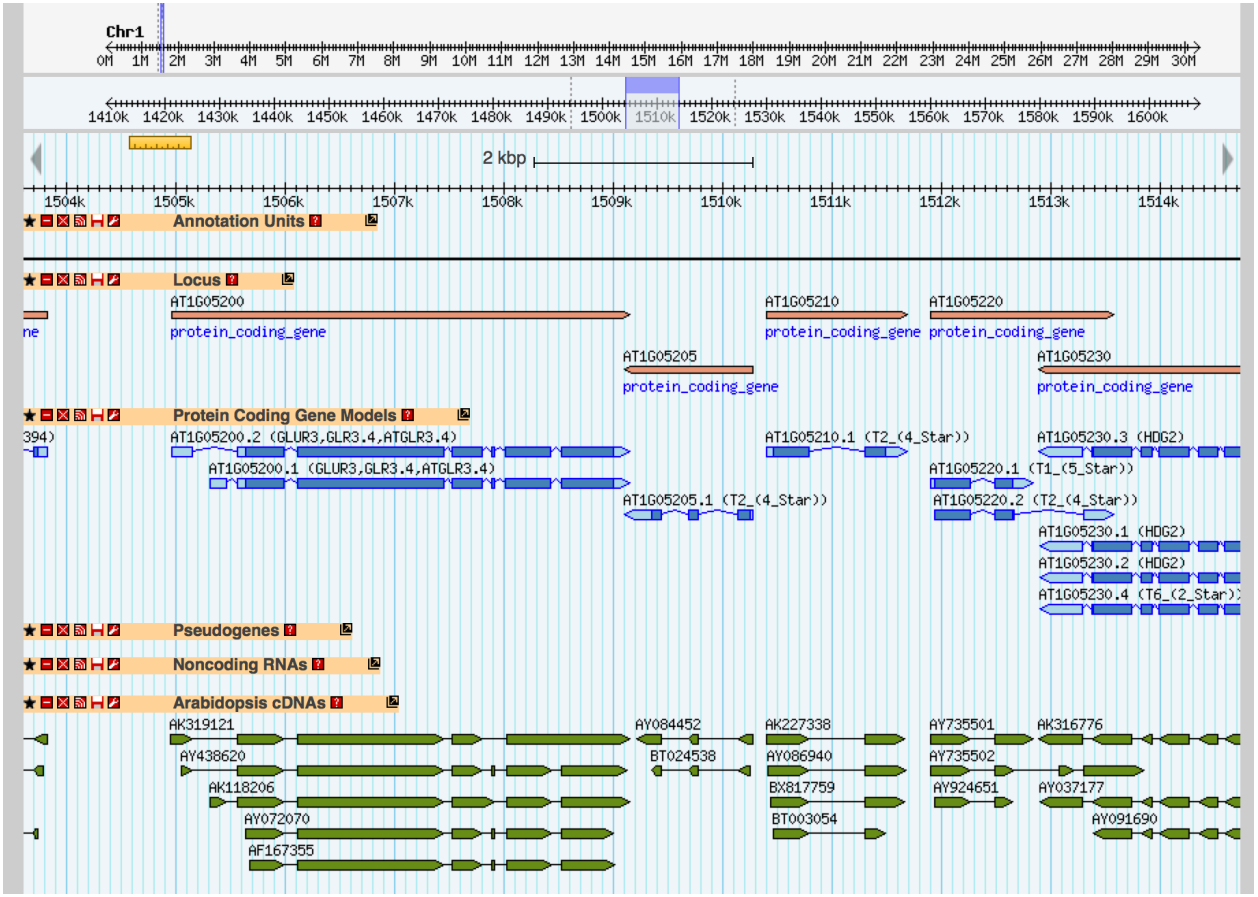
Genome annotation



Genome annotation

- Information itself (e.g., this gene encodes a cytochrome P450 protein, with exons at…)
- Annotation process (operational definition)
- Data management
  - formatting
  - storage
  - distribution
  - representation

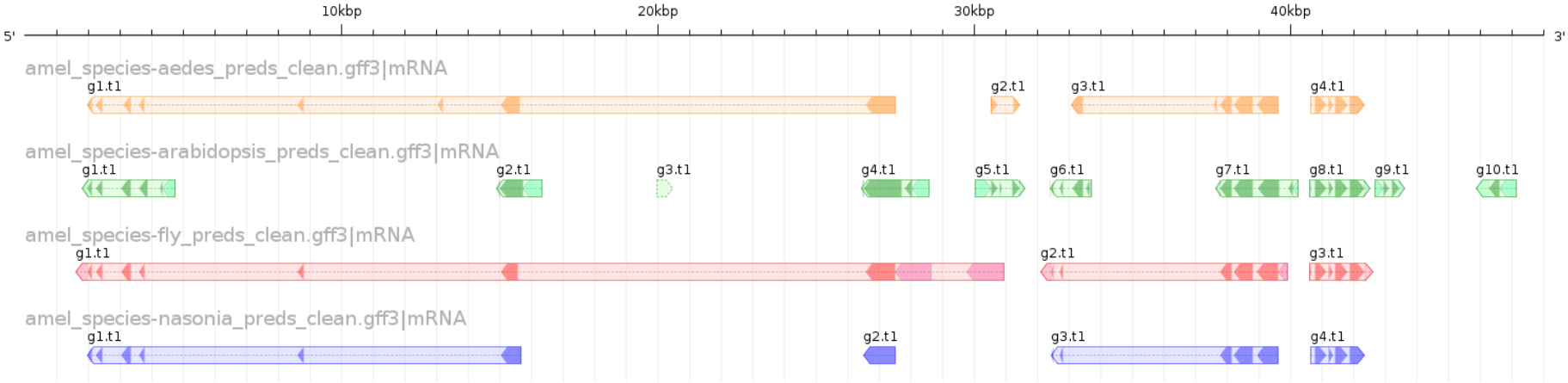
Methods for gene finding

- *Ab initio* gene prediction
- Gene prediction by spliced alignment

Ab initio gene prediction

- *Ab initio*: "from first principles"
- Requires only a genomic sequence
- Uses statistical model of genome composition to identify most probable location of start/stop codons, splice sites
- Popular implementations
  - Augustus
  - GeneMark
  - SNAP

Ab initio gene prediction



Prediction by spliced alignment

- Utilizes experimental (transcript) and/or homology (reference proteins) data
- Spliced alignment of sequences reveals gene structure
  - matches = exons
  - gaps = introns
- Popular implementations
  - GeneSeqer
  - Exonerate
  - GenomeThreader

Comparison of prediction methods

Ab initio	Spliced alignment
Do not require extrinsic evidence	Requires transcript and/or protein sequences
Does not benefit from additional transcript data	Accuracy improves with additional transcript data
More likely to recover complete gene structures	More likely to recover accurate internal exon/intron structure

Issues with gene prediction

- Accuracy (best methods achieve ≈80% at exon level)
- Parameters matter (species-specific codon usage)
- Comparison and assessment

Recurring theme in genomics

- Once I have a result, how to I assess its reliability?
- How do I compare it to alternative results?

Recurring theme in genomics

"Why, when you only had one result, did you think that was the correct one?"


Why don't we have both?

Manual annotation

- Visually inspect gene predictions, spliced alignments
- Determine reliable consensus gene structure
- Available software
  - Apollo: http://apollo.berkeleybop.org
  - yrGATE: http://goblinx.soic.indiana.edu/src/yrGATE

yrGATE-GDB001.
(NEW_ANNOT)

Annotation Class [Select...]

Project [Select...]

Working Group [Select...]

Genome Location

Genome Segment: PdomSCFr1.2-(

start: 13309 end: 21726 Change Location

Strand forward reverse strand Reset mRNA structure

User Defined Exons

20043 21226
(Gene models (Maker))

Portals and Tools

CpGAT

GeneMark GENSCAN

Genome Threader

Manual Entry

start

end add

Clear User-Defined Exons Table

mRNA (6397 nucleotides)

```
GTCGATAGAGGGCACCCAGAGAAGCGACACTAGATGACGTAATGA
TAAACTACAGCTGATCACTAATATCTCATTGGTTAACATTTACAC
CAATAGGAAAGACGCGTTGCACGTTGCAGTAAGGGCACGAATAAT
ACTGAAGGATTTTCGTCCACTTATTTTTAATCCGGACGCCAAACA
AAAACAGACGTTTTATTAGTCTCATTTATACCGCATGTAGTATTA
AGGGATAAATACTCTTTATTACCAAACCACGTCTCTCGGATTCAT
TAACAAAAACAATAACTAAAATCTTATGTGGTGGAAATTATAAGT
TCTGATAAAATAGCAAGGCGCATCCTCCACGATAAAGTTACATTA
CCAGTATTGCTCAATCAGTGGTTTACATGCCGTTAGTGGTTCGTA
GACAAATTTTAGTGGTCTGTTGCACTTAGGAGAAATACTGATATA
```

blastn blastx tblastx miRBASE

Protein Coding Region *Use the ORF finder!*

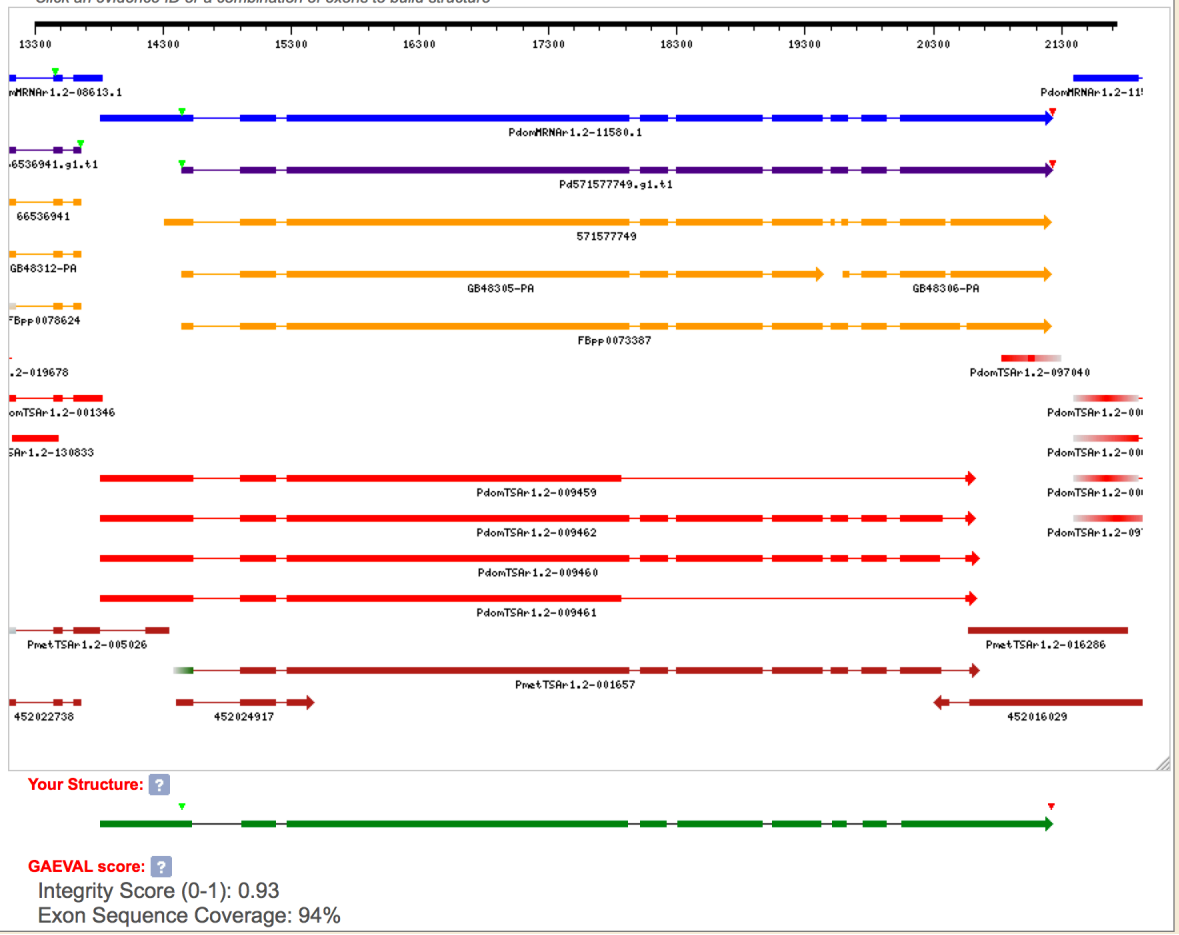
Start 14449 End 21226 ORF Finder

Protein (1918 amino acids)

```
MATTDSKAPLRTVKRVQFGILSPDEIRRMSVTDMGIRFPETMEGG
RPKLGGLMDPRQGVIDRNSRCQTCAGNMTECPGHFGHIDLAKPVF
```

Evidence Plot Change plot width to 850 pixels *Track Color Codes*

Click an evidence ID or a combination of exons to build structure



Your Structure:

GAEVAL score:

Integrity Score (0-1): 0.93

Exon Sequence Coverage: 94%

“Combiner” tools

- Maker: http://www.yandell-lab.org/software/maker.html

- EVidenceModeler: http://evidencemodeler.sourceforge.net

Evaluating annotations

- Comparison
  - ParsEval[1]: http://standage.github.io/AEGeAn
- Quality assessment
  - Annotation Edit Distance[2] (Maker)
  - GAEVAL (PlantGDB)

[1]Standage and Brendel (2012) *BMC Bioinformatics*, **13**:187.
[2]Eilbeck *et al* (2009) *BMC Bioinformatics,* **10**:67.

Recommendations / Considerations

- Automated annotation
- Manual refinement
- Assessment and filtering for particular analyses
- Be very skeptical
- Remember: no “one true” assembly / annotation

xGDBvm

- Pre-installed on iPlant cloud (free for academics!)
  - Search for xGDBvm image
- Includes an EVM pipeline for automated annotation
- Includes yrGATE for manual annotation
- Visualization, search, access control
- More info: http://goblinx.soic.indiana.edu

xGDBvm demo

Polistes dominula example