Differentiating between error and information in DNA sequence data

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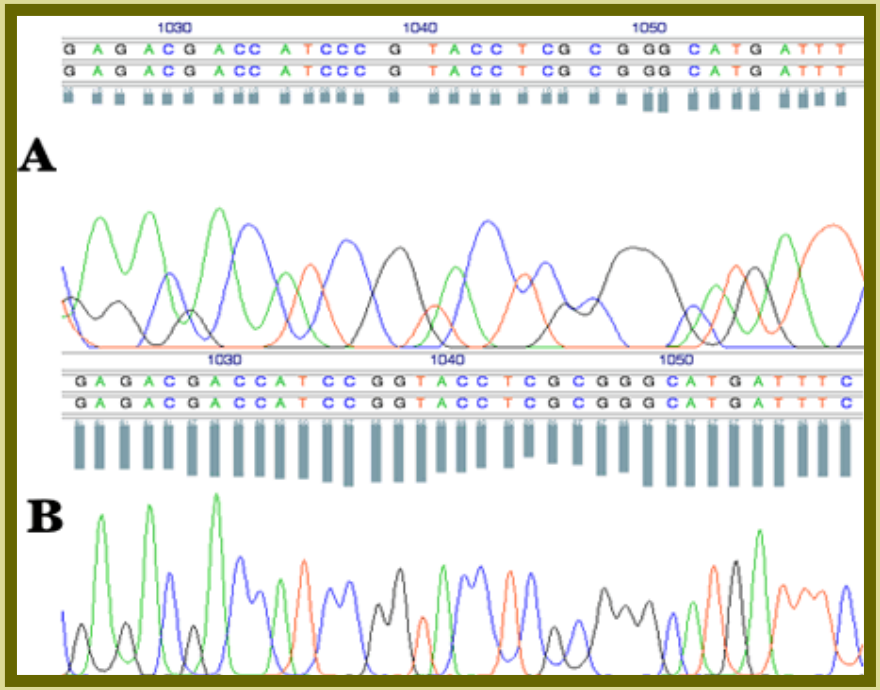
Introduction / Background

- DNA sequencing

Introduction / Background

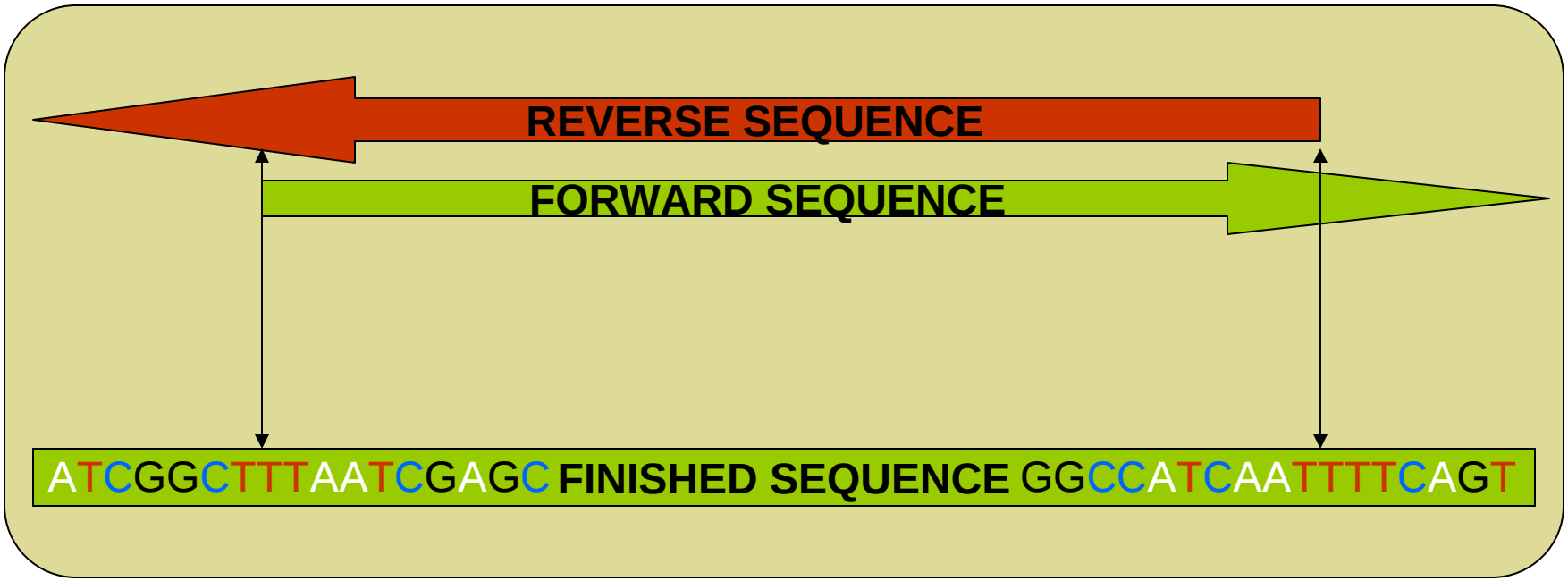
- Phred scores



Source: http://www.nucleics.com/images/site_images/CounterTrace-before-after.gif

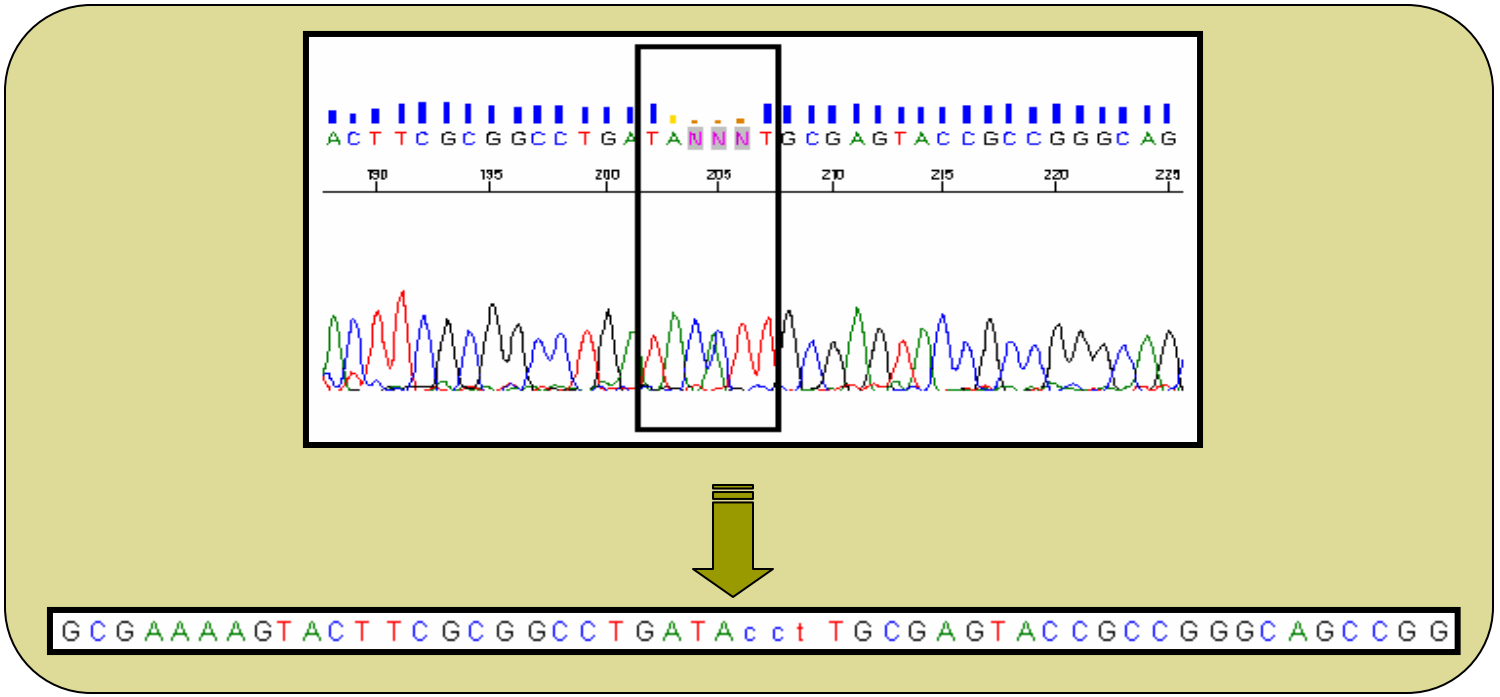
Introduction / Background

- Problem being addressed



Introduction / Background

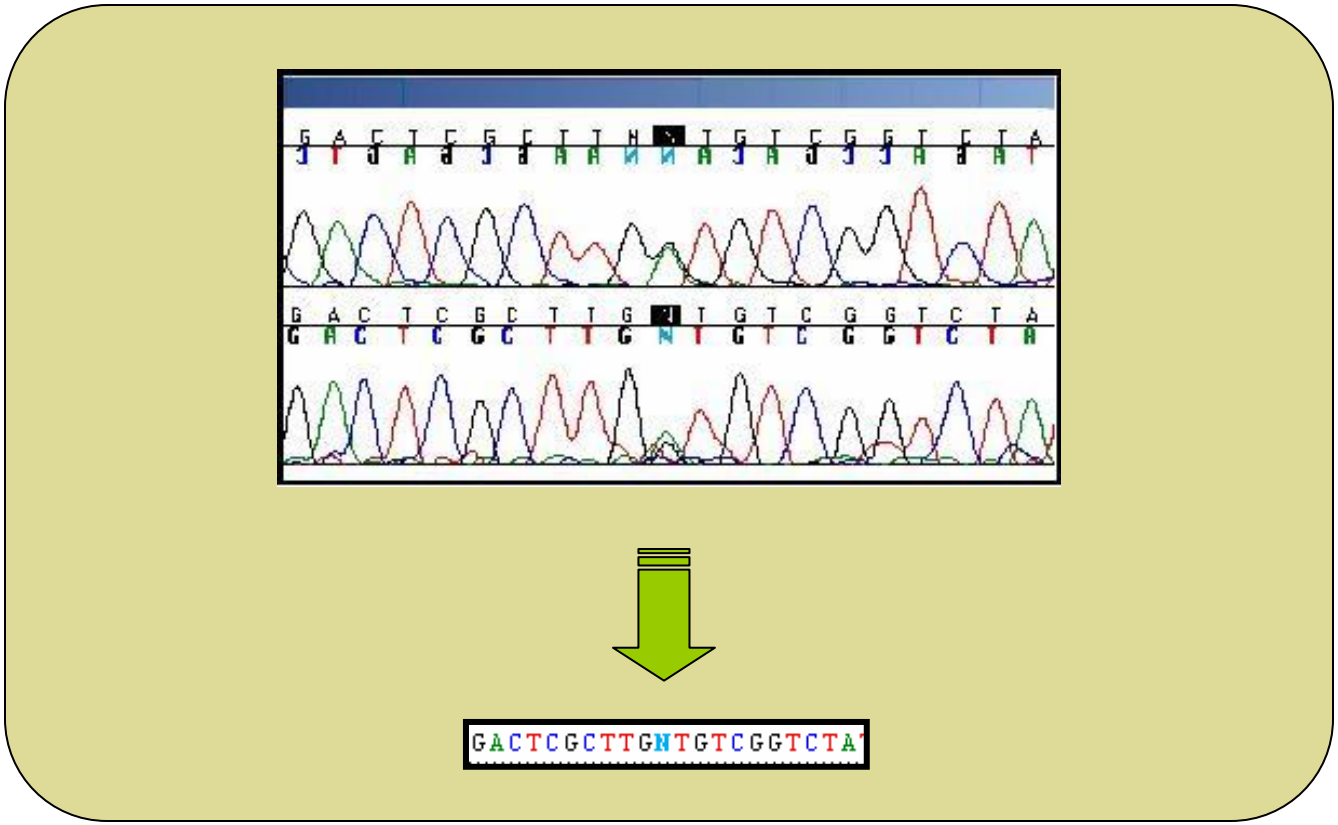
- Problem being addressed



Source: Applied Biosystems Sequence Scanner Software v. 1.0

Introduction / Background

- Problem being addressed



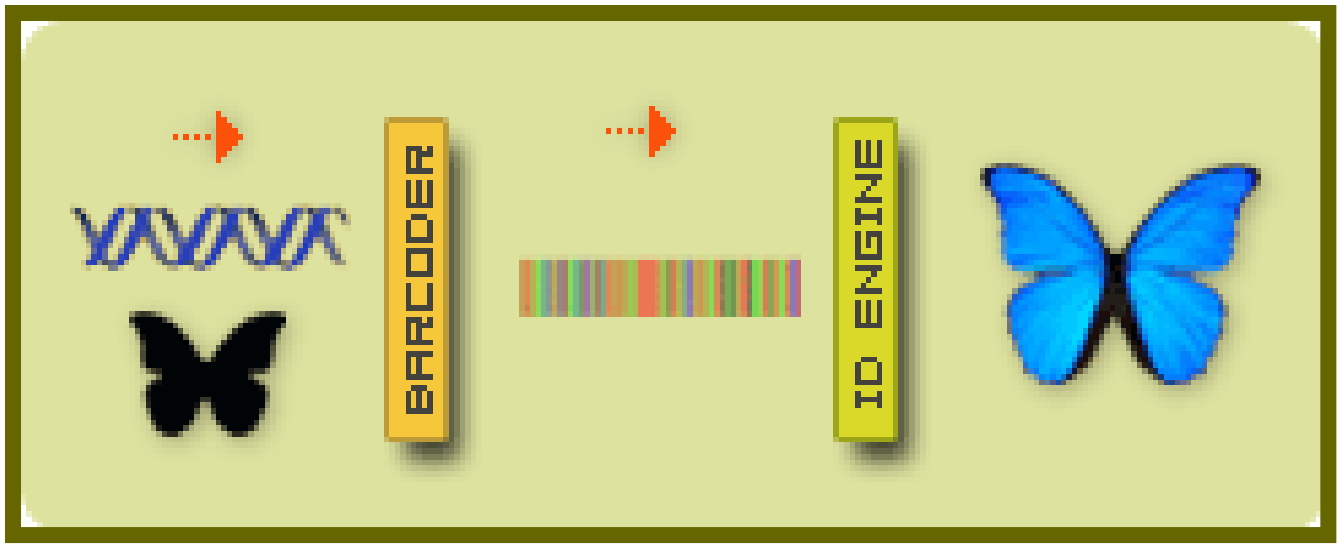
Source: Gene Codes Corporation Sequencher Software v. 4.5

Introduction / Background

- Why is this important?
  - Sequences with low variability are particularly influenced by sequencing errors in analyses of sequence variation due to their low signal-to noise ratio

Introduction / Background

- DNA Barcoding



Source: BOLD http://www.barcodinglife.org/views/login.php

Introduction / Background

- DNA Barcoding
  - *Astraptes fulgerator*

P.N.A.S. (2004) **101**: 14812 – 14817

Model

- DNA sequence data

Bats of Guyana

CAGTTGAAGCTGGTGTTGGGACTGGTTGAACCGTATATCCAC
CAGTTGAAGCTGGTGTTGGAACTGGTTGAACCGTATATCCAC
CCGTCGAAGCAGGGGTAGGTACTGGTTGAACCGTGTACCCCC
CCGTCGAAGCAGGGGTAGGTACTGGTTGAACCGTGTACCCCC
TGGTAGAAGCTGGGGCTGGTACCGGATGGACAGTATACCCAC
TGGTAGAAGCTGGGGCTGGTACCGGATGGACAGTATACCCAC
CAGTCGAAGCCGGAGTAGGAACTGGCTGAACTGTTTATCCCC
CAGTCGAAGCCGGAGTAGGAACTGGCTGAACTGTTTATCCCC
CAGTCGAGGCTGGAGTAGGGACTGGCTGAACTGTTTATCCCC
CAGTCGAGGCTGGAGTAGGGACTGGCTGAACTGTTTATCCCC
CAGTCGAAGCAGGAGTAGGTACCGGCTGAACAGTATACCCAC
CAGTCGAAGCAGGAGTAGGTACCGGCTGAACAGTATACCCGC
CAGTTGAGGCTGGAGTAGGTACAGGCTGAACAGTCTACCCTC
CAATTGAAGCAGGCGTTGGCACCGGCTGAACCGTCTACCCCC
CAATTGAAGCAGGCGTTGGCACCGGCTGAACCGTCTACCCCC

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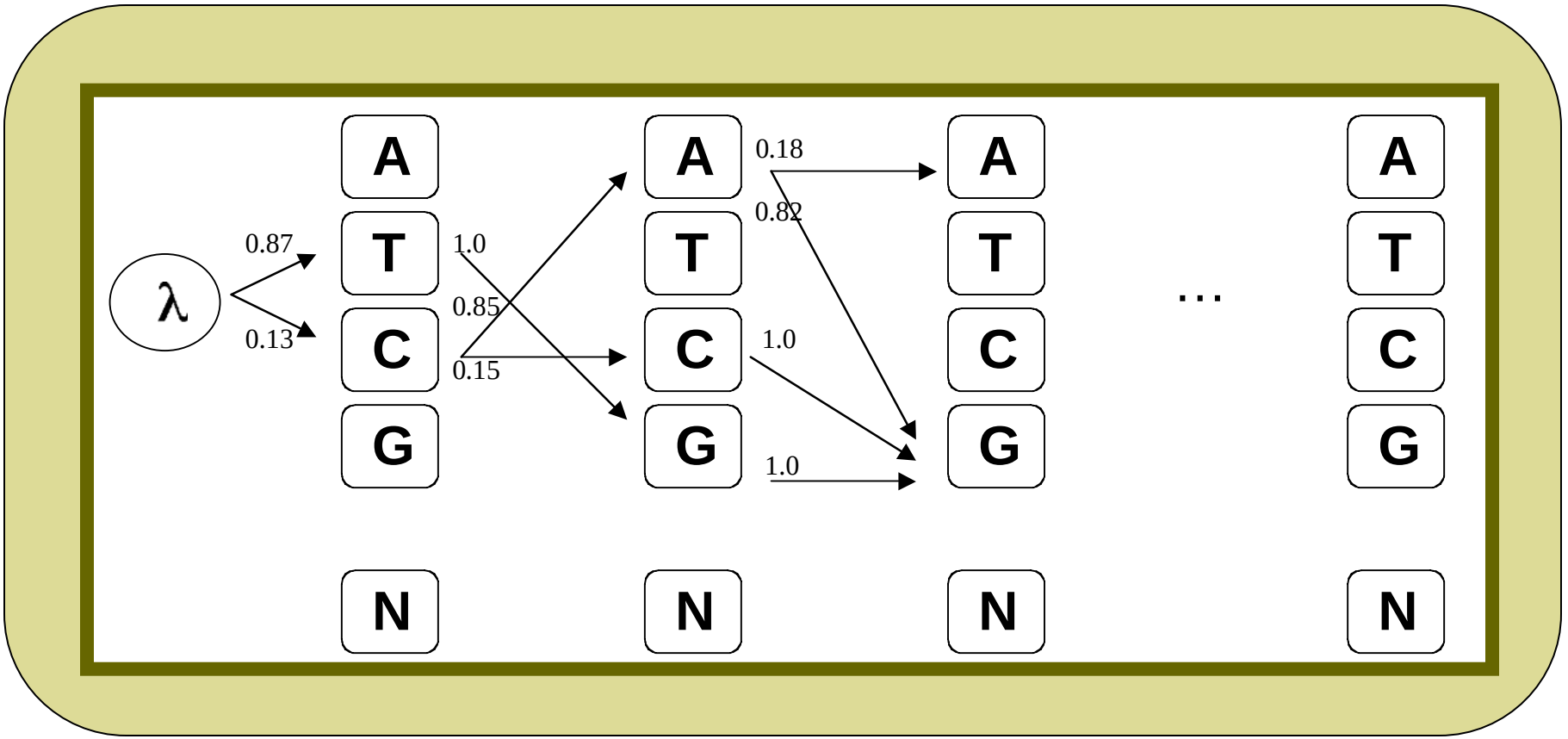
M. E. Notes (2007) **7**: 184 – 190

Model

- Representing DNA sequence data using a *profile Hidden Markov Model (pHMM)*



Model

- Probability of other sequence given the model

- Ex 1: *Ametrida centurio*

➡ **CAGTTGAAGCTGGTGTTGGGACTGGTTGAACCGTATATCCACC**

➡ **$P(O|\lambda) = 3.461 \times 10^{-9}$**

- Ex 2: *Boophis rappiodes*

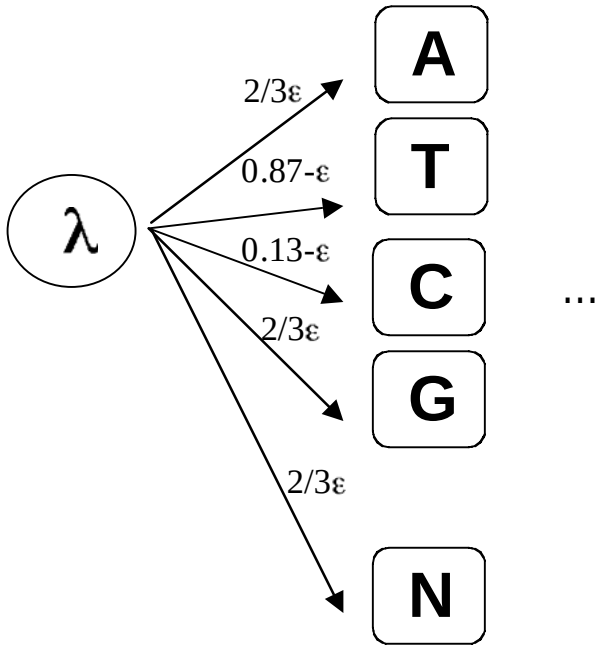
➡ **CCACCTTCAATGACCCAGTACCAAACCCCTCTTTTTGTGTGATC**

➡ **$P(O|\lambda) = 0$**

Model

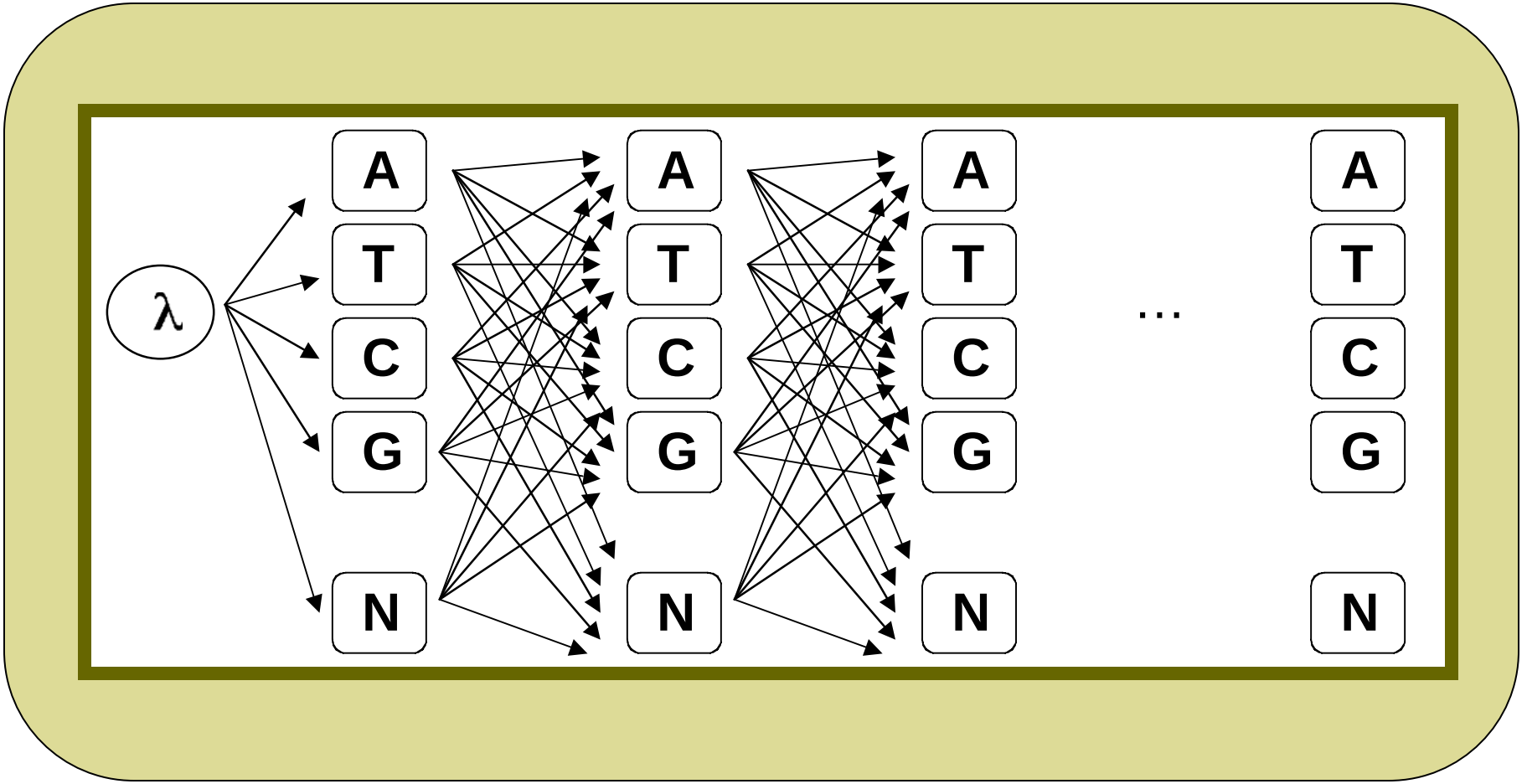
- Modifications to the model

- ε-padding
  - To allow for all possible sequences to have a positive probability according to the model
  - Can be adjusted to increase or decrease the sensitivity of the model to external sequences



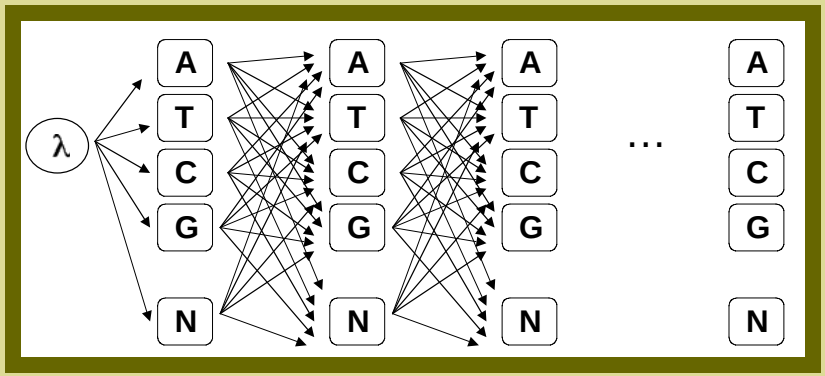
Model

- Profile Hidden Markov Models (pHMM)



Testing the Model

- Test the accuracy of the pHMM
  - Estimate accuracy directly by the pHMM's ability to detect error introduced into a set of DNA sequences

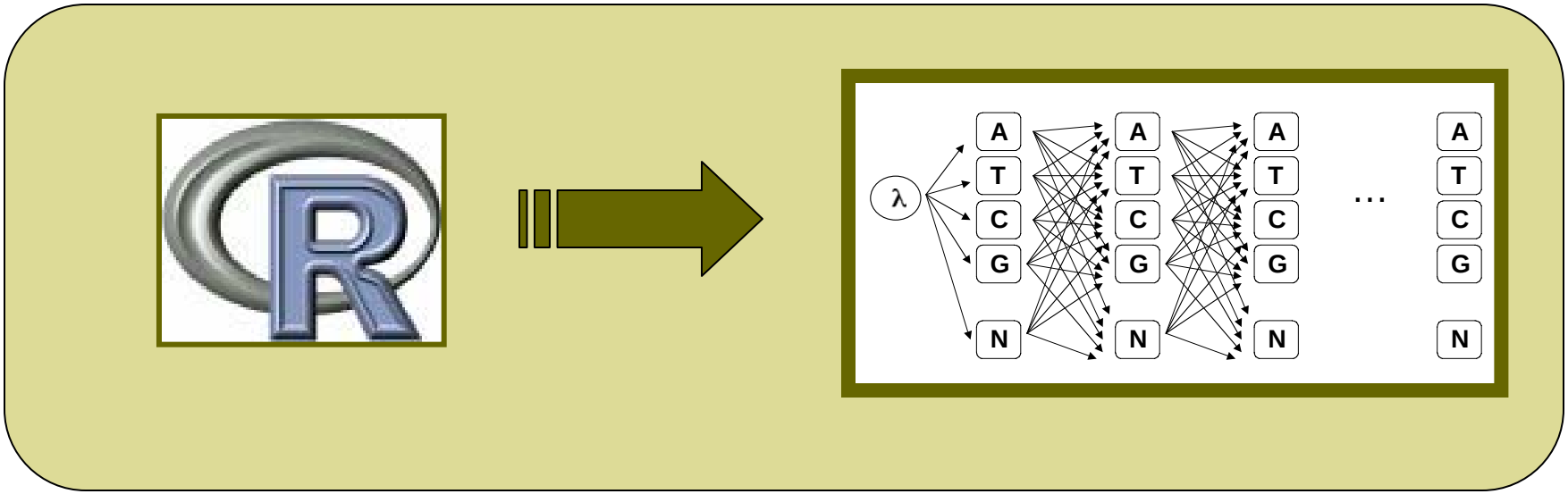


Methods

1. Detection of shallow sequence divergences in a set of sequences
2. Removal of the sequence(s) of interest
3. Construction of a pHMM from the remaining sequences
4. Evaluate the probability of nucleotide variation at all sites in the sequence(s) of interest
5. Sites detected are then recommended for re-examination

Methods

- The R programming environment was selected in which to build the pHMM of sequence data



Methods

- Example

```
$Rho
                [,1]        [,2]        [,3]        [,4]
[1,] 1.762655e-09 0.0001610240 7.992953e-01 8.425386e-01
[2,] 5.732607e-01 0.1424176100 8.142005e-09 3.906765e-13
[3,] 1.432232e-01 0.2814073574 6.207791e-04 7.020717e-06
[4,] 2.835161e-01 0.5760140085 2.000839e-01 1.574544e-01

$tpm
                [,1]        [,2]        [,3]        [,4]
[1,] 9.628128e-01 3.718670e-02 5.260536e-07 2.401726e-10
[2,] 1.831845e-01 8.166392e-01 1.395701e-04 3.666461e-05
[3,] 1.565513e-04 8.277518e-04 7.974409e-01 2.015748e-01
[4,] 2.625085e-06 6.299285e-05 5.919335e-02 9.407410e-01
```

```
$ispd
[1] 0.74884766 0.15199885 0.02250537 0.07664812

$log.like
[1] -13.20095
```

Significance

- Another level of error detection post - sequence editing
  - This level takes sequence history into consideration after the unbiased error estimates of the program PHRED

Other Applications

- Determining the suitability of sequences for analysis of sequence variability

Fishes of Australia

TGACTCCTTCCTCCCTCTTTCCTTCTGCTCCTAGCTTCTTCAGGAGTTGA
TGACTCCTTCCTCCCTCTTTCCTTCTGCTCCTAGCTTCTTCAGGAGTTGA
TGACTTCTTCCCCCCTCTTTCCTTCTGCTCCTAGCTTCTTCAGGAGTTGA
TGACTTCTTCCCCCCTCTTTCCTTCTGCTCCTAGCTTCTTCAGGAGTTGA
TGACTCCTTCCCCCCTCTTTCCTTCTGCTCCTAGCTTCTTCAGGAGTTGA
TGACTCCTTCCCCCCTCTTTCCTTCTGCTTCTAGCTTCTTCAGGAGTTGA
TGACTCCTTCCCCCCTCTTTCCTTCTGCTCCTAGCTTCTTCAGGAGTTGA
TGACTCCTTCCCCCTTCTTTCCTTCTGCTCCTAGCTTCTTCAGGAGTTGA
TGACTCCTTCCCCCTTCTTTCCTTCTGCTCCTAGCTTCTTCAGGAGTTGA
TGACTCCTTCCCCCTTCTTTCCTTCTGCTCCTAGCTTCTTCAGGAGTTGA
TGACTCCTTCCCCCCTCTTTCCTTCTGCTCCTAGCTTCTTCAGGAGTTGA
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TGACTCCTTCCTCCCTCTTTCCTTCTGCTCCTAGCTTCTTCAGGAGTTGA
TGACTCCTTCCTCCCTCTTTCCTTCTGCTCCTAGCTTCTTCAGGAGTTGA
TGACTCCTTCCTCCCTCTTTCCTTCTGCTCCTAGCTTCTTCAGGAGTTGA
TGACTCCTTCCTCCCTCTTTCCTTCTGCTCCTAGCTTCTTCAGGAGTTGA

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Phil. Trans. R. Soc. B. (2005) **360**: 1847– 1857

Other Applications

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Fishes of Australia

CCTTCCTCCCTCTTTCCTTCTGCTCCTAGCTTCTTCAGGAGTTGA
TGACTCCTTCCTCCCTCTTTCCTTCTGCTCCTAGCTTCTTCAGGAG
TGACTTCTTCCCCCCTCTTTCCTTCTGCTCCTAGCTTC
TGACTTCTTCCCCCCTCTTTCCTTCTGCTC
TGACTCCTTCCCCCCTCTTTCCTTCTGCTC
TGACTCCTTCCCCCCTCTTTCCTTCTGCTTCTAGCTTCTTCAGGAGTTGA
TGACTCCTTCCCCCCTCTTTCCTTCTGCTCCTAGCTTCTTCAGGAGTTGA
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TGACTCCTTCCCCCTTCTTTCCTTCTGCTCCTAGCTTCTTCAGGAGTTGA
TGACTCCTTCCCCCTTCTTTCCTTCTGCTCCTA
CCTTCCCCCCCTCTTTCCTTCTGCTC
TGACTCCTTCCCCCCTCTTTCCTTCTGCTCCTAGCTTCTTCAGGAGTTGA
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TGACTCCTTCCTCCCTCTTTCCTTCTGCTCCTAGCTTCTTCAGGAGTTGA

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TGACTTCTTCCCCCCTCTTTCCTTCTGCTCCTAGCTTCT
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TCTTTCCTTCTGCT
TGACTCCTTCCTCCCTCTTTCCTTCTGCT
TGACTCCTTCCTCCCTCTTTCCTTCTGCTCCTAGCTTCTTCAGGAGTTGA

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Other Applications

- Search for patterns of informative sites in sequences

Fishes of Australia

TGACTCCTTCCTCCCTCTTTCCTTCTGCTCCTAGCTTCTTCAGGAGTTGA
TGACTCCTTCCTCCCTCTTTCCTTCTGCTCCTAGCTTCTTCAGGAGTTGA
TGACTTCTTCCCCCCTCTTTCCTTCTGCTCCTAGCTTCTTCAGGAGTTGA
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Acknowledgements

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Committee

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Dr. Paul Hebert
Dr. Dan Ashlock

Committee Members:
Dr. Alex Smith
Dr. Gary Umphrey