Pre-mRNA Secondary Structure Prediction Aids Splice Site Recognition

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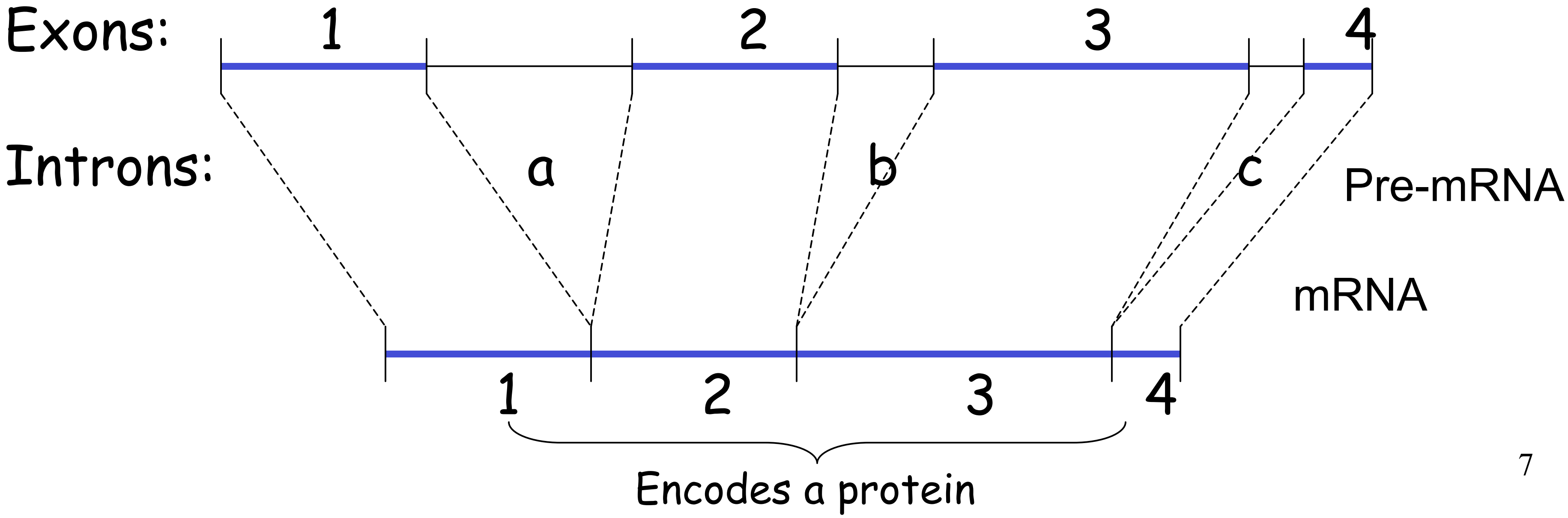
University of Washington Computational
Molecular Biology Group

Architecture of a Gene

- pre-mRNA's transcribed from most genes contain *introns*, which must be *spliced* out to form useful mRNAs



Characteristics of human genes

(Nature, 2/2001, Table 21)

	Median	Mean	Sample (size)
Internal exon	122 bp	145 bp	RefSeq alignments to draft genome sequence, with confirmed intron boundaries (43,317 exons)
Exon number	7	8.8	RefSeq alignments to finished sequence (3,501 genes)
Introns	1,023 bp	3,365 bp	RefSeq alignments to finished sequence (27,238 introns)
3' UTR	400 bp	770 bp	Confirmed by mRNA or EST on chromo 22 (689)
5' UTR	240 bp	300 bp	Confirmed by mRNA or EST on chromo 22 (463)
Coding seq	1,100 bp	1340bp	Selected RefSeq entries (1,804)*
(CDS)	367 aa	447 aa	
Genomic extent	14 kb	27 kb	Selected RefSeq entries (1,804)*

* 1,804 selected RefSeq entries were those with full-length unambiguous alignment to finished sequence

Cell, Vol. 92, 315–326, February 6, 1998
Mechanical Devices of the Spliceosome:
Motors, Clocks, Springs, and Things
Jonathan P. Staley and Christine Guthrie

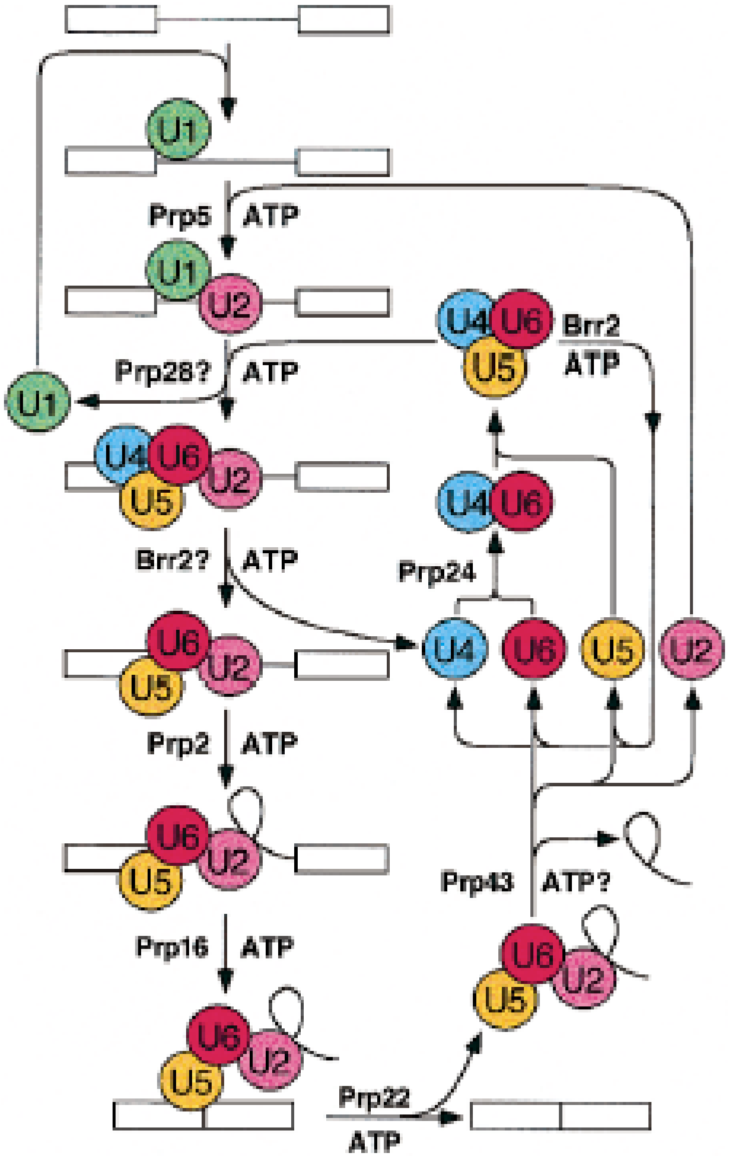


Figure 2. Spliceosome Assembly, Rearrangement, and Disassembly Requires ATP, Numerous DExD/H box Proteins, and Prp24

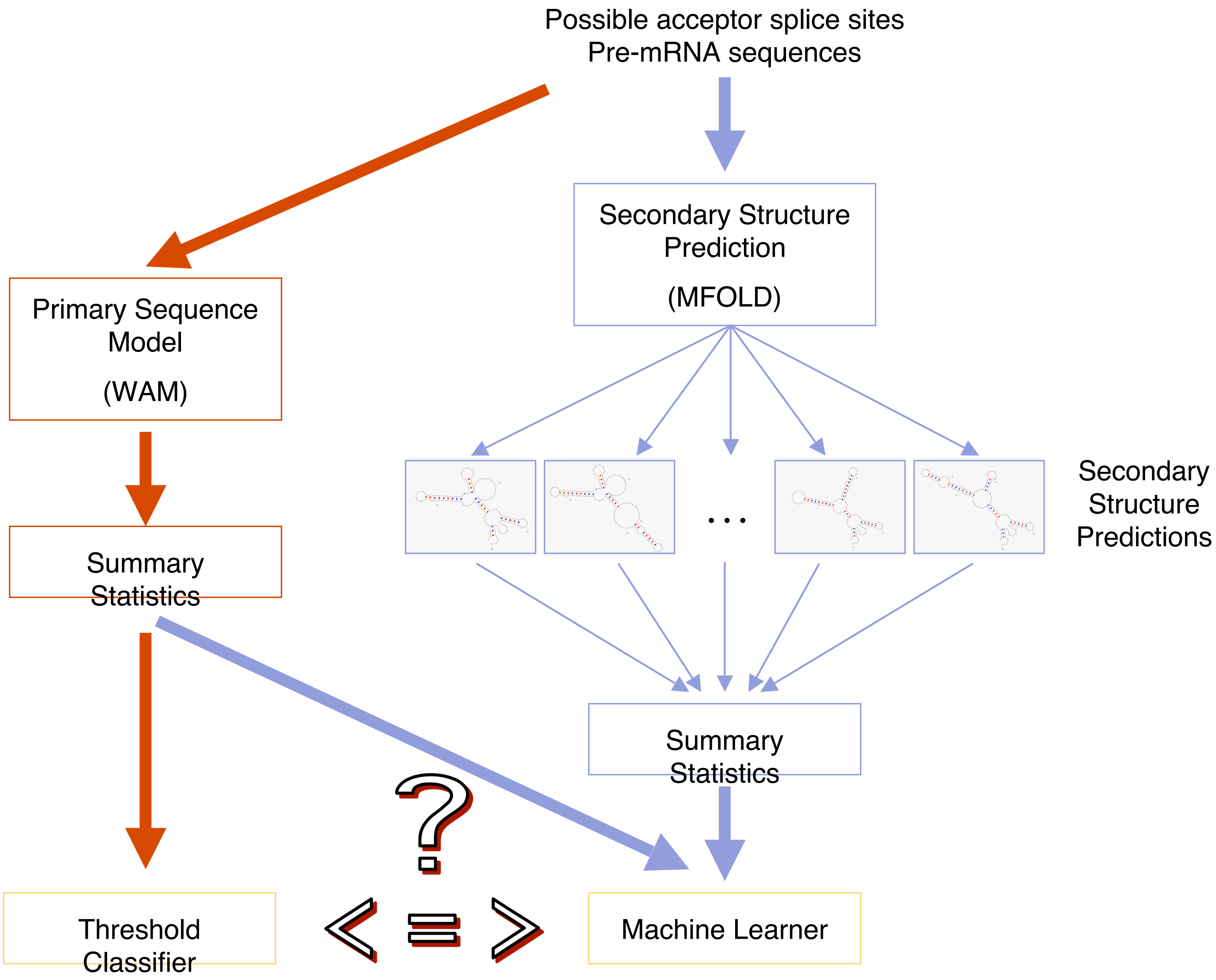
The snRNPs are depicted as circles. The pathway for *S. cerevisiae* is shown. (See Table 1.)

Relevance of Splice Prediction

- Splice site prediction is critical to eukaryotic gene prediction.
  - – Average human gene has 8.8 exons
  - – Genes with over 175 exons known
  - – Current primary sequence models do not display the same discriminatory power that cells exhibit *in vivo*
  - – Small per-site error rate compounds

Hypothesis

- Secondary structure contains information useful for predicting splice site location.
- This information is in addition to primary sequence information.
  - Specific instances of secondary structure variation affecting the splicing process.



Possible acceptor splice sites
Pre-mRNA sequences

Primary Sequence Model

(WAM)

Summary Statistics

Secondary Structure Prediction

(MFOLD)

. . .

Secondary Structure Predictions

Summary Statistics

Threshold Classifier

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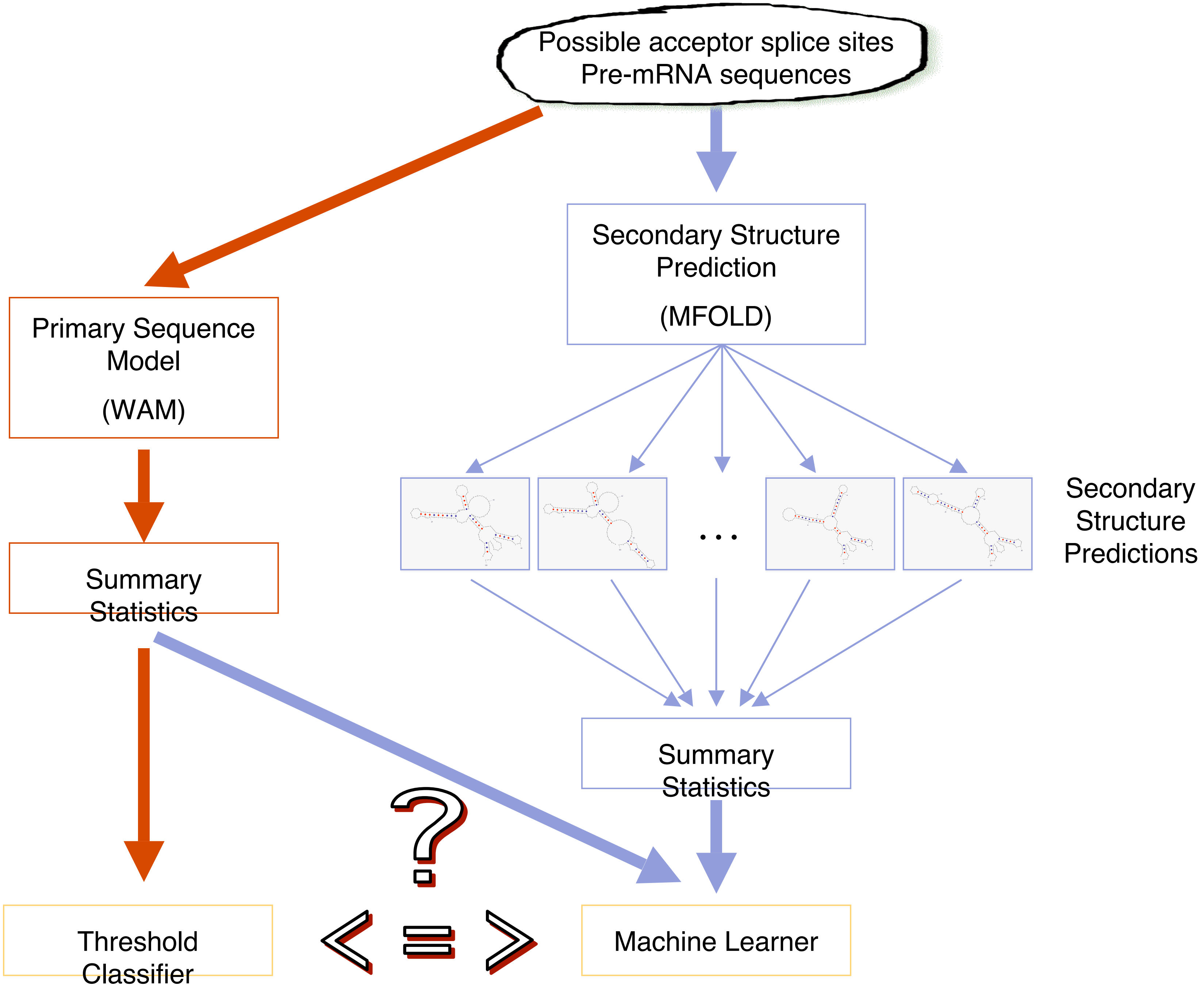
Machine Learner



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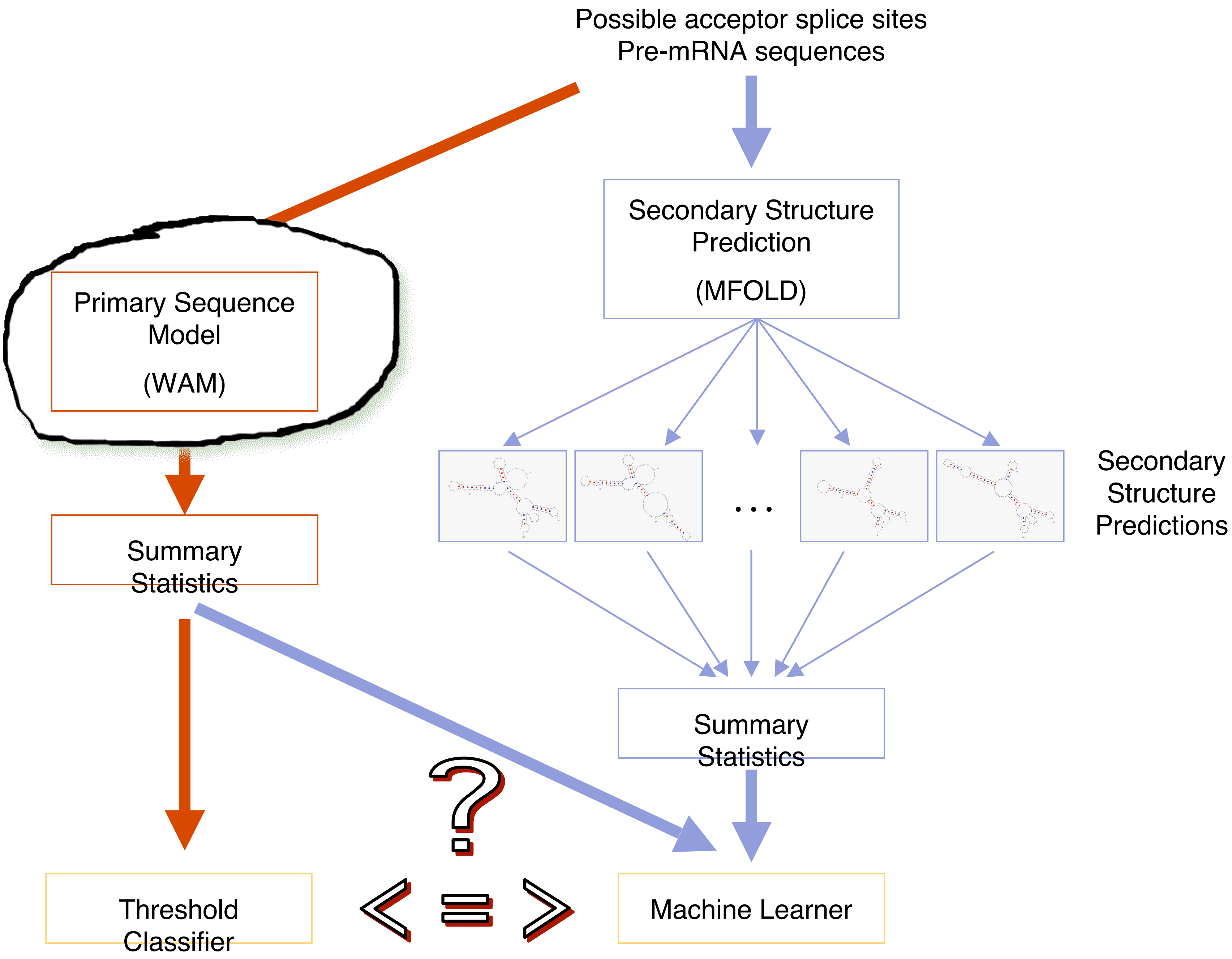
Data Set

- Drawn from 462 unrelated, annotated, multi-exon human genes with standard splicing. (Reese 97)
- 1,980 acceptor splice sites (3’ end of intron)
- 1,980 non-sites selected randomly
  - Aligned to an “AG” consensus
  - Located within 100 bases of an annotated acceptor splice site.



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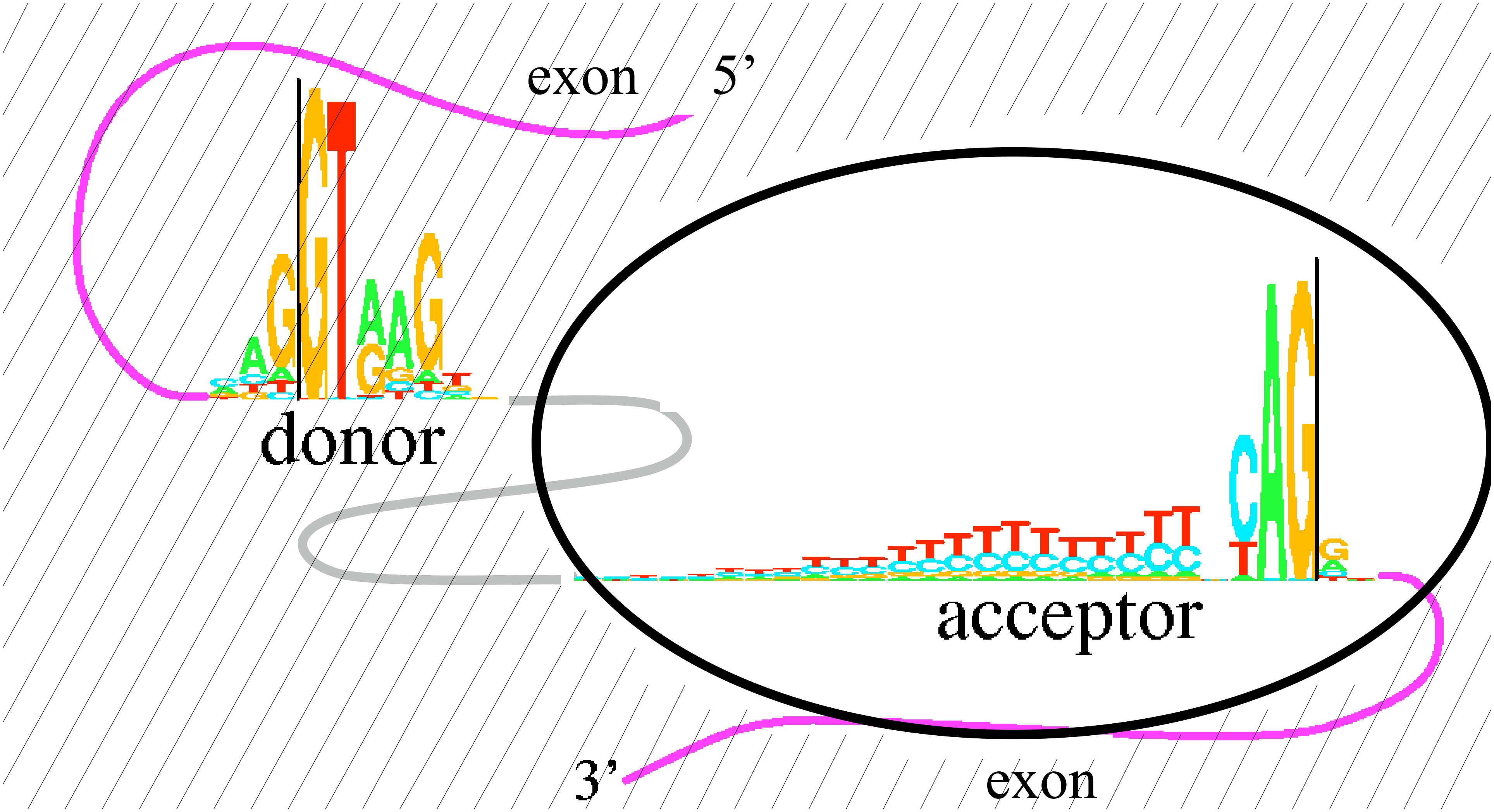
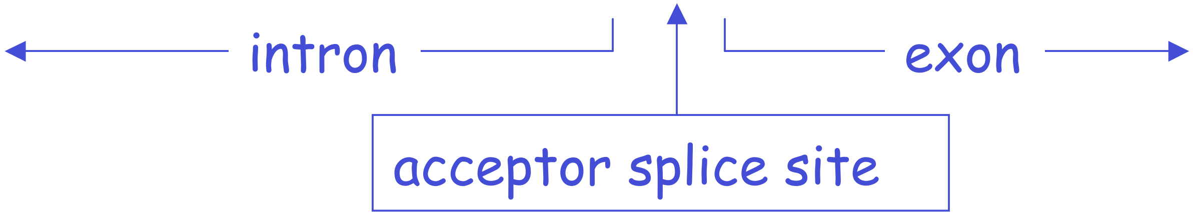
Machine Learner

What's in the Primary Sequence?



What's in the Primary Sequence?

	-4	-3	-2	-1	+1	+2	+3
A	22	4	**100**	0	25	25	27
C	33	74	0	0	13	21	27
G	22	0	0	**100**	52	22	24
T	22	21	0	0	9	32	23



intron

exon

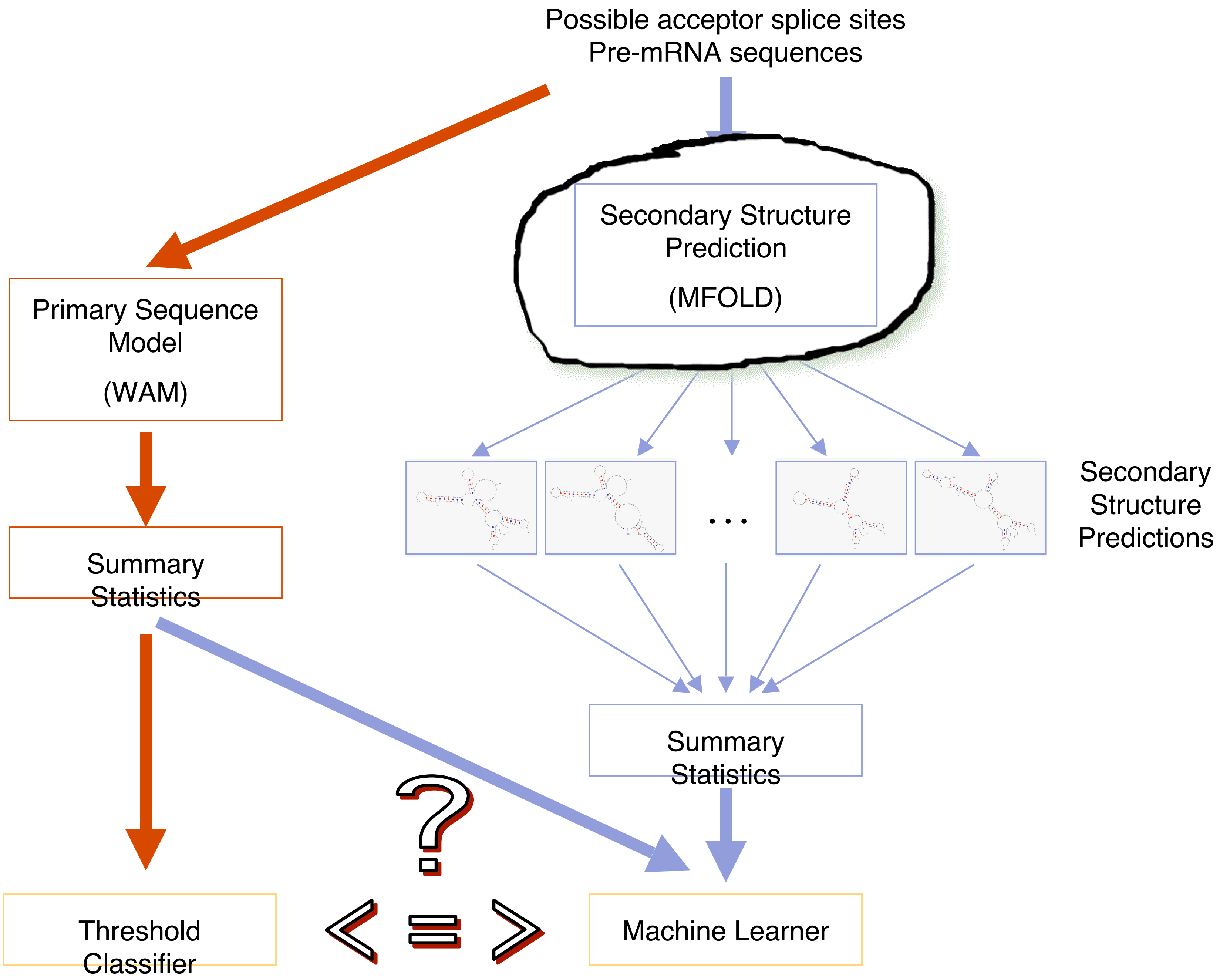
acceptor splice site

Weight Matrix Model (0th order Markov Model)

Sequence-based Metric

- 1^{st} order Weight Array Matrix (WAM) / Markov Model
  - $P_i(N_i=\{A,C,G,U\} \mid N_{i-1}=\{A,C,G,U\})$
- Training
  - Generate two conditional probability tables for positions (–21,+3), one from positive examples and one from negative examples.
- Testing
  - For each sequence, x, calculate its likelihood ratio:

$$\log_{10}\left(\frac{P_{WAM}^{+}(x)}{P_{WAM}^{-}(x)}\right)$$



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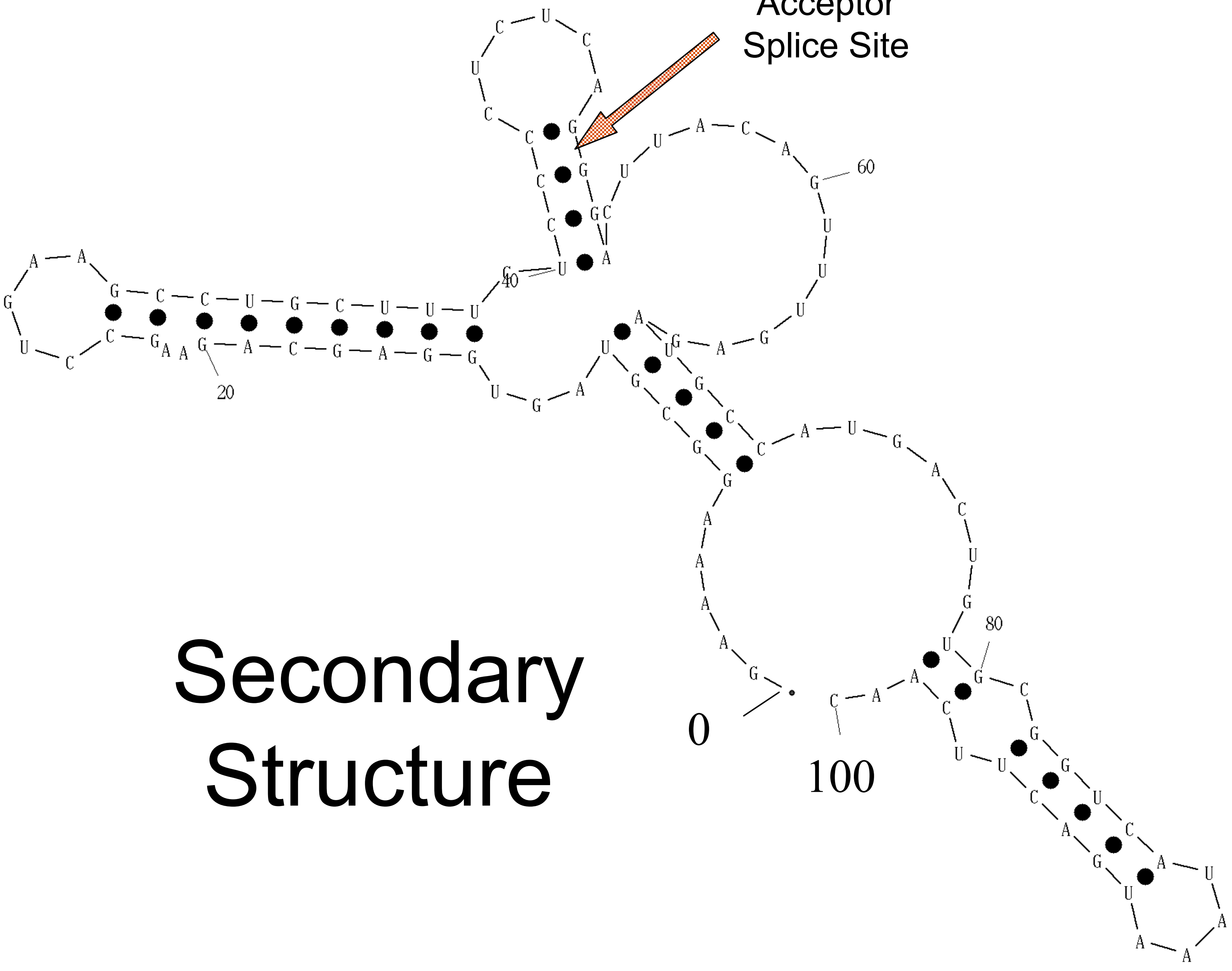
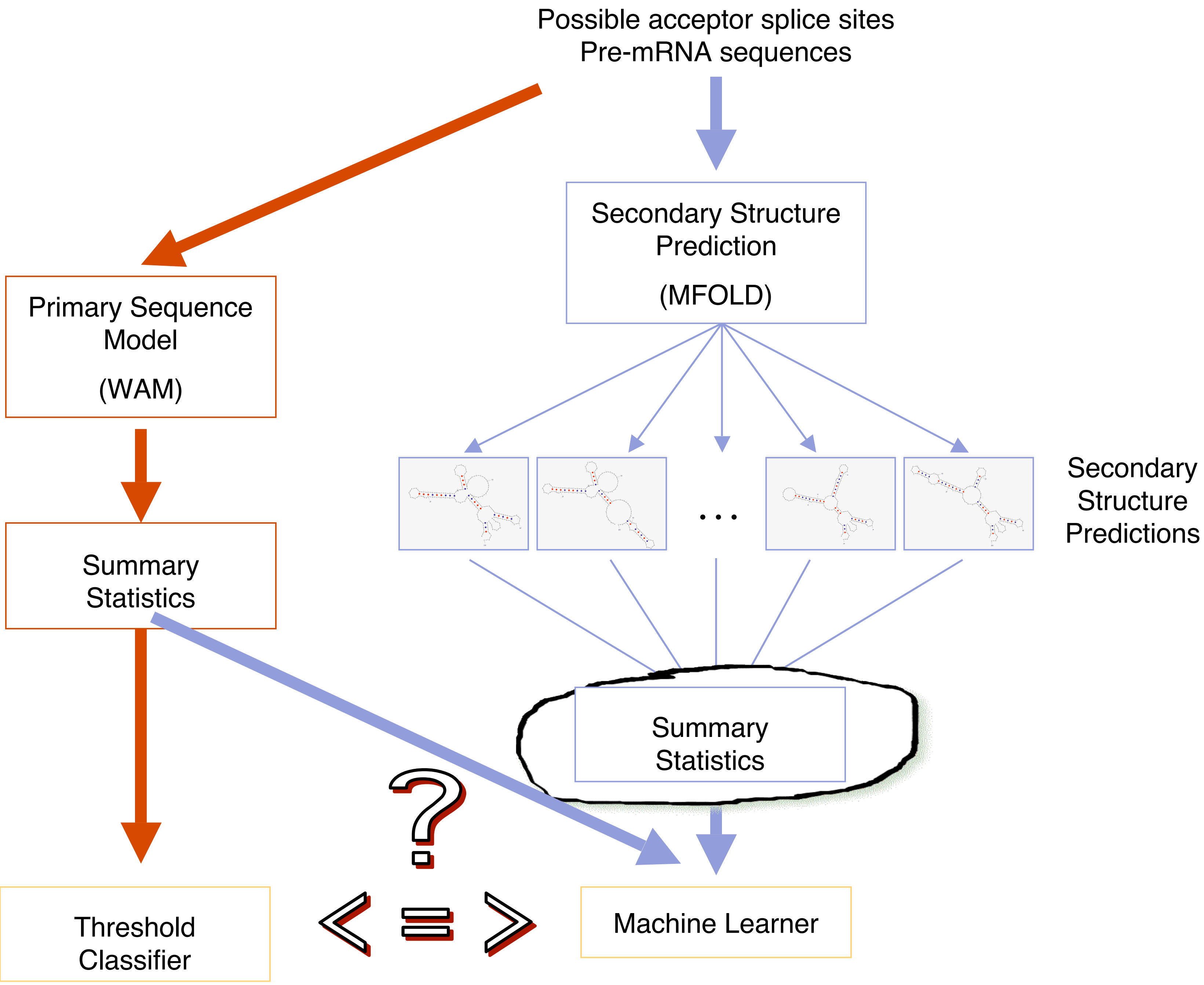
Secondary Structure



Acceptor Splice Site



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Secondary Structure Statistics

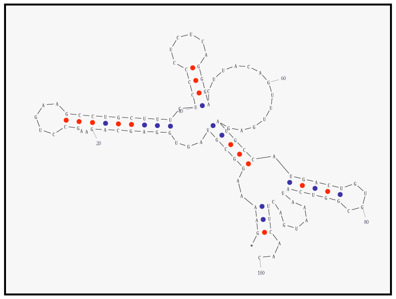
- Optimal Folding Energy
- Max Helix score
- Neighbor Pairing Correlation Model

1. Optimal Folding Energy

...CUGCUUUCUCCCCUCUCAGGGACUUACAGUUUGAGAUGC...

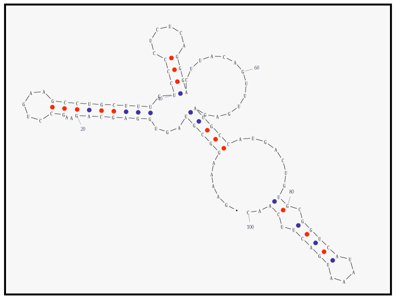
Secondary
Sequence Prediction

(MFOLD)



Free Energy

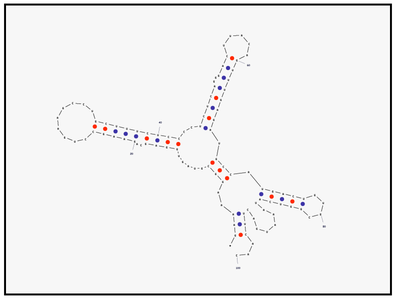
-35.2 kcal/mole



Free Energy

-34.0 kcal/mole

...

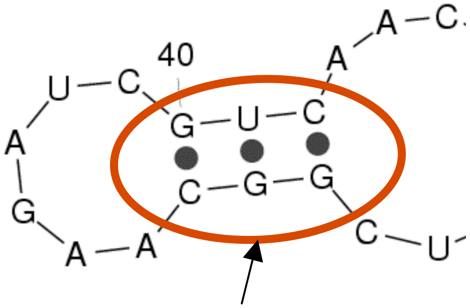


Free Energy

-2.0 kcal/mole

2. Max Helix

What is the highest probability that a helix will form nearby?

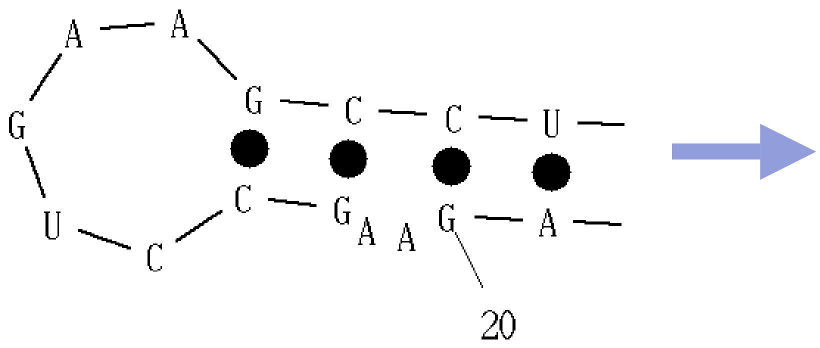


Helix

- Calculate $P_{HStart,x}$
- Calculate $P_{HEnd,x}$

$$MaxHelix_i = \max_{x \in (i-5,i+5)} (P_{HStart,x}, P_{HEnd,x})$$

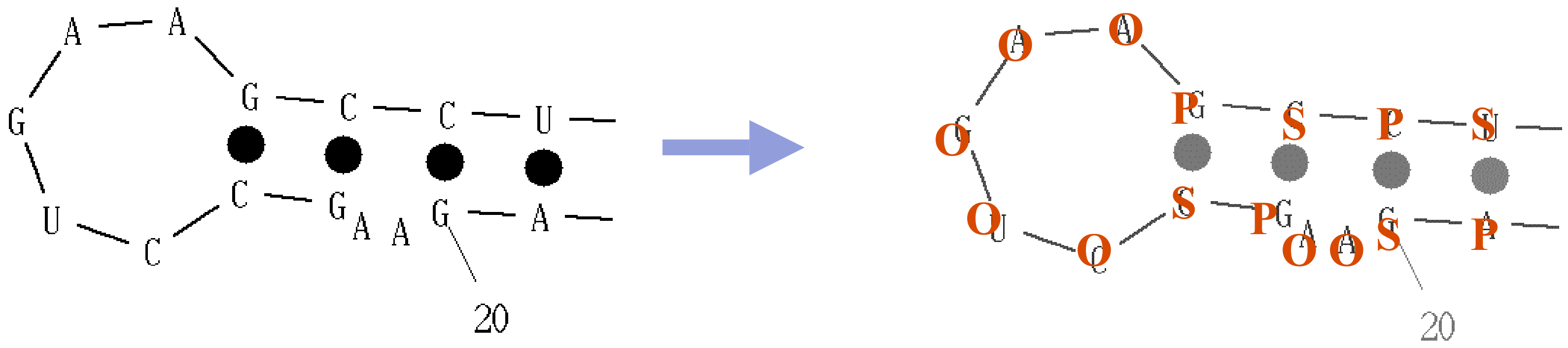
3. Neighbor Pairing Correlation Model



Change the pre-mRNA alphabet from nucleotides to structural symbols

O	Unpaired base
P	Paired base
S	Paired and stacked base

3. Neighbor Pairing Correlation Model



Change the pre-mRNA alphabet from nucleotides to structural symbols

O Unpaired base

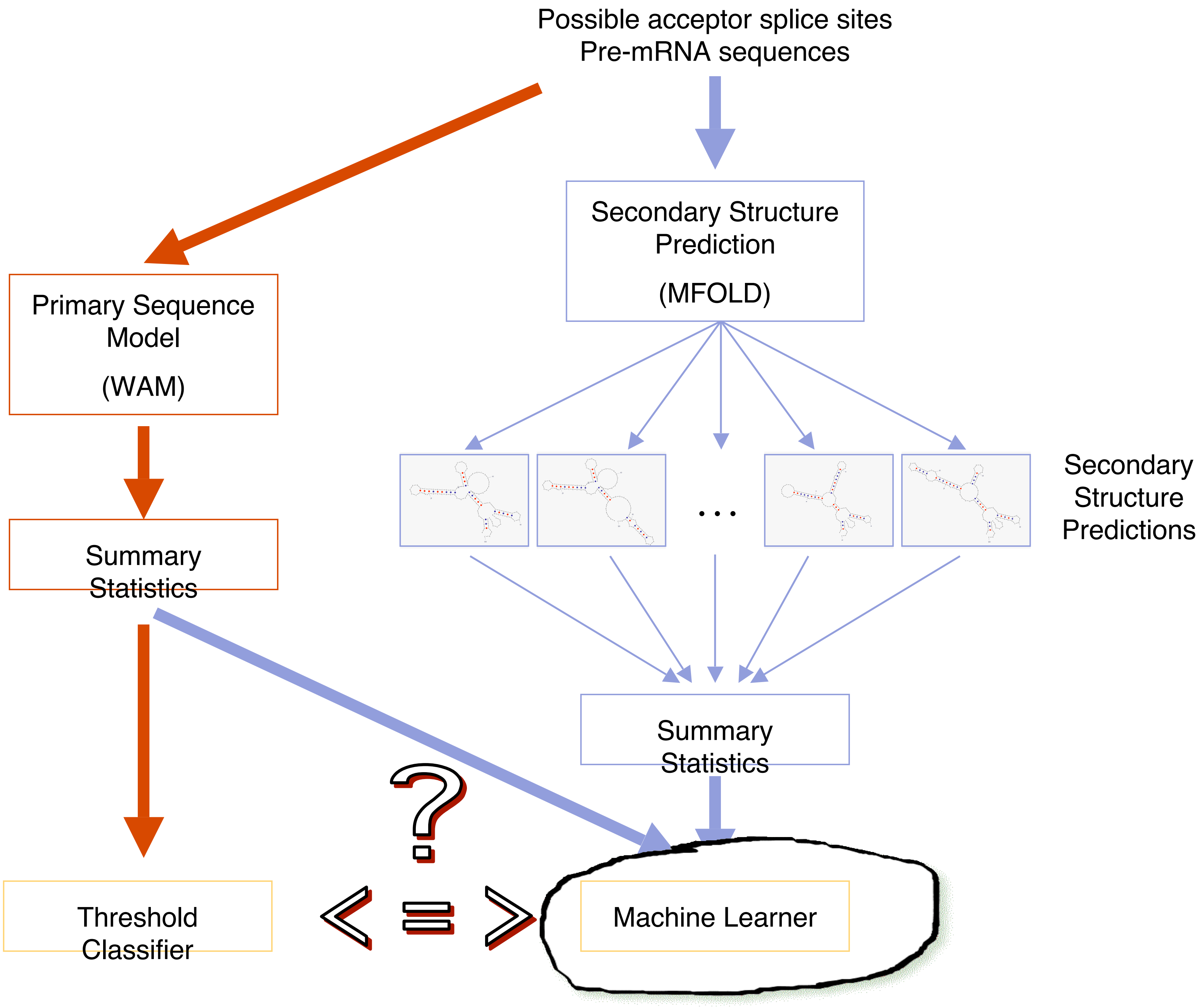
P Paired base

S Paired and stacked base

3. Neighbor Pairing Correlation Model

- 2^{nd} order Markov Model
  - $P_i(N_i=\{O,P,S\} \mid N_{i-1}=\{O,P,S\} \wedge N_{i-2}=\{O,P,S\})$
- Training
  - Generate two conditional probability tables for positions (–50,+3), one from positive examples and one from negative examples.
- Testing
  - For each sequence, x, calculate its log likelihood ratio:

$$\log_{10}\left(\frac{P^{+}_{NPCM}(x)}{P^{-}_{NPCM}(x)}\right)$$



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Secondary Structure Predictions

. . .

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Threshold Classifier

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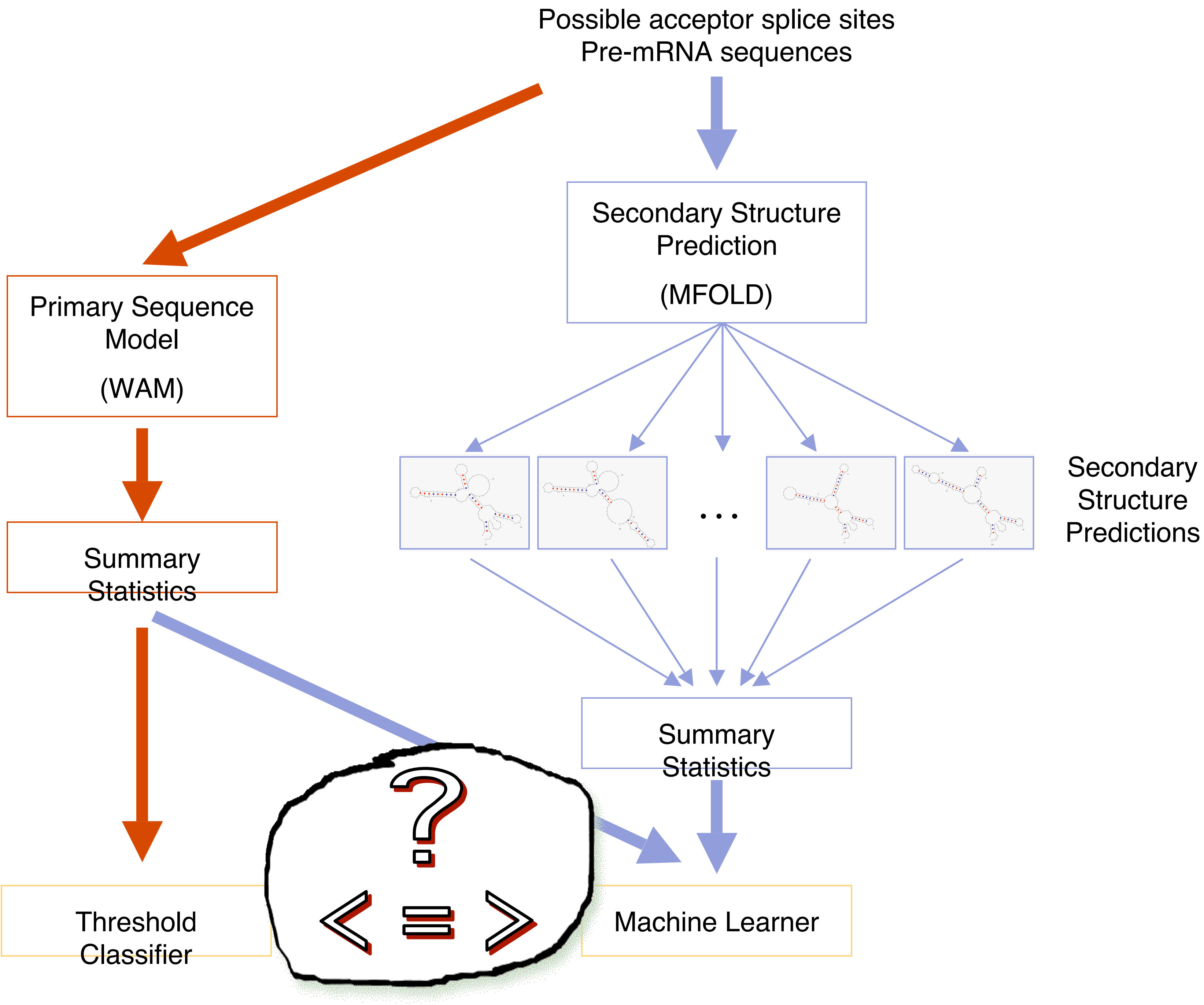
Machine Learner

Machine Learners

- Decision Trees
  - Quinlan’s C4.5
- Support Vector Machines
  - Noble’s svm 1.1
  - Radial Basis Kernel degree 2
- Both take a vector of statistics and produce a yes/no binary classifier.



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Results

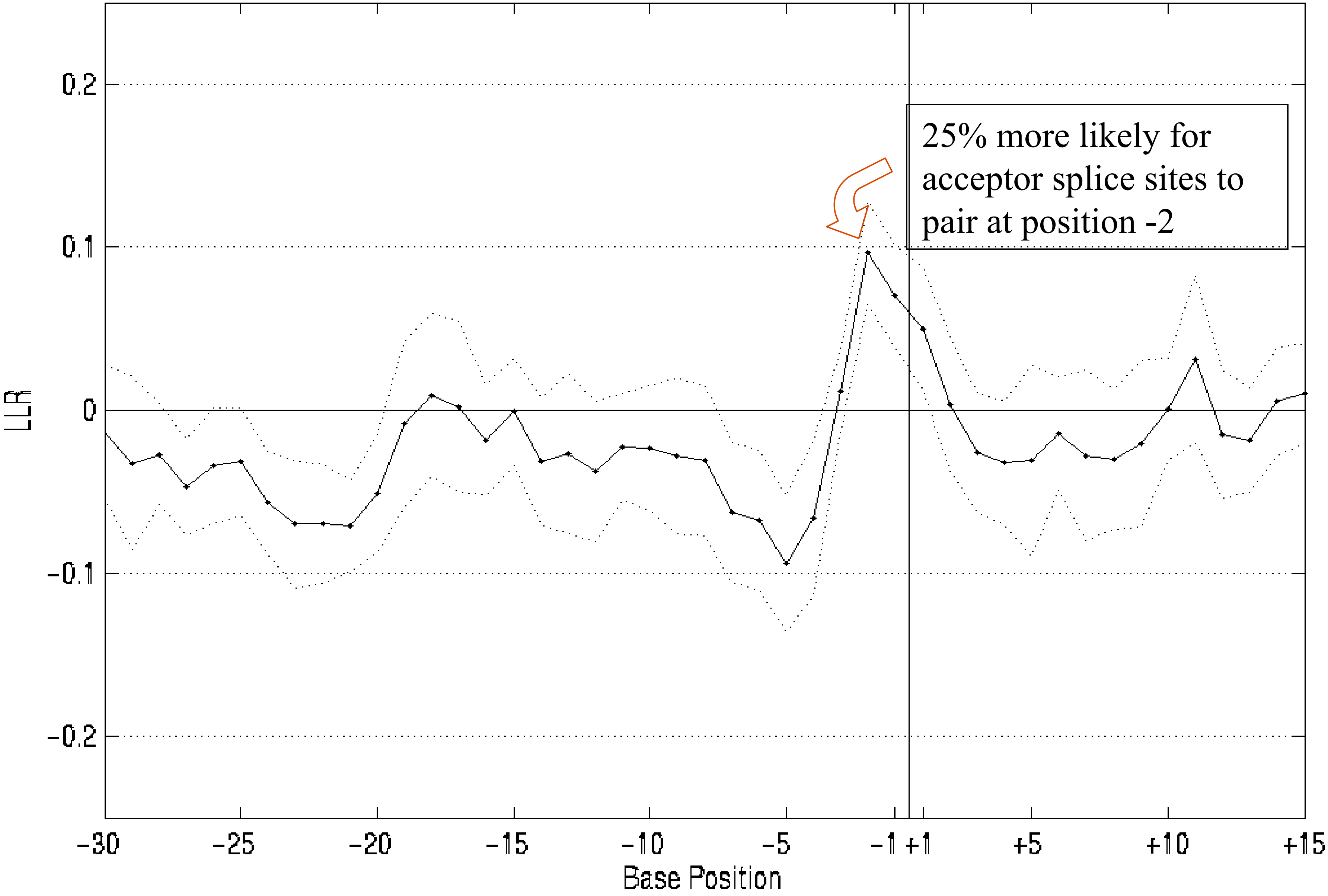
(Decision Trees)

Features	Mean Accuracy (%)	% Error Reduction	p
WAM (baseline)	92.73		
WAM,OFE	93.13	5.5	0.066
WAM,OFE,NPCM	93.16	5.9	0.022
WAM,OFE,MH	93.21	6.6	0.009
WAM,OFE,NPCM,MH	93.13	5.5	0.016

WAM = Weight Array Matrix (Primary Sequence Method)
OFE = Optimal Free Energy
MH = Max Helix
NPCM = Neighbor Pairing Correlation Matrix

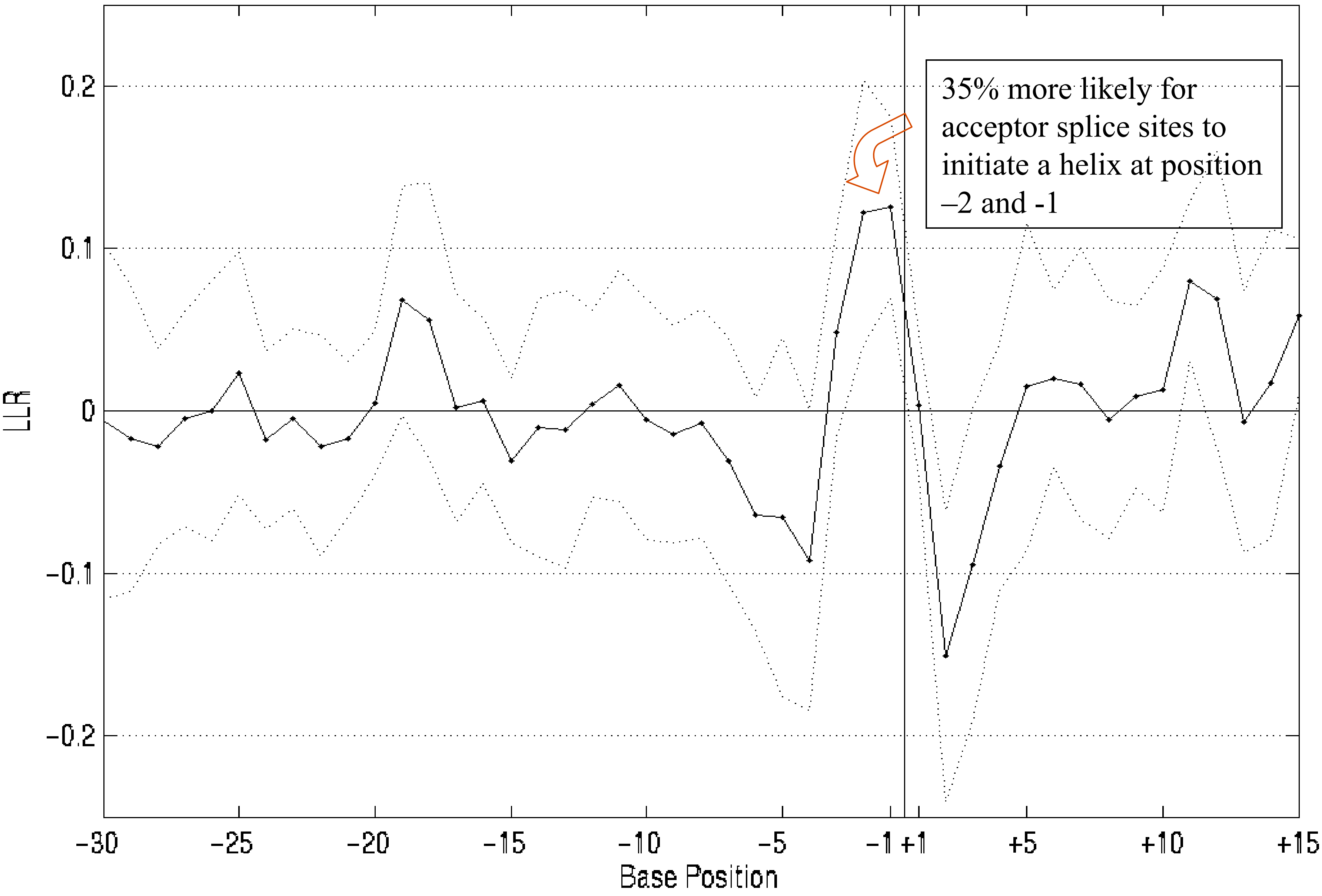
Wilcoxon p-value under 10-fold cross-validation

LLR of Base Pairing



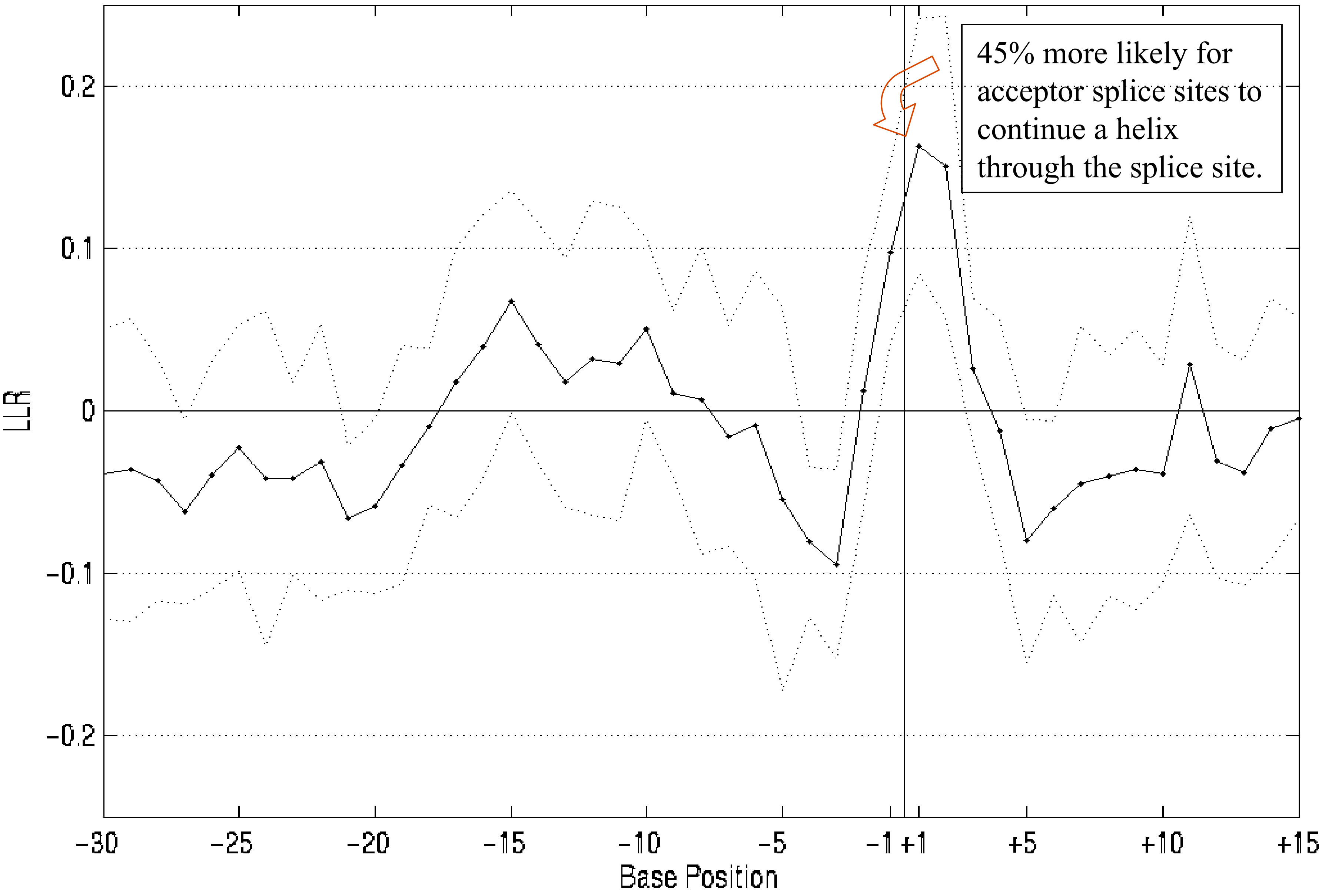
25% more likely for acceptor splice sites to pair at position -2

LLR of Helix Initiation



35% more likely for acceptor splice sites to initiate a helix at position –2 and -1

LLR of Helix Continuation



45% more likely for acceptor splice sites to continue a helix through the splice site.

Helix Formed at Splice Site

	Acceptor	Non-Acceptor
Pr(No Helix)	0.37	0.48
Pr(Helix)	0.63	0.52
Pr(Folds Left)	0.35	0.26
Pr(Folds Right)	0.28	0.26

Conclusions

- Secondary structure statistics correlate with splice site location.
- Our models (Max Helix, NPCM) can represent some of the relevant secondary structure.
- These models capture correlations that current primary sequence models don’t capture.

Future Work

- Other organisms
  - *Oryza sativa* (rice) in progress
- Donor splice sites
- Other features?
- More structure models
  - Stochastic Context Free Grammars?

Acknowledgements

- Don Paterson
- Ken Yasuhara
- Jeff Stoner
- Kevin Chu

More Info

http://www.cs.washington.edu/homes/ruzzo