Fast detection of conserved complementary motifs using gapped-seed associative arrays

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- RNA structure prediction appears to be **a completely different business at different scales of RNA sequence length**
- short RNAs, $\leq$ 200nts, thermodynamic model works fine
- long RNAs, kilobases and megabases
  - The requirement of nested RNA structure is the major limitation
  - Only a small corner of the search space is explored
  - $O(n^k)$, $k \geq 3$ is irrelevant as soon as the model is incomplete

Talk outline

- A novel **ultra-fast** method for detecting conserved complementary motifs
  - Dictionary (n-mer $\mapsto$ where it occurs)
  - Complementarity and conservation = intersection of dictionaries
  - **Exhaustive transcriptome-wide** search in linear time
  - Does not require multiple sequence alignment as an input
  - No limit on the distance between complementary motifs
- In application to **RNA (intra-molecular) secondary structure**
  - RNA structures associated with alternative splicing
  - in fruit flies[1]
  - in placental mammals[2]
  - in nematodes
  - (**morning session**)
- In application to **RNA-RNA interaction prediction**
  - non-coding RNAs as possible trans-regulators of pre-mRNA splicing
  - long non-coding RNAs (lncRNAs) and snoRNAs
  - non-coding segments of protein-coding genes
  - (**evening session**)

[1] Raker *et al*, NAR 37(14):4533-44, 2009

[2] Pervouchine *et al*, RNA 18(1):1-15, 2012

Intermolecular RNA (binary) Interaction Search

- Intermolecular RNA Interaction Search = IRIS[1]
  - intra- and inter-molecular structure simultaneously
  - thermodynamic model, dynamic programming, $O(n^3 m^3)$
  - no loop models for RNA structure with pseudoknots
- IRIS + binary search = **IRBIS** (Snow Leopard)
  - No dynamic programming
  - **Conservation** is a powerful and restrictive filter
  - Nearly exact matches, internal loops 2×2
- Workflow
  - Genomic annotation (reference genome)
  - Transcriptome segmentation (by exon boundaries)
  - Boundaries projected to other genomes (blastZ)
  - Orthologous segments
  - **Binary gapped-seed search**
  - Candidate selection
  - Extension, alignment, and visualization
- `http://genome.crg.es/~dmitri/irbis.html`
- soon at `https://github.com/pervouchine/irbis/`

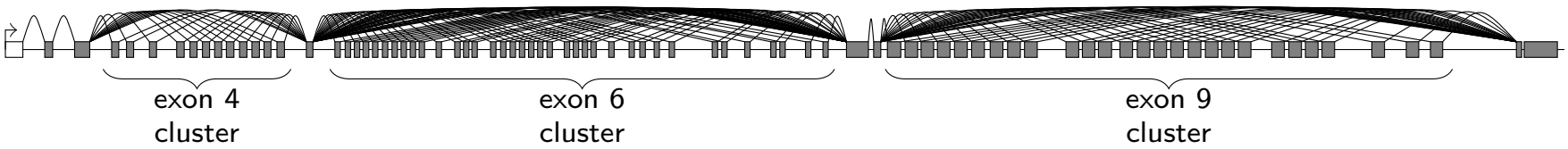
[1] D. Pervouchine, Genome Informatics 15(2), 2004

Part I. Gallery

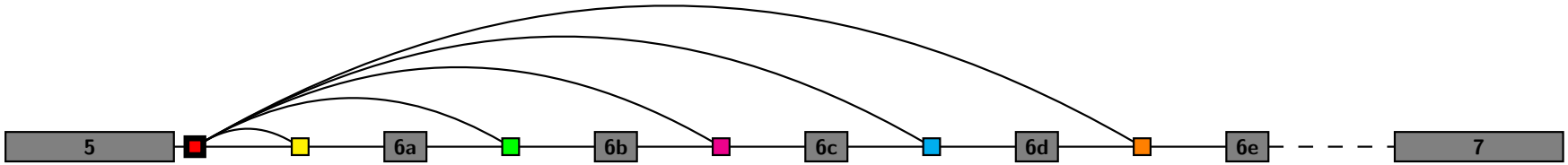
Part II. Algorithm

Part III. Results

Gallery: Mutually exclusive splicing in *Dscam* gene



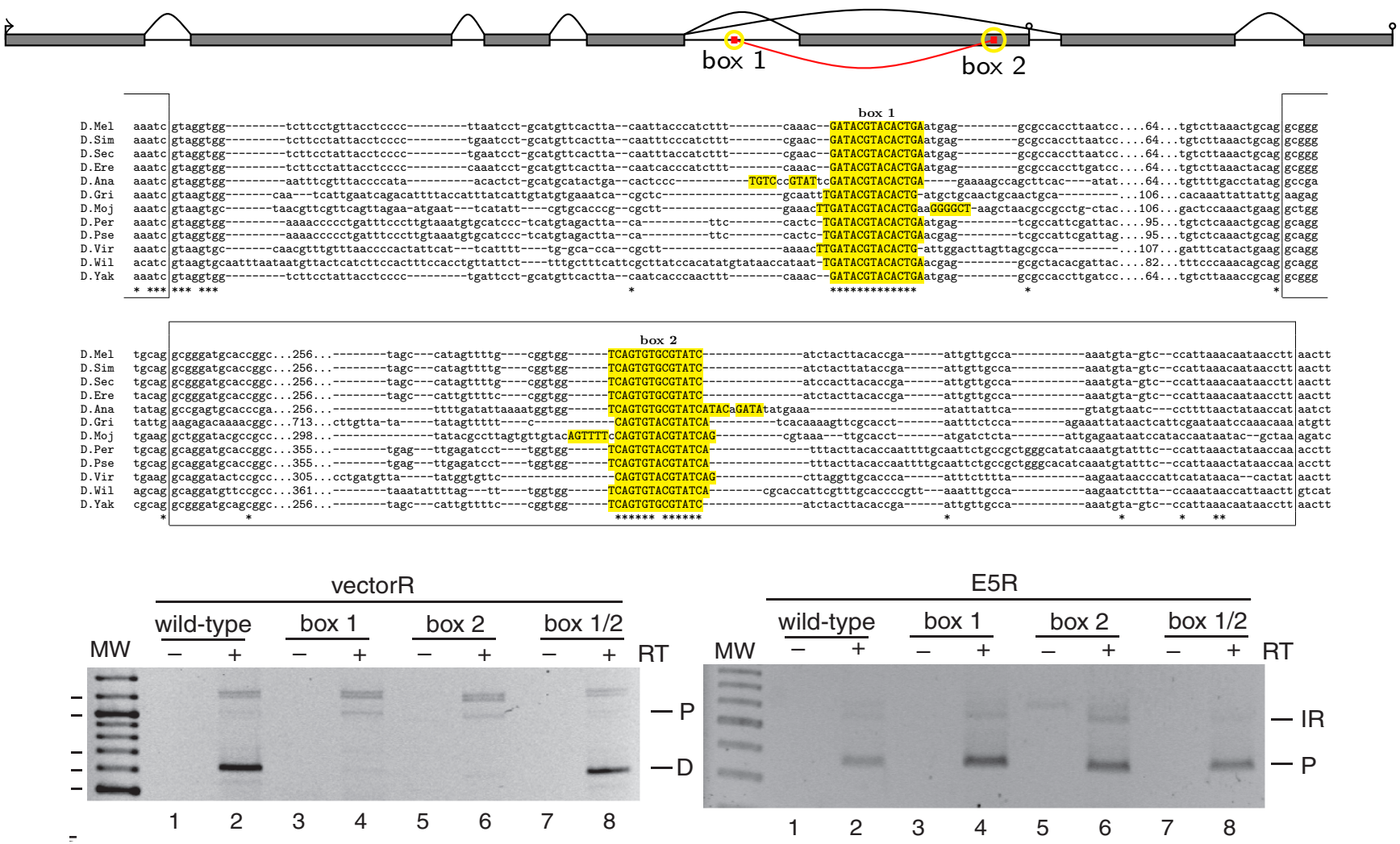
- 12 exons in Exon 4 cluster
- 37 exons in Exon 6 cluster
- 27 exons in Exon 9 cluster
- $12 \times 37 \times 27 \simeq 12,000$ alternative transcripts
- **One and only one** exon from each cluster is included



- Mutually exclusive base-pairing $\Longrightarrow$ mutually exclusive exon choice (May *et al* 2011, Graveley 2005)
- These base-pairings span over 10–15 Kb!

Gallery: splicing and polyadenylation in NMNAT gene

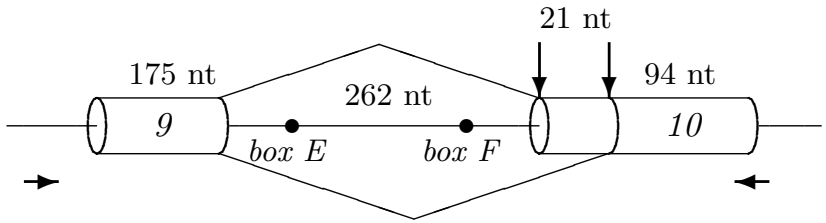
VA Raker, AA Mironov, MS Gelfand, DD Pervouchine, NAR 2009

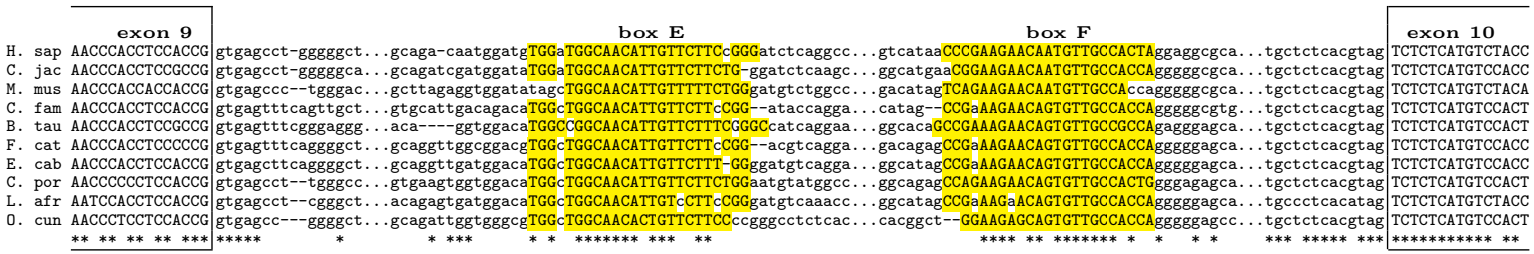


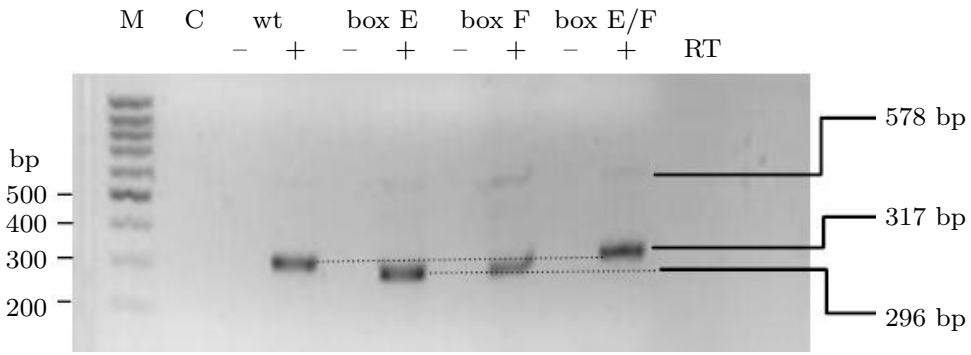
RNA structure affects **both** splicing and polyadenylation

Gallery: Splicing factor 1 (SF1)

Pervouchine *et al*, RNA 2012



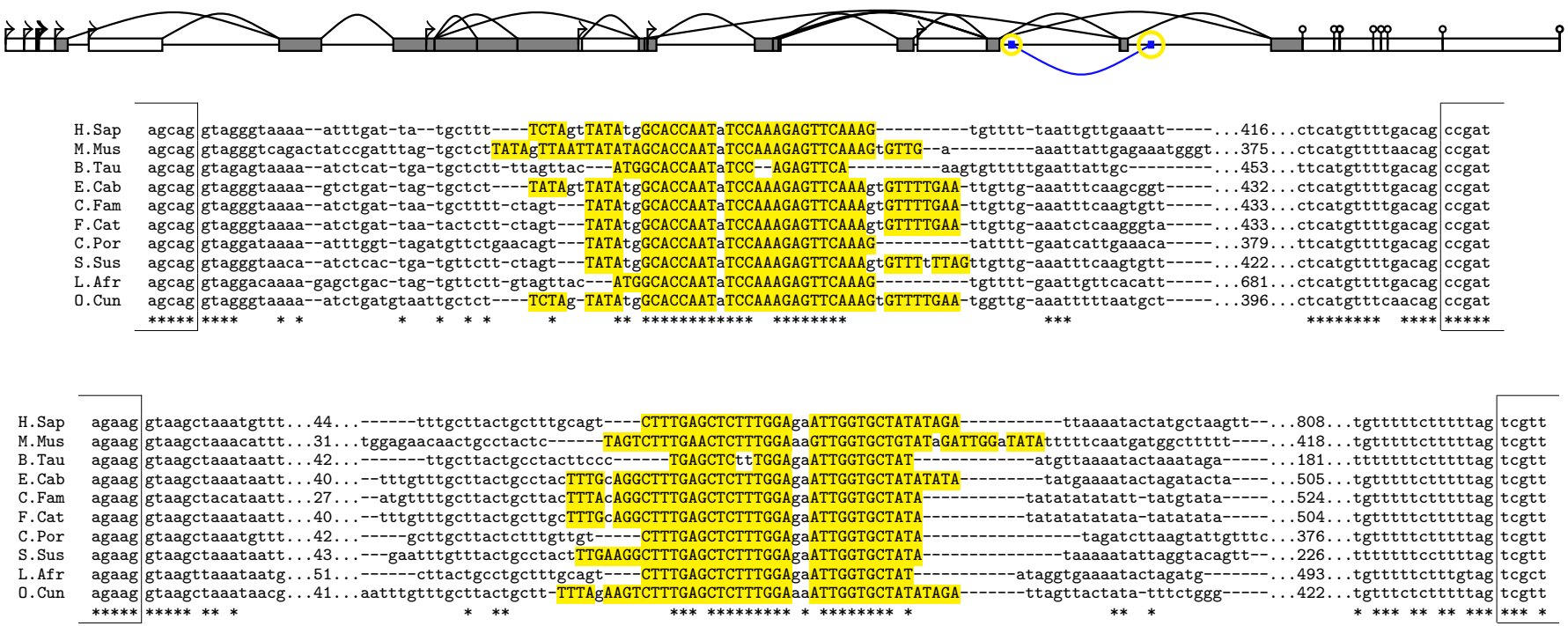




- Intron between exon 9 and 10 contains premature stop codon
- ESTs from breast and uterine adenocarcinoma cell lines support distal acceptor

Gallery: human splicing factor SRSF7

Pervouchine *et al*, RNA 2012



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Complementarity: no gapsgapped seeds

Sequence A

ATACGAGTCTGATCATTTACGGTCTTATACCGGTCTTATAC

ATACGAGTCT
TACGAGTCTG
ACGAGTCTGA

n-mer	position
ACCGGTCTTA	28
ACGAGTCTGA	2
ACGGTCTTAT	18
AGTCTGATCA	5
ATACCGGTCT	26
ATACGAGTCT	0
ATCATTTACG	11
ATTTACGGTC	14
CATTTACGGT	13
CCGGTCTTAT	29
CGAGTCTGAT	3
CGGTCTTATA	19, 30
CTGATCATTT	8
CTTATACCGG	23
GAGTCTGATC	4
GATCATTTAC	10

ATACGAGTCT

ATACGAGT
ATAC AGTC
ATAC GTCT
TACGAGTCTG
TACGAGTC
TACG GTCT
TACG TCTG
ACGAGTCTGA
ACGAGTCT
ACGA TCTG
ACGA CTGA

n-mer	position:gap

Sequence B

TACCTCGATGCAGAAATCGTCGAGACTCGTATCATTCGAGC

TACCTCGATG >> CATCGAGGTA
ACCTCGATGC >> GCATCGAGGT
CCTCGATGCA >> TGCATCGAGG

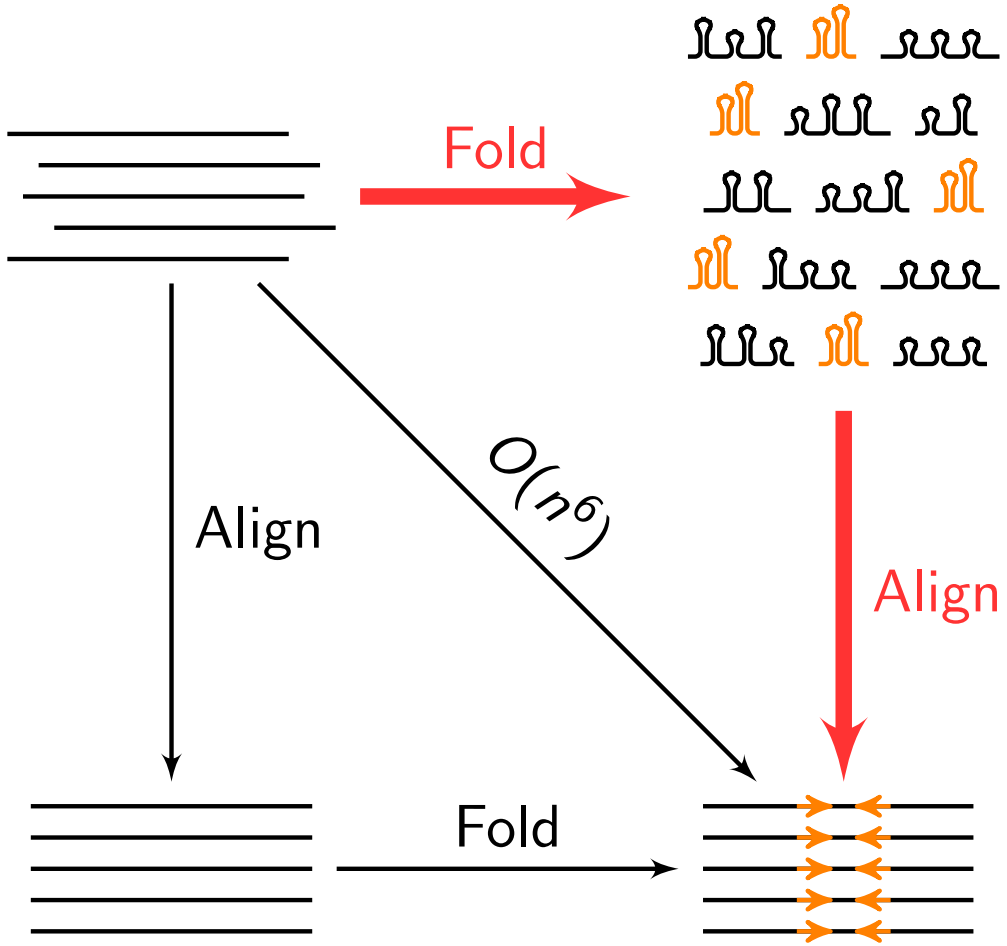
n-mer	position
AATGATACGA	26
ACGAGTCTCG	20
ACGATTTCTG	10
AGTCTCGACG	17
ATACGAGTCT	22
ATGATACGAG	25
ATTTCTGCAT	7
CATCGAGGTA	0
CGAATGATAC	28
CGACGATTTC	12
CGAGTCTCGA	19
CGATTTCTGC	9
CTCGAATGAT	30
CTCGACGATT	14
CTGCATCGAG	3
GAATGATACG	27

TACCTCGATG >> CATCGAGGTA

CATCGAGG
CATC AGGT
CATC GGTA
ACCTCGATGC >> GCATCGAGGT
GCATCGAG
GCAT GAGG
GCAT AGGT
CCTCGATGCA >> TGCATCGAGG
TGCATCGA
TGCA CGAG
TGCA GAGG

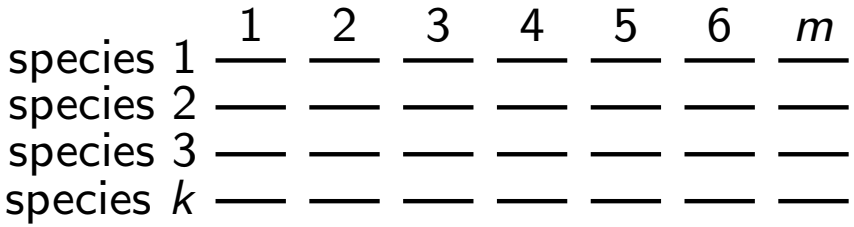
n-mer	position:gap

Align *vs.* Fold: a non-commutative diagram



- $n = 8$, $4^8 = 65535$ words, dictionary size = 8,837,747
- Min number of complementary pairs = 1,191,817,686 (best case)
- For 16 mammals, at least 4 bytes per pair = 342.1 Gb of RAM

Intersection: dropping non-conserved n-mers



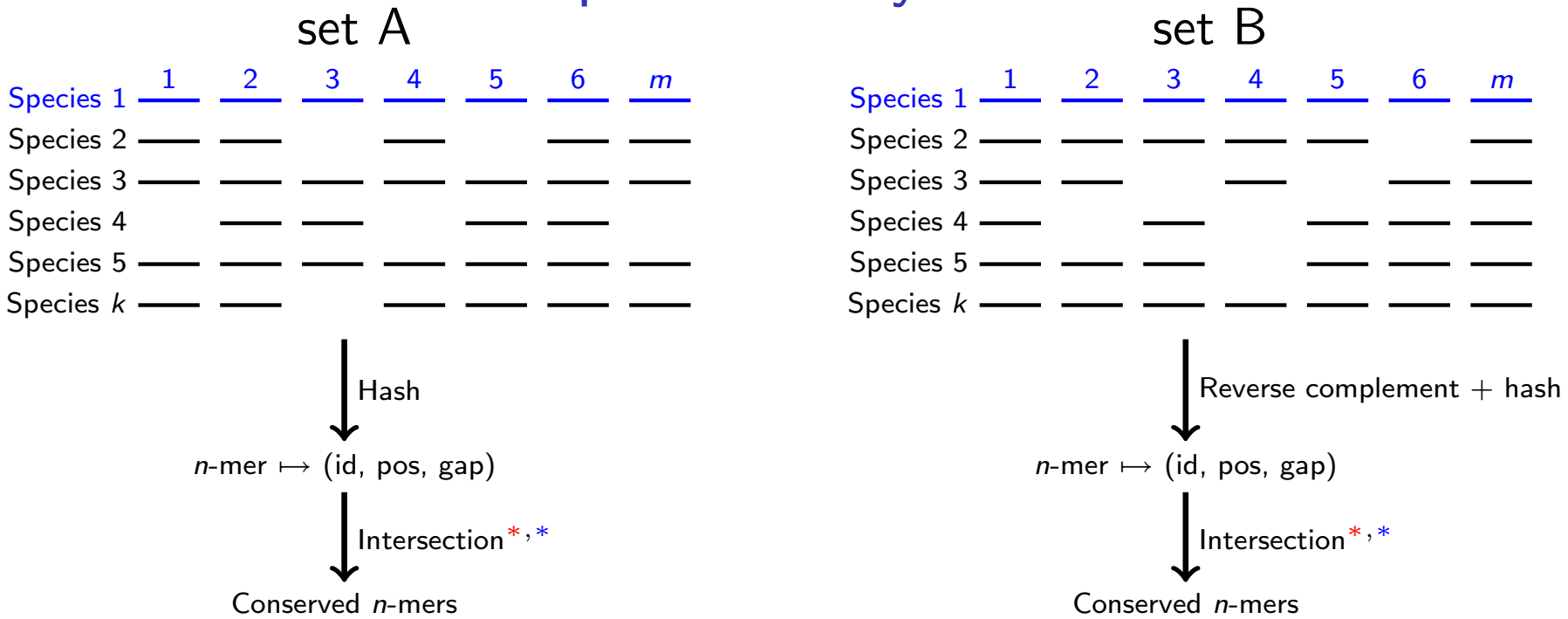
n-mer		segment_id:position:gap
AAAAAAAA	Species 1	1:100:0, 2:100:1, 7:200:1, ...
	Species 2	2:150:1, 4:200:0, 7:100:2...
	...	
	Species k	2:500:0, 7:300:1, 8:400:1...
AAAAAAAC	...	...

blue = pointed at
red = min element
gray = discarded
green = retained

- $i_1{:}p_1{:}g_1 \leq i_2{:}p_2{:}g_2 \iff i_1 < i_2$ or $i_1 = i_2$ & $p_1 < p_2$ or $i_1 = i_2$ & $p_1 = p_2$ & $g_1 \leq g_2$
- $i_1{:}p_1{:}g_1 \simeq i_2{:}p_2{:}g_2 \iff i_1 = i_2$ & $|p_1 - p_2| < M$

for *each n-mer* **do**
  initialize pointers $r_1 = r_2 = \cdots = r_k = 0$;
  while $x = \min\{x_{r_1}, x_{r_2}, \ldots, x_{r_k} | \leq\}$ *is defined* **do**
    compute $c =$ the number of j such that $x \simeq x_{r_j}$;
    keep $x_{r_1}, x_{r_2}, \ldots, x_{r_k}$ if $c >$ threshold;
  end
end

Conservation and complementarity



* seed pattern: 4-2-4; at most 1 GT and at least 2 GC base pairs per seed; sum of weights $\geq 75\%$;
* (id, pos, gap)$\simeq$(id$'$, pos$'$, gap$'$) $\iff$ id=id$'$ & |pos-pos$'$| $< M$
** induced by Cartesian product

One more thing: binary relationship $\mathcal{R} \subseteq A \times B$

Constrain the Cartesian product by a binary relationship $\mathcal{R} \subseteq A \times B$

$A = B =$ segments of protein-coding genes

- $x\mathcal{R}y$ iff $x = y$: local RNA structure
- $x\mathcal{R}y$ iff x and y belong to the same gene: long-range RNA structure within one gene (not necessarily at annotated splicing events)
- **Raker et al, NAR 2009**: $x\mathcal{R}y$ only if the intron $x \longrightarrow y$ is annotated
- **Pervouchine et al, RNA 2012**: $x\mathcal{R}y$ if x and y belong to the same gene

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- The input to the pipeline: $(A, B, \mathcal{R})$
- $A = B =$ windows around splice sites: RNA structures around splice sites
- $A =$ miRNAs, $B =$ 3'-UTRs, $\mathcal{R} = A \times B$: miRNA targets
- $A =$ snoRNAs, $B =$ windows around splice sites: snoRNA splicing targets
- $A =$ lncRNA segments vs. $B =$ windows around splice sites... **(today at 6pm)**

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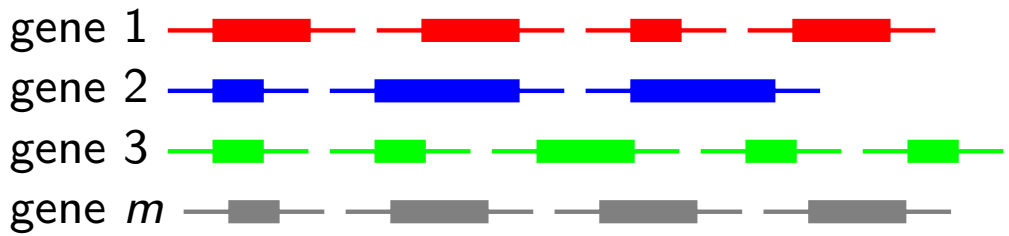
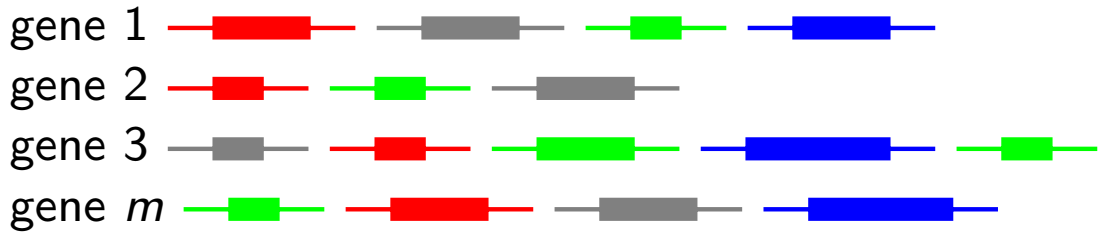
Statistical control

- Original set of genes

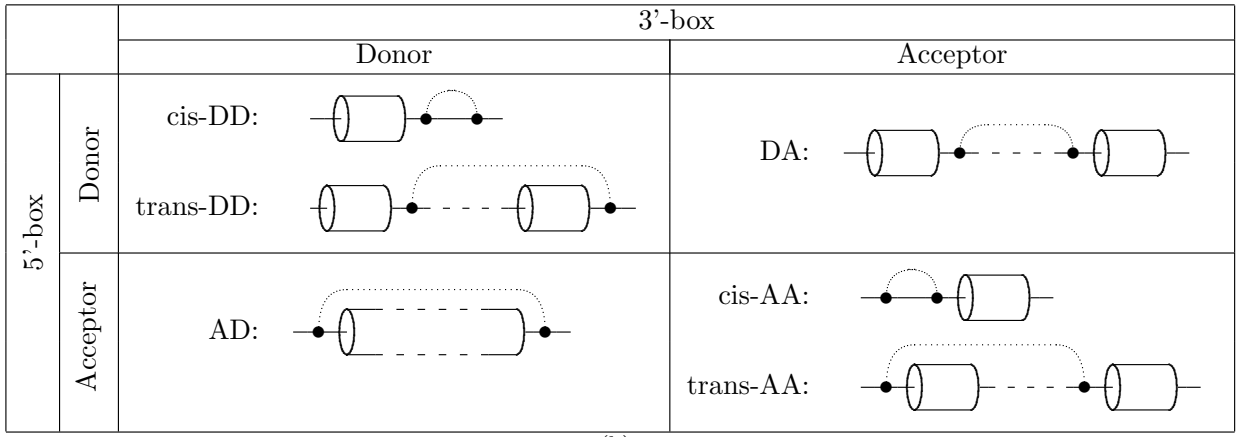


- Control: "re-wired" set



- Look at introns; length reduced to 1000 nts
- Estimate False Positive Rate (FPR)
- Blocking by GC content and/or sequence conservation rate

False positive rate



Repeats	Arrangement	Search	Control	Control GC	Control GC+Cons
Not masked	trans-DD	161	42.5±7.1 (26%±4%)	50.1±7.8 (31%±5%)	72.4±7.5 (45%±5%)
	trans-AA	132	57.0±8.2 (43%±6%)	47.7±7.4 (36%±6%)	60.9±7.1 (46%±5%)
	DA	211	60.1±4.2 (28%±2%)	61.6±4.3 (29%±2%)	76.0±4.1 (36%±2%)
	AD	212	62.6±4.1 (30%±2%)	58.1±4.0 (27%±2%)	80.5±4.7 (38%±2%)
Masked	trans-DD	114	34.2±4.4 (30%±4%)	36.0±4.2 (32%±4%)	27.6±3.5 (24%±3%)
	trans-AA	108	43.1±4.6 (40%±4%)	42.2±4.5 (39%±4%)	43.5±4.1 (40%±4%)
	DA	167	47.4±3.1 (28%±2%)	43.8±3.2 (26%±2%)	50.6±3.0 (30%±2%)
	AD	174	44.7±3.3 (26%±2%)	47.0±3.2 (27%±2%)	42.9±2.9 (25%±2%)

It is not unlikely to find a pair of conserved complementary *n*-mers next to splice sites of mammalian genes

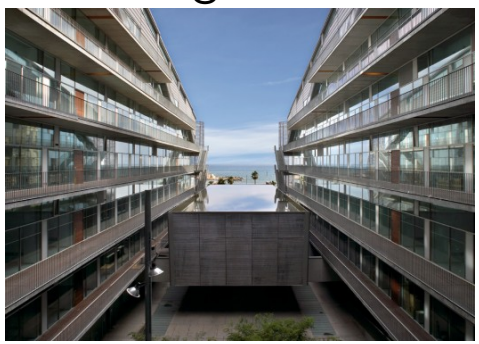
Summary

- IRBIS: a conceptually novel (and computationally realistic) framework for predicting conserved RNA structures and RNA-RNA interactions on genome-wide scale
- Hash table (dictionary) is a natural instrument for simultaneously detecting motif conservation and complementarity
- Implemented as a C++ library

- The set of genes/introns with complementary boxes differs from simple random samples of the same size in many important ways
- Even with FPR as high as 50%, **there is a strong statistical evidence for many stable long-range RNA structures to be conserved and functionally important**

Acknowledgments

Centre de Regulació Genòmica



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Thank you for your attention

(continued for RNA-RNA interactions)