RIsearch2

Large-scale RNA–RNA interaction prediction

Anne Wenzel

Center for non-coding RNA in Technology and Health
Department of Veterinary Clinical and Animal Sciences
Faculty of Health and Medical Sciences
University of Copenhagen

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RTH
CENTER FOR NON-CODING RNA
IN TECHNOLOGY AND HEALTH

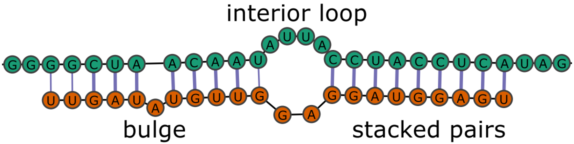
UNIVERSITY OF COPENHAGEN
MEDICAL SCIENCES

Motivation

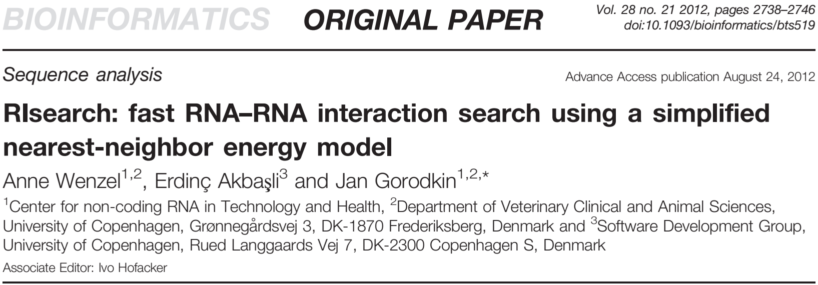
Regulatory, non-coding RNAs

- often function by forming a duplex with other RNAs
- many identified but unknown targets
- their interactome provides insight to function

Goal: Predict RNA–RNA duplexes *in silico* on genome-wide scale



Recap: RIsearch



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Sequence analysis

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RIsearch: fast RNA–RNA interaction search using a simplified nearest-neighbor energy model

Anne Wenzel[1,2], Erdinç Akbaşli[3] and Jan Gorodkin[1,2,*]

[1]Center for non-coding RNA in Technology and Health, [2]Department of Veterinary Clinical and Animal Sciences, University of Copenhagen, Grønnegårdsvej 3, DK-1870 Frederiksberg, Denmark and [3]Software Development Group, University of Copenhagen, Rued Langgaards Vej 7, DK-2300 Copenhagen S, Denmark

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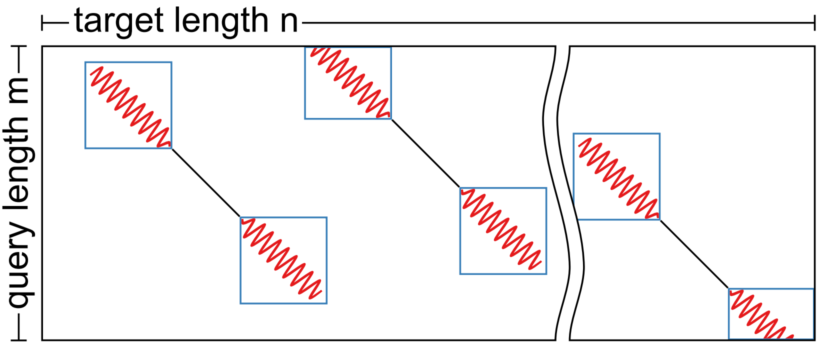
- dinucleotide scoring matrix in Smith–Waterman-like algorithm
- approximates Nearest Neighbor energy model
- computed energies deviate from full model, BUT
- candidates are ranked similar
- fast method for predicting near-complementary duplexes

Search space



- RIsearch: DP over $\boxed{m \times n}$
- idea: seed-and-extend (DP on either end over $\boxed{l^2}$)
- GUUGle + RIsearch performs very well for miRNAs
- Now: one stop shop → RIsearch2

RIsearch2: outline

Suffix array enables very fast seed detection

- build generalized suffix array of the target (entire human genome stored in 47 GiB)
- build partial suffix array of the query (according to seed settings)
- match suffix arrays (allowing wobble pairs)

Extend seeds with DP as before

- DP matrix anchored at first/last position of seed

RIsearch2: building the target index

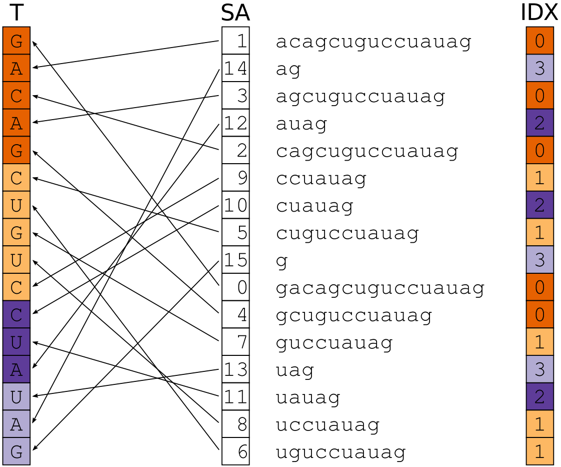
```
>targetSeqA
gacag                gacagcuguccuauag   T
>targetSeqB          0000011111222333  IDX
cua
```

T	SA		IDX
G	0	gacagcuguccuauag	0
A	1	acagcuguccuauag	0
C	2	cagcuguccuauag	0
A	3	agcuguccuauag	0
G	4	gcuguccuauag	0
C	5	cuguccuauag	1
U	6	uguccuauag	1
G	7	guccuauag	1
U	8	uccuauag	1
C	9	ccuauag	1
C	10	cuauag	2
U	11	uauag	2
A	12	auag	2
U	13	uag	3
A	14	ag	3
G	15	g	3

RIsearch2: building the target index

```
>targetSeqA
gacag
>targetSeqB
cua
```

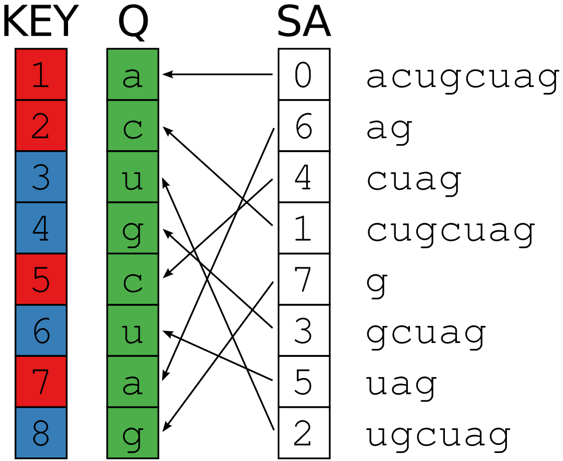
```
gacagcuguccuauag    T
0000011111222333    IDX
```



T	SA		IDX
G	1	acagcuguccuauag	0
A	14	ag	3
C	3	agcuguccuauag	0
A	12	auag	2
G	2	cagcuguccuauag	0
C	9	ccuauag	1
U	10	cuauag	2
G	5	cuguccuauag	1
U	15	g	3
C	0	gacagcuguccuauag	0
C	4	gcuguccuauag	0
U	7	guccuauag	1
A	13	uag	3
U	11	uauag	2
A	8	uccuauag	1
G	6	uguccuauag	1

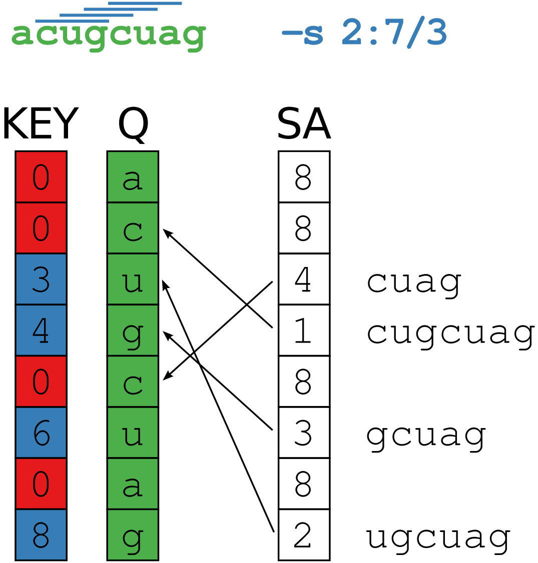
RIsearch2: building (partial) query SA

acugcuag   -s 2:7/3



KEY	Q	SA	
1	a	0	acugcuag
2	c	6	ag
3	u	4	cuag
4	g	1	cugcuag
5	c	7	g
6	u	3	gcuag
7	a	5	uag
8	g	2	ugcuag

RIsearch2: building (partial) query SA



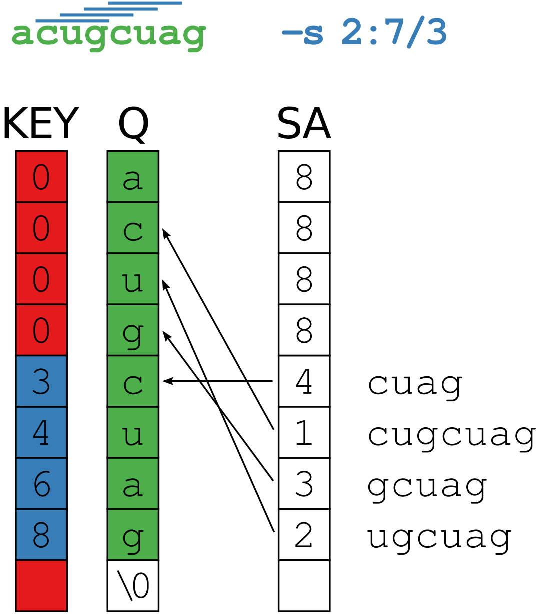
acugcuag

-s 2:7/3

KEY	Q	SA	
0	a	8	
0	c	8	
3	u	4	cuag
4	g	1	cugcuag
0	c	8	
6	u	3	gcuag
0	a	8	
8	g	2	ugcuag

RIsearch2: building (partial) query SA

acugcuag   -s 2:7/3

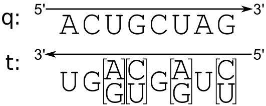


RIsearch2: parallel matching SAs

KEY	Q	SA	
0	a	8	
0	c	8	
0	u	8	
0	g	8	
3	c	4	cuag
4	u	1	cugcuag
6	a	3	gcuag
8	g	2	ugcuag
	\0		

SA		IDX	T
1	acagcuguccuauag	0	G
14	ag	3	A
3	agcuguccuauag	0	C
12	auag	2	A
2	cagcuguccuauag	0	G
9	ccuauag	1	C
10	cuauag	2	U
5	cuguccuauag	1	G
15	g	3	U
0	gacagcuguccuauag	0	C
4	gcuguccuauag	0	C
7	guccuauag	1	U
13	uag	3	A
11	uauag	2	U
8	uccuauag	1	A
6	uguccuauag	1	G

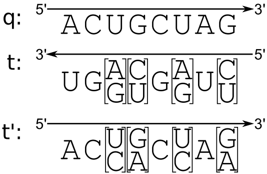
RIsearch2: parallel matching SAs



KEY	Q	SA	
0	a	8	
0	c	8	
0	u	8	
0	g	8	
3	c	4	cuag
4	u	1	cugcuag
6	a	3	gcuag
8	g	2	ugcuag
	\0		

SA		IDX	T
1	acagcuguccuauag	0	G
14	ag	3	A
3	agcuguccuauag	0	C
12	auag	2	A
2	cagcuguccuauag	0	G
9	ccuauag	1	C
10	cuauag	2	U
5	cuguccuauag	1	G
15	g	3	U
0	gacagcuguccuauag	0	C
4	gcuguccuauag	0	C
7	guccuauag	1	U
13	uag	3	A
11	uauag	2	U
8	uccuauag	1	A
6	uguccuauag	1	G

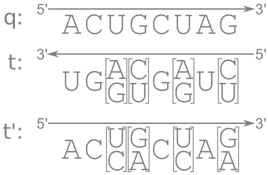
RIsearch2: parallel matching SAs



KEY	Q	SA	
0	a	8	
0	c	8	
0	u	8	
0	g	8	
3	c	4	cuag
4	u	1	cugcuag
6	a	3	gcuag
8	g	2	ugcuag
	\0		

SA		IDX	T
1	acagcuguccuauag	0	G
14	ag	3	A
3	agcuguccuauag	0	C
12	auag	2	A
2	cagcuguccuauag	0	G
9	ccuauag	1	C
10	cuauag	2	U
5	cuguccuauag	1	G
15	g	3	U
0	gacagcuguccuauag	0	C
4	gcuguccuauag	0	C
7	guccuauag	1	U
13	uag	3	A
11	uauag	2	U
8	uccuauag	1	A
6	uguccuauag	1	G

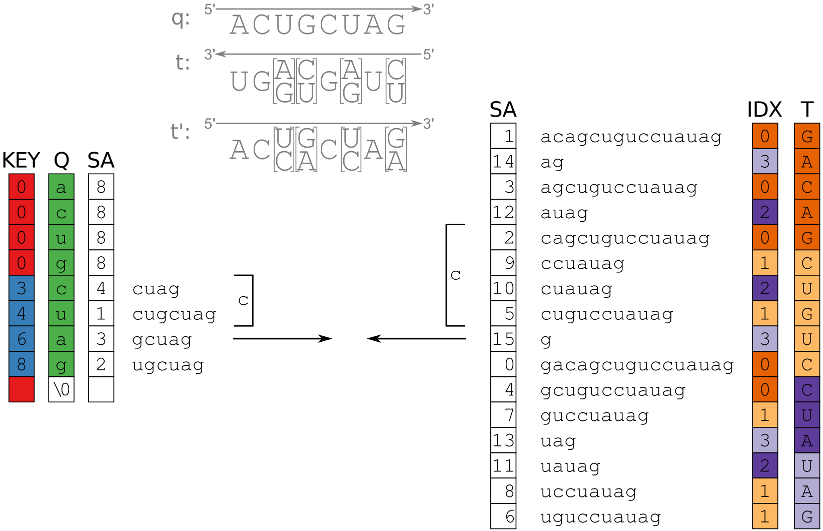
RIsearch2: parallel matching SAs



KEY	Q	SA	
0	a	8	
0	c	8	
0	u	8	
0	g	8	
3	c	4	cuag
4	u	1	cugcuag
6	a	3	gcuag
8	g	2	ugcuag
	\0		

SA		IDX	T
1	acagcuguccuauag	0	G
14	ag	3	A
3	agcuguccuauag	0	C
12	auag	2	A
2	cagcuguccuauag	0	G
9	ccuauag	1	C
10	cuauag	2	U
5	cuguccuauag	1	G
15	g	3	U
0	gacagcuguccuauag	0	C
4	gcuguccuauag	0	C
7	guccuauag	1	U
13	uag	3	A
11	uauag	2	U
8	uccuauag	1	A
6	uguccuauag	1	G

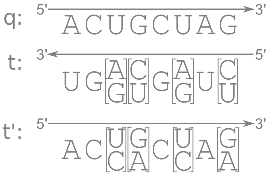
RIsearch2: parallel matching SAs



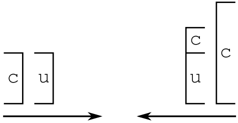
KEY	Q	SA	
0	a	8	
0	c	8	
0	u	8	
0	g	8	
3	c	4	cuag
4	u	1	cugcuag
6	a	3	gcuag
8	g	2	ugcuag
	\0		

SA		IDX	T
1	acagcuguccuauag	0	G
14	ag	3	A
3	agcuguccuauag	0	C
12	auag	2	A
2	cagcuguccuauag	0	G
9	ccuauag	1	C
10	cuauag	2	U
5	cuguccuauag	1	G
15	g	3	U
0	gacagcuguccuauag	0	C
4	gcuguccuauag	0	C
7	guccuauag	1	U
13	uag	3	A
11	uauag	2	U
8	uccuauag	1	A
6	uguccuauag	1	G

RIsearch2: parallel matching SAs

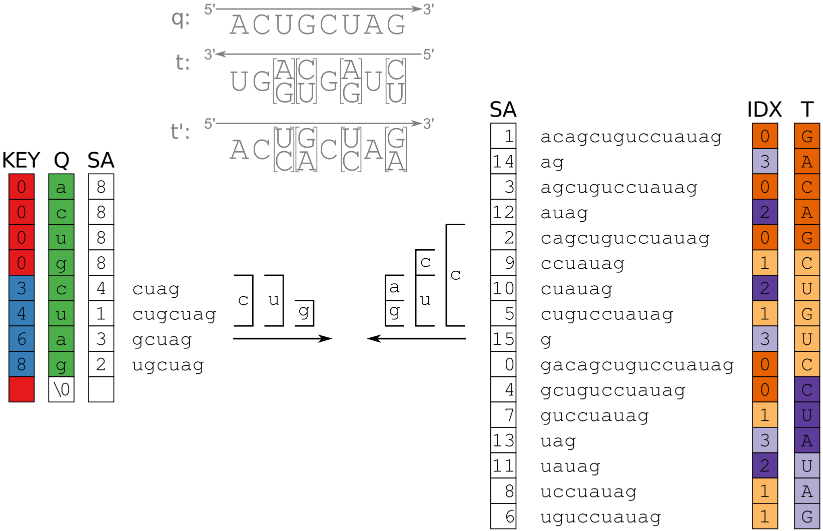

q: 5' ACUGCUAG 3'
t: 3' UG A/G C/U G A/G U C/U 5'
t': 5' AC U/C G/A C U/C A G/A 3'

KEY	Q	SA	
0	a	8	
0	c	8	
0	u	8	
0	g	8	
3	c	4	cuag
4	u	1	cugcuag
6	a	3	gcuag
8	g	2	ugcuag
	\0		



SA		IDX	T
1	acagcuguccuauag	0	G
14	ag	3	A
3	agcuguccuauag	0	C
12	auag	2	A
2	cagcuguccuauag	0	G
9	ccuauag	1	C
10	cuauag	2	U
5	cuguccuauag	1	G
15	g	3	U
0	gacagcuguccuauag	0	C
4	gcuguccuauag	0	C
7	guccuauag	1	U
13	uag	3	A
11	uauag	2	U
8	uccuauag	1	A
6	uguccuauag	1	G

RIsearch2: parallel matching SAs


q: 5' ACUGCUAG 3'
t: 3' UG A/G C/U G A/G U C/U 5'
t': 5' AC U/C G/A C U/C A G/A 3'
c
u
g
a
g
c
u
c

KEY	Q	SA	
0	a	8	
0	c	8	
0	u	8	
0	g	8	
3	c	4	cuag
4	u	1	cugcuag
6	a	3	gcuag
8	g	2	ugcuag
	\0		

SA		IDX	T
1	acagcuguccuauag	0	G
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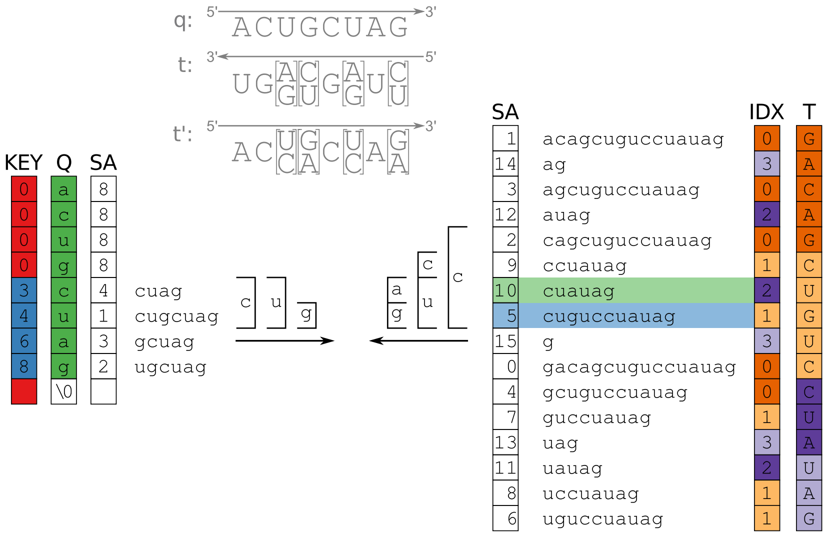
3	agcuguccuauag	0	C
12	auag	2	A
2	cagcuguccuauag	0	G
9	ccuauag	1	C
10	cuauag	2	U
5	cuguccuauag	1	G
15	g	3	U
0	gacagcuguccuauag	0	C
4	gcuguccuauag	0	C
7	guccuauag	1	U
13	uag	3	A
11	uauag	2	U
8	uccuauag	1	A
6	uguccuauag	1	G

RIsearch2: parallel matching SAs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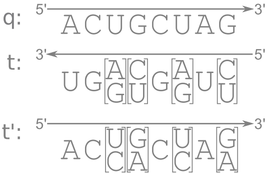
t: 3' UG A/G C/U G A/G U C/U 5'
t': 5' AC U/C G/A C U/C A G/A 3'
c
u
g
a
g
c
u
c

KEY	Q	SA	
0	a	8	
0	c	8	
0	u	8	
0	g	8	
3	c	4	cuag
4	u	1	cugcuag
6	a	3	gcuag
8	g	2	ugcuag
	\0		

SA		IDX	T
1	acagcuguccuauag	0	G
14	ag	3	A
3	agcuguccuauag	0	C
12	auag	2	A
2	cagcuguccuauag	0	G
9	ccuauag	1	C
10	cuauag	2	U
5	cuguccuauag	1	G
15	g	3	U
0	gacagcuguccuauag	0	C
4	gcuguccuauag	0	C
7	guccuauag	1	U
13	uag	3	A
11	uauag	2	U
8	uccuauag	1	A
6	uguccuauag	1	G

RIsearch2: parallel matching SAs



KEY	Q	SA	
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0	c	8	
0	u	8	
0	g	8	
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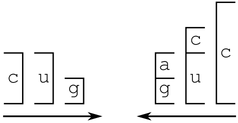
4	u	1	cugcuag
6	a	3	gcuag
8	g	2	ugcuag
	\0		

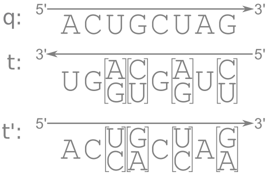


SA		IDX	T
1	acagcuguccuauag	0	G
14	ag	3	A
3	agcuguccuauag	0	C
12	auag	2	A
2	cagcuguccuauag	0	G
9	ccuauag	1	C
10	cuauag	2	U
5	cuguccuauag	1	G
15	g	3	U
0	gacagcuguccuauag	0	C
4	gcuguccuauag	0	C
7	guccuauag	1	U
13	uag	3	A
11	uauag	2	U
8	uccuauag	1	A
6	uguccuauag	1	G

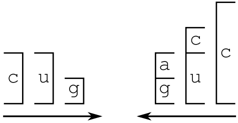
T gacagcuguccuauag

IDX 0000011111222333

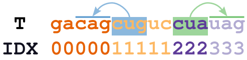
RIsearch2: parallel matching SAs


q: 5' ACUGCUAG 3'
t: 3' UG A G C U G A G U C U 5'
t': 5' AC U C G A C U C A G A 3'

KEY	Q	SA	
0	a	8	
0	c	8	
0	u	8	
0	g	8	
3	c	4	cuag
4	u	1	cugcuag
6	a	3	gcuag
8	g	2	ugcuag
	\0		



SA		IDX	T
1	acagcuguccuauag	0	G
14	ag	3	A
3	agcuguccuauag	0	C
12	auag	2	A
2	cagcuguccuauag	0	G
9	ccuauag	1	C
10	cuauag	2	U
5	cuguccuauag	1	G
15	g	3	U
0	gacagcuguccuauag	0	C
4	gcuguccuauag	0	C
7	guccuauag	1	U
13	uag	3	A
11	uauag	2	U
8	uccuauag	1	A
6	uguccuauag	1	G



RIsearch2: Benchmark

100 miRNAs vs. repeat-masked human genome

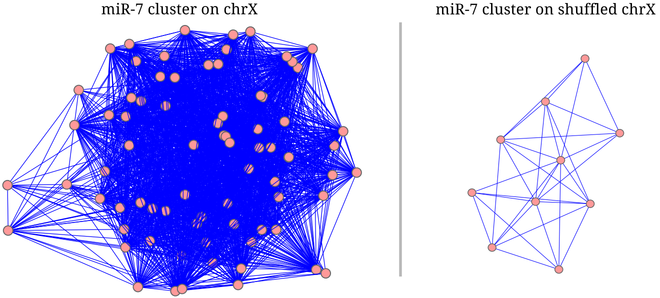
variant	run time [h:mm:ss]
RIsearch	
1 optimal per query–target pair (miR–chr-strand)	65:58:21
suboptimals $\leq -10\,\mathrm{kcal/mol}$	184:47:47
RIsearch2 with different seed sizes anywhere in the query	
-s 8	2:28:39
-s 7	6:36:24
-s 6	19:10:45
RIsearch2 with seeds position-constrained in the query	
-s 1:8/6 (1–6 / 2–7 / 3–8)	4:05:43
-s 2:7 (2–7)	1:29:08
-s 2:7/5 (2–6 / 3–7)	8:49:09

This is single-core time, additionally RIsearch2 is multi-threaded.

Application 1: miRNA sponge discovery

`RIsearch2` is especially efficient when constrained to seed region

- screen all human mature miRNAs against the human genome
- identify genomic regions with high binding site density
- select candidates based on difference real vs. shuffled



miR-7 cluster on chrX

miR-7 cluster on shuffled chrX

Pan *et al.*, in prep.

Application 2: search for off-targets

Off-targeting is a problem in probe/siRNA design

siRNAs can effect transcripts other than the intended target

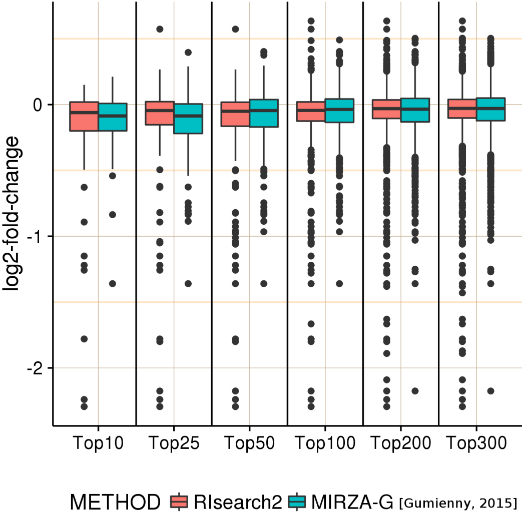
- (near-)perfect complementarity : silencing
- imperfect binding to 3' UTR : miRNA-like effect

Approach:

- use `RIsearch2` to screen for potential off-targets
- combine with accessibility profiles and transcript abundance
- partition function to quantify off-target volume (per siRNA)
- compute off-targeting probabilities (per transcript)

Application 2: search for off-targets (cont.)

Predicted off-targets are down-regulated upon siRNA transfection (combined results for 6 siRNAs [Burchard, 2009])



[Gumienny, 2015]

Acknowledgements

`RIsearch2` coders (in descending order of recentness of their contributions):

Ferhat Alkan
Oana Palasca
Peter Kerpedjiev
Anders F Rudebeck

Peter F. Stadler
Ivo L. Hofacker

Jan Gorodkin and our whole group at RTH

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DANISH COUNCIL FOR
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