



# ROSO: A software to design optimized oligonucleotide probes for DNA micro-arrays

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*Biologie Fonctionnelle, Insectes et Interactions*



# The transcriptome « plate-forme » Génopole Rhône-Alpes



Spotting



Scanning



Analyzing

- *to give the opportunities for regional laboratories to make and use their own DNA chips.*

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69622 Villeurbanne Cedex

# *SITRANS: information system for transcriptome analysis*

*Welcome !*

User

Password

What would you like to do?

Stock Management

Enter data for a new stored oligonucleotide set

Enter data for a new stored slide set

Datas Management

Enter data for a new experiment set

Proceeding with a data capture

Consultation

View existing datas

Downloads

- Gene file
- Oligonucléotides file
- Spots file
- Spoflevel file

LISI

Laboratoire de Recherche en Ingénierie des Systèmes d'Information  
INSA Bât Blaise Pascal  
69 621 Villeurbanne Cedex

<http://pbil.univ-lyon1.fr/roso/loadWkgFiles.php>

Netscape: Run ROSO

Précédente Suivante Recharger Accueil Rechercher Guide Images Imprimer Sécurité Arrêter

Adresse <http://pbil.univ-lyon1.fr/roso/loadWkgFiles.php> Infos connexes



PBIL  
11011001

ROSO  
a friend for your design



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### Send directly your formatted files

The software require FASTA formatted files.

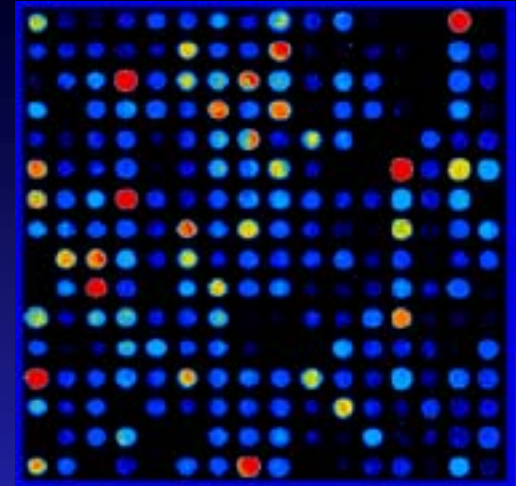
- Interest sequences file:  [Parcourir...](#)
- Interest hits file:  [Parcourir...](#)
- External hits file:  [Parcourir...](#)

[Send files](#) [CLEAR](#)


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- **Introduction: what we would like to do...**
- **Data set selection**
- **Probe specificity: search of similarities**
- **Probe localisation: physical and biological constraints**
- **Probe sensitivity: thermodynamic analysis**
- **Probe sensitivity: secondary structures**
- **Conclusion: running ROSO on the web**



- n genes of interest (“internal” genes)
- n probes :
  - 30-70 bases (synthesized oligonucleotides)
  - Specific :
    - as compared with the n “internal“ genes
    - as compared with the N “external“ genes
  - homogeneous and optimal chemical properties:
    - $T_M$
    - secondary structures
    - %GC, zippering...



**WWW-Query**  
BBE contribution to [PBIL](#) in Lyon, France

[Species](#) [Send](#) [Modify](#) [Retrieve](#) [Releases](#) [Help](#)

Databank:

Selection criteria:

1.	<input type="text" value="DEFAULT"/>	<input type="text" value="Species"/>	<input type="text" value="mus"/>
2.	<input type="text" value="AND"/>	<input type="text" value="Molecule"/>	<input type="text" value="MRNA"/>
3.	<input type="text" value="AND"/>	<input type="text" value="Keyword"/>	<input type="text"/>
4.	<input type="text" value="AND"/>	<input type="text" value="Keyword"/>	<input type="text"/>

List name:

## GenBank: "sp=mus" et "m=MRNA"

[Modify](#) [Retrieve](#) [Analyze](#)

### 2598493 matching sequences found

- [AA000002](#) mg27f05.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone IMAGE:425025 5', mRNA sequence.
- [AA000003](#) mg27f06.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone IMAGE:425027 5', mRNA sequence.
- [AA000004](#) mg27f07.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone IMAGE:425029 5' similar to gb:X67083 M.musculus chop-10 mRNA (MOUSE);, mRNA sequence.
- [AA000005](#) mg27g05.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone IMAGE:425048 5' similar to gb:D14531 60S RIBOSOMAL PROTEIN L9 (HUMAN);, mRNA sequence.
- [AA000006](#) mg27g06.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone IMAGE:425050 5' similar to gb:X71491 VACUOLAR ATP SYNTHASE SUBUNIT E (HUMAN);, mRNA sequence.
- [AA000007](#) mg27g07.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone IMAGE:425052 5', mRNA sequence.

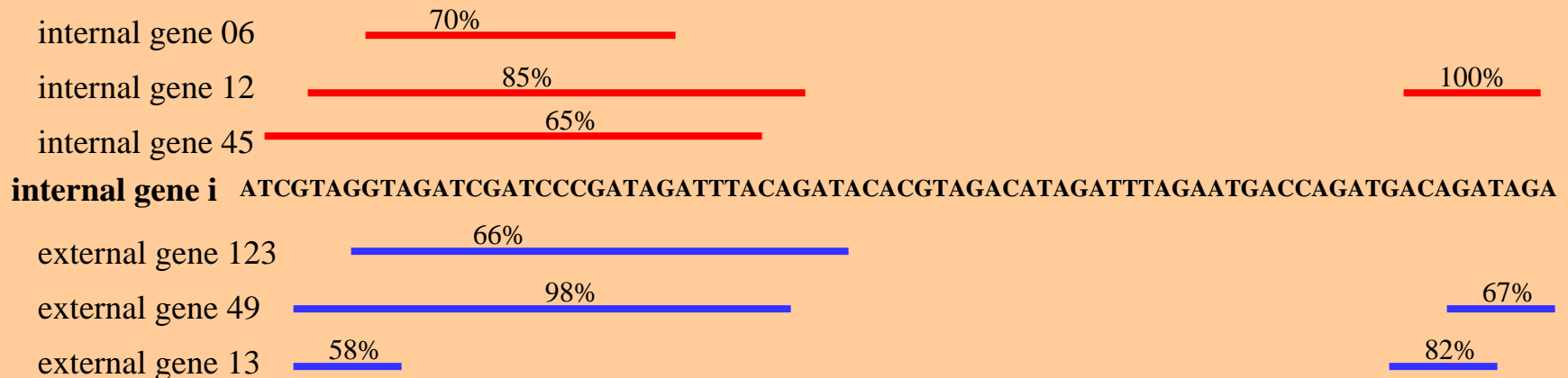
## Problems:

- Alternative splicing, terminating...
- Paralogous families
- Data set redundancy (human, mouse)

# Probe specificity: search of similarities



Probes are selected in specific regions, i.e. regions with no homology with both internal and external genes (n+N). Homologous regions will be determined (blast) and eliminated.





## The Blast software:

W : word size (default 11)

f : Threshold for extending hits (default 0)

q : mismatch penalty (default -3)

r : match reward (default 1)

W=11, q= -3 : minimal homology detected 80% (100 pb)

W=7, q= -2 : minimal homology detected 70% (20 pb)

## Example:

ACGTAGTGACGATAGAATGATGACGATGATGACAGTAATGTGTCGTGAAC  
ACG**G**AGTGACG**C**TAGAATGAT**C**ACGATGAT**A**ACAGTAAT**T**TGTCGTGAAC

Homology = 90% (W=11 : no match)



## 20 bp on the *groES* gene of *E. coli*

tggcgaagtgaagccgctgg

## Blastn (W=11, q= -3, default options)

```
>gi|18028157|gb|AF325453.1|AF325453 Escherichia coli strain ATCC700336 GroES and GroEL mRNAs, complete
      cds
      Length = 2009

Score = 40.1 bits (20), Expect = 0.004
Identities = 20/20 (100%)
Strand = Plus / Plus

Query: 1   tggcgaagtgaagccgctgg 20
          |||
Sbjct: 155 tggcgaagtgaagccgctgg 174
```



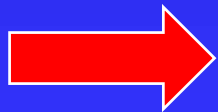
one central mutation (95% homology with *groEL*):  
tggcgaagta**a**agccgctgg

## Blastn (W=11, q= -3, default option)

```
>gi|15619767|gb|AE008627.1|AE008627 Rickettsia conorii Malish 7, section 59 of 114 of the complete genome
      Length = 10162

Score = 30.2 bits (15), Expect = 1.5
Identities = 15/15 (100%)
Strand = Plus / Plus

Query: 6  aagtaaagccgctgg 20
         |||
Sbjct: 36 aagtaaagccgctgg 50
```



*The biggest homology is not found by Blast*





- **Physical constraints:**

- PolydT priming: probes should be designed close to the 3' end
- Random priming: Probes should not be designed close to the 3' end

--> 100 to 750 bp from 3' end

- **Biological constraints:**

- exons / introns
- alternative splicing
- UTR are more variable than coding regions



- $T_M$  = Melting temperature  
50% of nucleic acids are double strand
- Hybridization ( $T_H$ ) is performed 5 to 10°C below  $T_M$ .
- $T_M$  depends of:
  - type and position of nucleotides
  - salt concentration (stringency)
  - nucleic acid concentration
  - denaturing agent (urea, formamid)

--> In order to compare absolute values of spot intensities, probes should share the same  $T_m$  however to compare relative values (Cy3/Cy5), it is less important.

## Calculating melting temperature (T<sub>m</sub>):

- **AT/GC method:**  $T_m = 2AT + 4GC$   
(very simple but really bad!)

- **GC method:**

ADN/ADN :  $T_m = 81.5 + 0.41(\%GC) + 16.6 \log\left(\frac{[K^+]}{1 + 0.7[K^+]}\right) - (\%mes) - 500 / N$

ADN/ARN :  $T_m = 79.8 + 0.58(\%GC) + 18.5 \log\left(\frac{[K^+]}{1 + 0.7[K^+]}\right) - (\%mes) - 820 / N$

(very good for probe > 50 bp)

- **Nearest Neighbor:**

$$T_m = \frac{\Delta H}{\Delta S + R \ln C_T} + 16.6 \log\left(\frac{[K^+]}{1 + 0.7[K^+]}\right) - 273.15$$

(very good for probe < 70 bp)

**These models give good predictions for liquide-liquide hybridization.**



$$T_m = \frac{\Delta H}{\Delta S + R \ln C_T} + 16.6 \log \left( \frac{[K^+]}{1 + 0.7[K^+]} \right) - 273.15$$

$R = 1.987 \text{ cal K}^{-1} \text{ mol}^{-1}$  (perfect gaz constant)

$C_T$  : nucleic acid concentration

$\Delta H$  and  $\Delta S$  : variations of enthalpy and entropy between single and double strand DNA

$$\Delta H = \Delta H_{\text{initiation}} + \Delta H_{\text{symétrie}} + \sum \Delta H_x$$
$$\Delta S_{1M [Na^+]} = \Delta S_{\text{initiation}} + \Delta S_{\text{symétrie}} + \sum \Delta S_x$$

$\Delta H$  or  $\Delta S$  initiation : function of GC

$\Delta H$  ou  $\Delta S$  symétrie : function of mismatch

$\Delta H_x$  ou  $\Delta S_x$  : nearest neighbor interactions

- initiation and symmetry:

	$\Delta H \text{ kcal.mol}^{-1}$	$\Delta S \text{ cal.mol}^{-1}$	$\Delta G \text{ kcal.mol}^{-1}$
Initiation (A, T, G,C)	0,1	-2,8	0,98
Initiation ( only A and T)	2,3	4,1	1,03
Symétrie	0	-1,4	-0,4

- interaction table:

		Right Base											
		A			T			C			G		
		$\Delta H$	$\Delta S$	$\Delta G$	$\Delta H$	$\Delta S$	$\Delta G$	$\Delta H$	$\Delta S$	$\Delta G$	$\Delta H$	$\Delta S$	$\Delta G$
Left Base	A	-7,9	-22,2	-1,00	-7,2	-20,4	-0,88	-8,4	-22,4	-1,44	-7,8	-21,0	-1,28
	T	-7,2	-21,3	-0,58	-7,9	-22,2	-1,00	-8,2	-22,2	-1,30	-8,5	-22,7	-1,45
	C	-8,5	-22,7	-1,45	-7,8	-21,0	-1,28	-8,0	-19,9	-1,42	-10,6	-27,2	-2,24
	G	-8,2	-22,2	-1,30	-8,4	-22,4	-1,4	-9,8	-24,4	-1,84	-8,0	-19,9	-1,84

(Santa Lucia, PNAS, 1995)

## Example : GCTAGC

$$\Delta H_{\text{total}} = \Delta H_{\text{initiation}} + \Delta H_{\text{symétrie}} + \Delta H_{\text{GC}} + \Delta H_{\text{CT}} + \Delta H_{\text{TA}} + \Delta H_{\text{AG}} + \Delta H_{\text{GC}}$$

$$\Delta H_{\text{total}} = 0.1 + 0 - 9.8 - 7.8 - 7.2 - 7.8 - 9.8 = - 42.3 \text{ kcal.mol}^{-1}$$

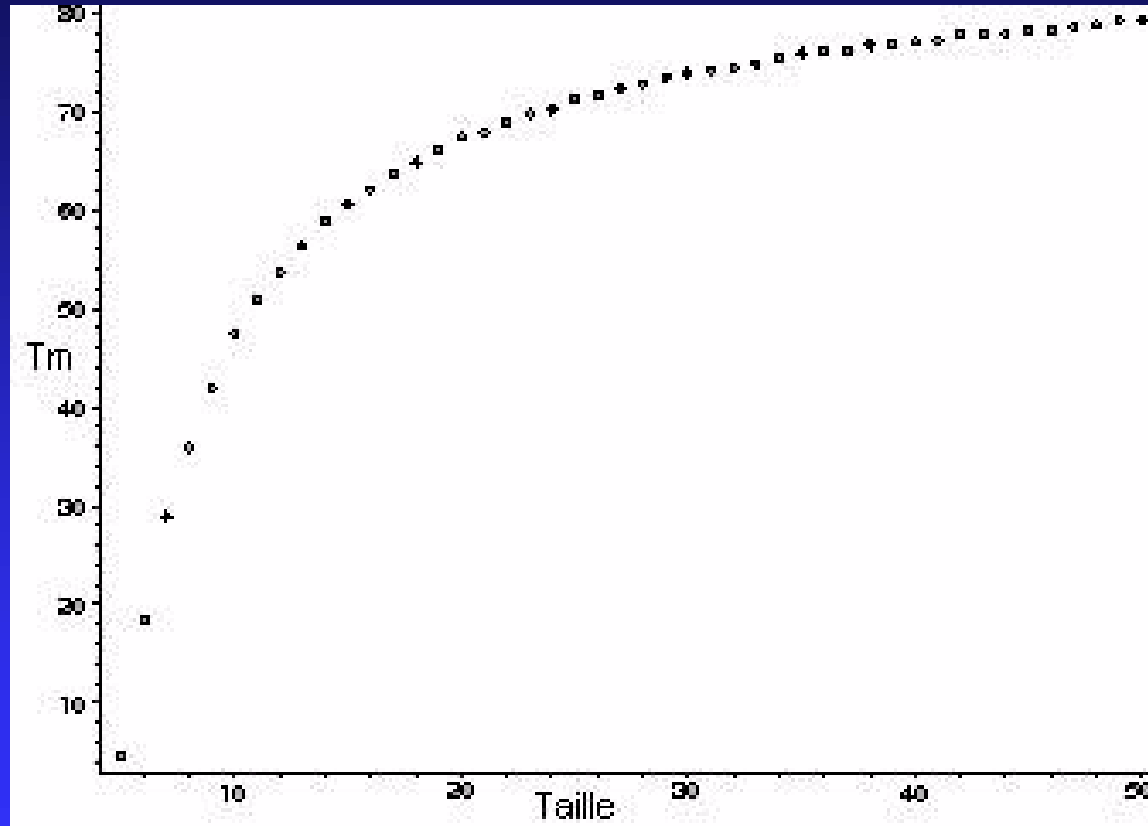
$$\Delta S_{\text{total}} = \Delta S_{\text{initiation}} + \Delta S_{\text{symétrie}} + \Delta S_{\text{GC}} + \Delta S_{\text{CT}} + \Delta S_{\text{TA}} + \Delta S_{\text{AG}} + \Delta S_{\text{GC}}$$

$$\Delta S_{\text{total}} = -2.8 - 1.4 - 24.4 - 21 - 21.3 - 21 - 24.4 = - 116.3 \text{ cal.mol}^{-1}$$

$$T_m = \frac{- 42300}{- 116.3 + 1.987 \ln 10^{-6}} + 16.6 \log \left( \frac{[1]}{1 + 0.7[1]} \right) - 273.15 = 28.7 \text{ } ^\circ\text{C}$$

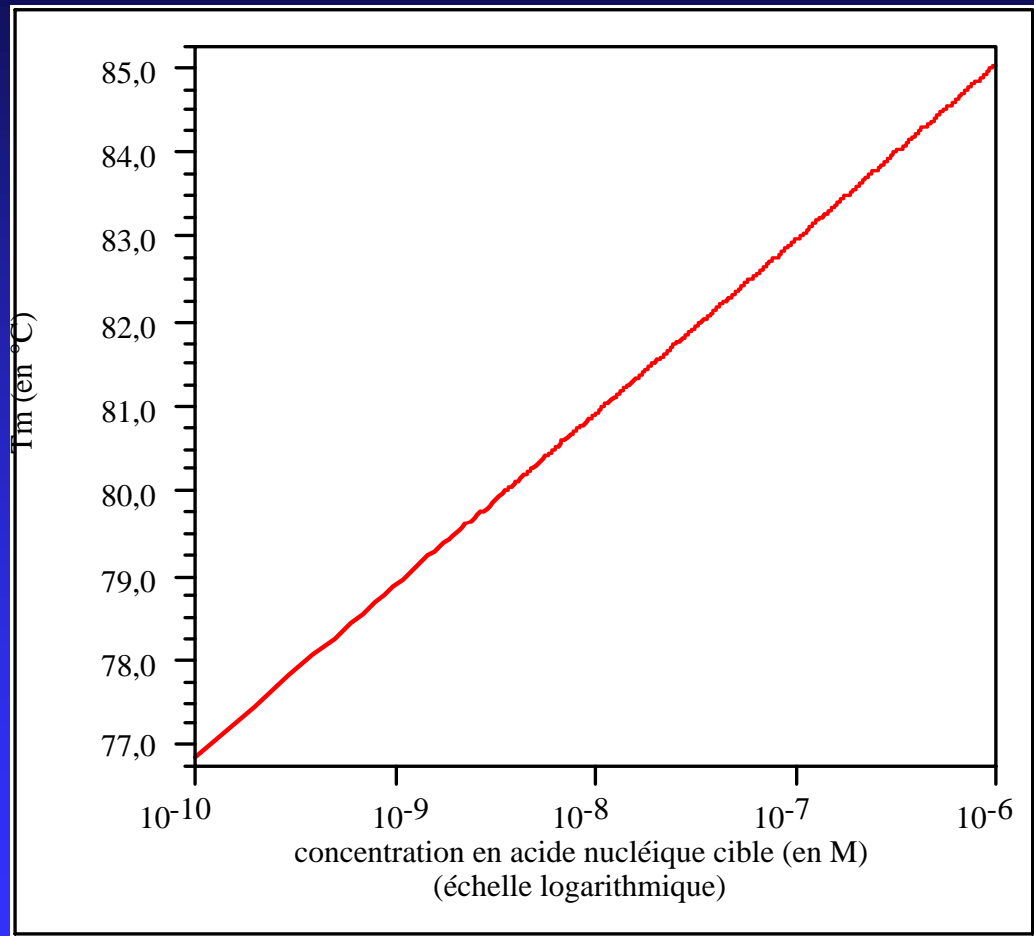
Mismatch can be considered in the formula but the table are not all published

$T_m$  (hybridization rate) is function of the probe size



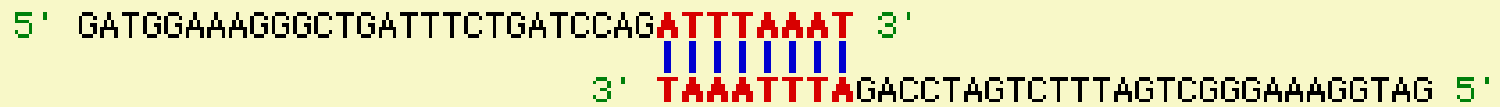
--> compromise between  $T_m$  and specificity

$T_m$  is function of DNA concentration

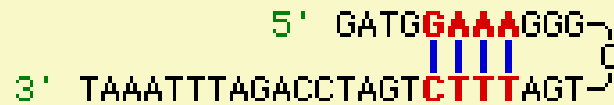


Two kinds of secondary structures:

Homoduplex



Hairpin



--> Stable secondary structures decrease hybridization rates

## A reference software: MFOLD

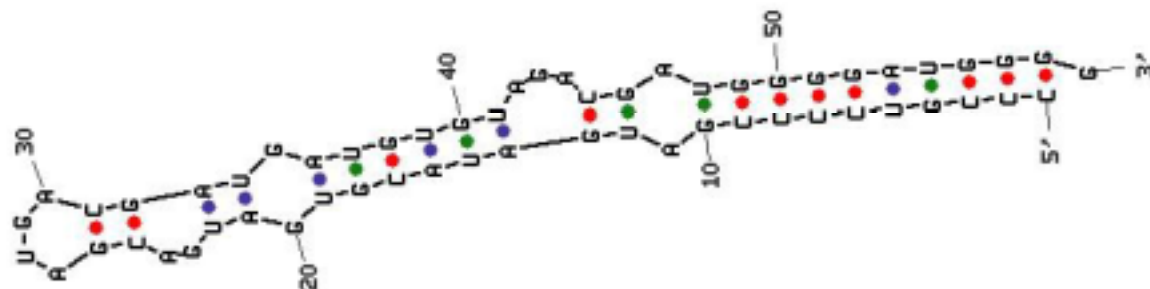
(<http://bioinfo.math.rpi.edu/~mfold/rna/form1.cgi>)

### MFOLD : Prediction of RNA secondary structure ([M. Zuker](#))

Sequence File (SEQ) ([format](#))

Sequence type (default = linear) (LC) ?  [default]  
RNA (default) or DNA (NA) ?  [default]  RNA

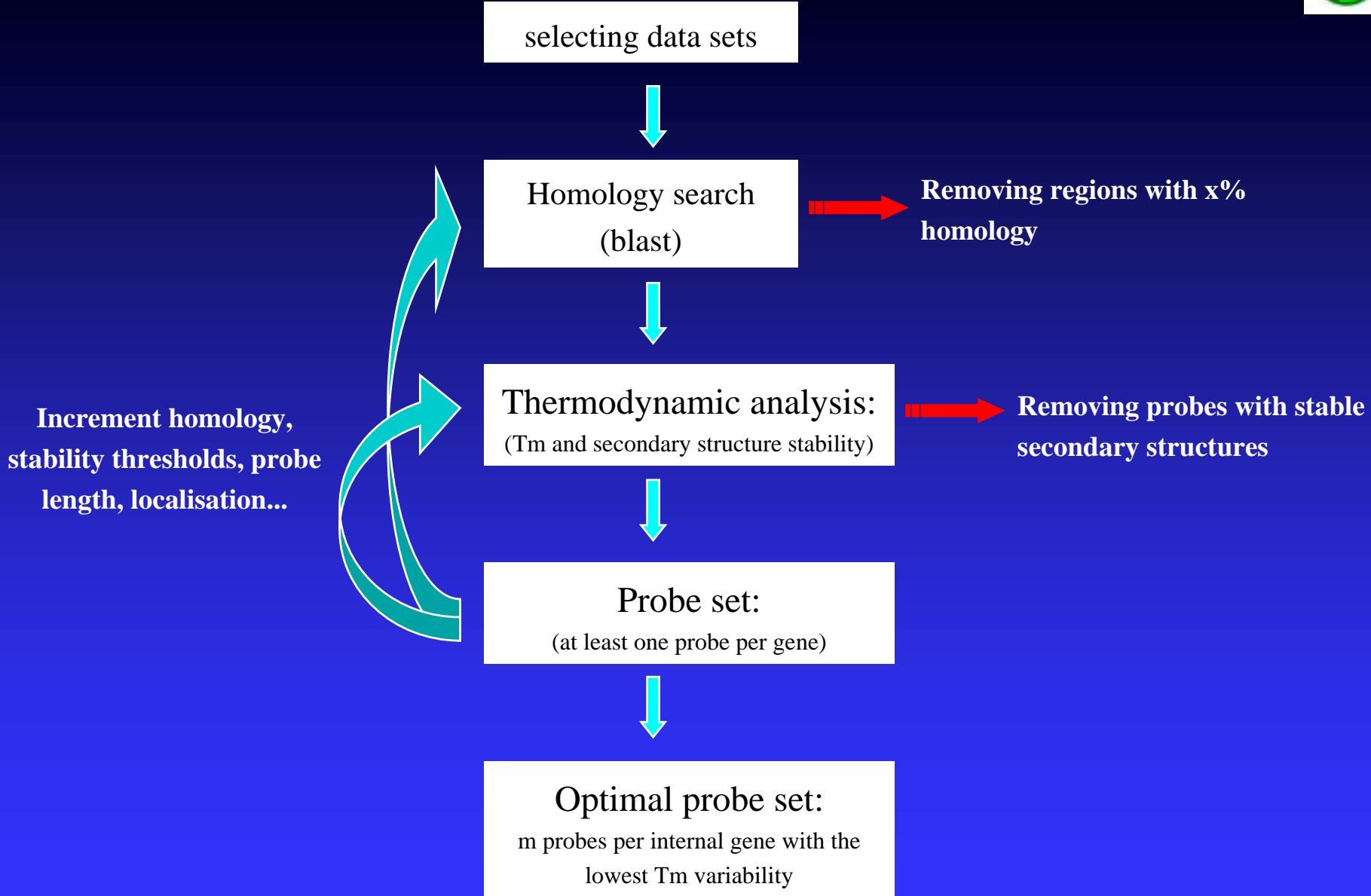
str\_graph by D. Stewart and M. Zuker  
© 2002 Washington University

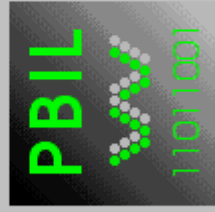


dG = -23.9 [initially -23.9] 02Mar27-13-15-5



- The formation free energy for the two distal pentamers has a key role in hybridization « zippering effect », probes with G or C at both ends are more stable.
- probe GC contents should not be outside 40-65% GC.
- motif such as  $N_4$  or GGG and CCC should be avoided.





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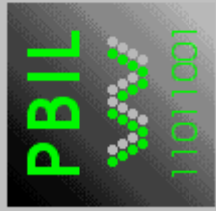
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a software to design optimized oligonucleotide probes  
for microarrays

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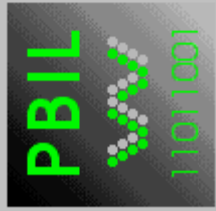
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## STEPS TO PROCESS

-  Please [load your FASTA files](#)
-  *This file will be used to run ELAST, and produce input files for ROSO.*
-  Change ROSO configuration
-  *ROSO allows you to set some parameters.*
-  Run ROSO.
-  *Depending on your input files, the process can take more or less time.*



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### Choice of input files

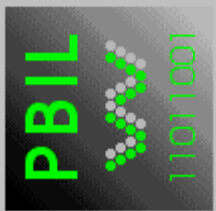
*WARNING: The software require FASTA formatted files.*

[Learn more about selecting and loading input files](#)

- Name of file with interest sequences:
- Name of database with external sequences:  or personal file with external sequences:
- Interest sequences present in the external sequences file ?  yes  no



The screenshot shows the ROSO web application interface. At the top left is the PBIL logo with the text 'PBIL' in green, a stylized 'W' made of dots, and '11011001' below it. To the right is the ROSO logo, a green circle with 'rOs' inside. Below the ROSO logo is the text 'ROSO a friend for your design' and 'BBE contribution to [PBIL](#) in Lyon, France'. A navigation menu contains buttons for HOME, ARTICLES, PRINCIPLES, STATISTICS, USE ROSO (highlighted in green), CREDENTIALS, COMMENTS, and HELP. Below the menu is the heading 'STEPS TO PROCESS' followed by a green circular icon with a refresh symbol and the text 'FASTA FILES SUCCESSFULLY LOADED'. Underneath is a link: 'Now you have to set [ROSO configuration](#).' followed by the text 'Run ROSO.' At the bottom right, there are three small circular icons: a refresh icon, a checkmark icon, and a network icon. The footer text reads: 'Laboratory of Functional Ecology Insects and Interactions (EFEI) Copyright-Roso Development Team 2012'.



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## Define the settings

[Learn more about the configuration](#)

### Research settings

- Bunch of four identical nucleotides in the probes allowed ? yes  no
- Overlaps between probes allowed ? yes  no
- Number of probes expected :  *(nucleotides must be upper than 1)*

### Probes settings

- EST interest sequences ? yes  no
- Probe type: identity  reverse
- Probe size:  *(nucleotides must be upper than 32)*

### Solution concentrations

- K+ concentration:  M
- Na+ concentration:  M
- Target concentration:  M



**Hybridization temperature**

- Hybridization temperature:  °C
- Melting temperature range for probes: Tm minimum:  °C and Tm maximum:  °C

**Threshold of secondary structures**

- Hairpin:  kcal/mol
- Homoduplex:  kcal/mol

**Selected area research**

- Selected area research ? Total  Reduced area
- Beginning on ? 5' end  3' end
- Research area size:  nucleotides

---

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Ralstonia.xls												
A	B	C	D	E	F	G	H	I	J	K	L	M
58	1788	runB1	acps-RS04136_AA-1119910-1120323	CCATTTATGCGATCGGACCGACATTCATCCAGATGAGGGCGTGGAGAGGGGTGA'	10	60.00%	95.27	83%	-2.17	-5.62		
59	3307	runB2	aerA-RS01833_AA-12817-11624	DGACGAGG6TCCGGTGTGGTACCGTGTGACCGCCACCTCCGGTGGGGCTGACACCGA	109	72.31%	100.32	69%	-5.33	-11.05		
60	7485	runA3	aerA-RS01833_AA-12817-11624	DGTGGCAGCGATTGGATGGTCTCGACGGGGCTGAAGCGGGTGAACAGGGTGAATC	1028	64.29%	97.03	82%	-6.07	-10.87		
61	3306	runB2	aerB-RS01832_AA-11595-8449	CTTGGCAGGTTTCAACGCGATGTTTCAACGAGACACGACCGTACCAGAGCCGTC	1535	64.62%	97.17	60%	-4.23	-7.07		
62	6208	runA2	aerB-RS01832_AA-11595-8449	GAGCGTGGTACTTCAAGGTGGCGTGAATGGGGTGGTGGGCTGTGGCGCAAGACG	2760	61.43%	95.86	80%	-5.17	-5.46		
63	12	runA1	aerR-RS01834_AA-13346-14002	CAACAGTGGGAGTGTGGTGGAGGACAGAGCCGATCCCTCAAGCGCCAGCAGGA	338	62.86%	96.45	70%	-3.72	-5.62		
64	1413	runB1	aerR-RS01834_AA-13346-14002	CAACAGTGGGAGTGTGGTGGAGGACAGAGCCGATCCCTCAAGCGCCAGCAGGA	338	62.86%	96.45	70%	-3.72	-5.62		
65	4697	runB2	ada-RS00768_AA-2778230-2779309	CAACAGTGGGAGTGTGGTGGAGGACAGAGCCGATCCCTCAAGCGCCAGCAGGA	702	63.08%	96.54	83%	-5.68	-6.54		
66	8587	runA3	ada-RS00768_AA-2778230-2779309	CAACAGTGGGAGTGTGGTGGAGGACAGAGCCGATCCCTCAAGCGCCAGCAGGA	702	63.08%	96.54	83%	-5.68	-6.54		
67	3062	runA1	ado-RS02395_AA-1340565-1341305	CACGAGCAGGTTCCCGAAGCGCCAGCAGTGAATGGGGTGGTGGGCGGAT	696	62.86%	96.45	83%	-9.43	-10.49		
68	9346	runA3	ado-RS02395_AA-1340565-1341305	CACGAGCAGGTTCCCGAAGCGCCAGCAGTGAATGGGGTGGTGGGCGGAT	696	62.86%	96.45	83%	-9.43	-10.49		
69	4441	runB2	add-RS03663_AA-2272333-2273367	ATGATACCGTCCGCAAGACAGTCCCTTGGCGAGCGTGTGGCGCAACACATGGAGAC	522	64.29%	97.03	83%	-5.87	-11.21		
70	6683	runA2	add-RS03663_AA-2272333-2273367	ATGATACCGTCCGCAAGACAGTCCCTTGGCGAGCGTGTGGCGCAACACATGGAGAC	522	64.29%	97.03	83%	-5.87	-11.21		
71	4990	runB2	adhA-RS00473_AA-3376654-3377676	CACGCGCAAGACCGAGCTGCACATCCACATGACAGTGGTGGTGGAGTGGAGT	38	61.54%	95.91	70%	-2.39	-6.64		
72	8814	runA3	adhA-RS00473_AA-3376654-3377676	CACGCGCAAGACCGAGCTGCACATCCACATGACAGTGGTGGTGGAGTGGAGT	38	61.54%	95.91	70%	-2.39	-6.64		
73	9987	runA5	adhC1-RS02044_AA-79046-80149	GCATCTGCCATACCGACTACTACAGCGTCTGGGGGGGACCGCGAAGGACTCTT	300	64.62%	97.17	70%	-2.52	-6.28		
74	6199	runB5	adhC1-RS02044_AA-79046-80149	GCATCTGCCATACCGACTACTACAGCGTCTGGGGGGGACCGCGAAGGACTCTT	300	64.62%	97.17	70%	-2.52	-6.28		
75	980	runA1	adhC1-RS02044_AA-79046-80149	GCATCTGCCATACCGACTACTACAGCGTCTGGGGGGGACCGCGAAGGACTCTT	300	64.62%	97.17	70%	-2.52	-6.28		
76	2739	runB1	adhC1-RS02044_AA-79046-80149	GCATCTGCCATACCGACTACTACAGCGTCTGGGGGGGACCGCGAAGGACTCTT	300	64.62%	97.17	70%	-2.52	-6.28		
77	6174	runB4	adhC1-RS02044_AA-79046-80149	GCATCTGCCATACCGACTACTACAGCGTCTGGGGGGGACCGCGAAGGACTCTT	300	64.62%	97.17	70%	-2.52	-6.28		
78	7429	runA2	adhC1-RS02044_AA-79046-80149	GCATCTGCCATACCGACTACTACAGCGTCTGGGGGGGACCGCGAAGGACTCTT	300	64.62%	97.17	70%	-2.52	-6.28		
79	9890	runA4	adhC1-RS02044_AA-79046-80149	GCATCTGCCATACCGACTACTACAGCGTCTGGGGGGGACCGCGAAGGACTCTT	300	64.62%	97.17	70%	-2.52	-6.28		
80	6025	runB2	adhC1-RS02044_AA-79046-80149	GCATCTGCCATACCGACTACTACAGCGTCTGGGGGGGACCGCGAAGGACTCTT	300	64.62%	97.17	70%	-2.52	-6.28		
81	6120	runB3	adhC1-RS02044_AA-79046-80149	GCATCTGCCATACCGACTACTACAGCGTCTGGGGGGGACCGCGAAGGACTCTT	300	64.62%	97.17	70%	-2.52	-6.28		
82	9746	runA3	adhC1-RS02044_AA-79046-80149	GCATCTGCCATACCGACTACTACAGCGTCTGGGGGGGACCGCGAAGGACTCTT	300	64.62%	97.17	70%	-2.52	-6.28		
83	3662	runB2	adhC2-RS04838_AA-652505-651402	GGCGGGGCGCACCGACGTCGCGCAAGATTCGTCAGCTGGTACATGGAAGGCAAGGCTT	947	63.08%	96.54	92%	-3.22	-7.72		
84	9958	runA5	adhC2-RS04838_AA-652505-651402	GGCGGGGCGCACCGACGTCGCGCAAGATTCGTCAGCTGGTACATGGAAGGCAAGGCTT	947	63.08%	96.54	92%	-3.22	-7.72		
85	6181	runB5	adhC2-RS04838_AA-652505-651402	GGCGGGGCGCACCGACGTCGCGCAAGATTCGTCAGCTGGTACATGGAAGGCAAGGCTT	947	63.08%	96.54	92%	-3.22	-7.72		
86	6134	runB4	adhC2-RS04838_AA-652505-651402	GGCGGGGCGCACCGACGTCGCGCAAGATTCGTCAGCTGGTACATGGAAGGCAAGGCTT	947	63.08%	96.54	92%	-3.22	-7.72		
87	6351	runA2	adhC2-RS04838_AA-652505-651402	GGCGGGGCGCACCGACGTCGCGCAAGATTCGTCAGCTGGTACATGGAAGGCAAGGCTT	947	63.08%	96.54	92%	-3.22	-7.72		
88	9602	runA3	adhC2-RS04838_AA-652505-651402	GGCGGGGCGCACCGACGTCGCGCAAGATTCGTCAGCTGGTACATGGAAGGCAAGGCTT	947	63.08%	96.54	92%	-3.22	-7.72		
89	9852	runA4	adhC2-RS04838_AA-652505-651402	GGCGGGGCGCACCGACGTCGCGCAAGATTCGTCAGCTGGTACATGGAAGGCAAGGCTT	947	63.08%	96.54	92%	-3.22	-7.72		
90	6071	runB3	adhC2-RS04838_AA-652505-651402	GGCGGGGCGCACCGACGTCGCGCAAGATTCGTCAGCTGGTACATGGAAGGCAAGGCTT	947	63.08%	96.54	92%	-3.22	-7.72		
91	656	runA1	adh-RS01181_AA-2570516-2568240	CGAGACCGATATCCACGGGCTGGTGGAGGAGAGATTGGGGTGGGTTGGGTA	2204	60.00%	95.27	60%	-2.48	-5.87		
92	2313	runB1	adh-RS01181_AA-2570516-2568240	CGAGACCGATATCCACGGGCTGGTGGAGGAGAGATTGGGGTGGGTTGGGTA	2204	60.00%	95.27	60%	-2.48	-5.87		
93	4669	runB2	adh-RS05765_AA-2740594-2741259	CGAGACCGATATCCACGGGCTGGTGGAGGAGAGATTGGGGTGGGTTGGGTA	385	64.62%	97.17	70%	-5.32	-8.58		
94	6759	runA2	adh-RS05765_AA-2740594-2741259	CGAGACCGATATCCACGGGCTGGTGGAGGAGAGATTGGGGTGGGTTGGGTA	385	64.62%	97.17	70%	-5.32	-8.58		
95	1274	runA1	aer-RS03168_AA-1559762-1558221	CGAGACCGATATCCACGGGCTGGTGGAGGAGAGATTGGGGTGGGTTGGGTA	476	61.43%	95.86	75%	-6.27	-4.86		
96	7473	runA2	aer-RS03168_AA-1559762-1558221	CGAGACCGATATCCACGGGCTGGTGGAGGAGAGATTGGGGTGGGTTGGGTA	476	61.43%	95.86	75%	-6.27	-4.86		
97	3128	runB1	aer-RS03168_AA-1559762-1558221	CGAGACCGATATCCACGGGCTGGTGGAGGAGAGATTGGGGTGGGTTGGGTA	1409	65.71%	97.62	81%	-3.01	-5.34		
98	8384	runA3	aga2-RS01458_AA-2329279-2327927	CGAGACCGATATCCACGGGCTGGTGGAGGAGAGATTGGGGTGGGTTGGGTA	1409	65.71%	97.62	81%	-3.01	-5.34		
99	4462	runB2	aga2-RS01458_AA-2329279-2327927	CGAGACCGATATCCACGGGCTGGTGGAGGAGAGATTGGGGTGGGTTGGGTA	1280	68.57%	98.79	60%	-7.11	-16.21		
100	1438	runB1	ahcY-RS02285_AA-107458-106037	CGAGACCGATATCCACGGGCTGGTGGAGGAGAGATTGGGGTGGGTTGGGTA	1279	69.23%	99.06	60%	-7.11	-16.21		
101	7284	runA2	ahcY-RS02285_AA-107458-106037	CGAGACCGATATCCACGGGCTGGTGGAGGAGAGATTGGGGTGGGTTGGGTA	633	57.14%	94.10	71%	-2.49	-5.87		
102	30	runA1	ahcY-RS02285_AA-107458-106037	CGAGACCGATATCCACGGGCTGGTGGAGGAGAGATTGGGGTGGGTTGGGTA	986	57.14%	94.10	78%	-5.20	-5.22		
103	3374	runB2	ahb-RS00391_AA-142057-143727	CGAGACCGATATCCACGGGCTGGTGGAGGAGAGATTGGGGTGGGTTGGGTA	1020	57.14%	94.10	85%	-3.97	-5.22		
104	3625	runA2	ahb-RS00391_AA-142057-143727	CGAGACCGATATCCACGGGCTGGTGGAGGAGAGATTGGGGTGGGTTGGGTA	3	63.08%	96.54	70%	-2.50	-6.26		
				CGAGACCGATATCCACGGGCTGGTGGAGGAGAGATTGGGGTGGGTTGGGTA	1271	64.29%	97.03	79%	-4.35	-10.65		



- **T<sub>m</sub>**

<http://www.cbs.dtu.dk/services/DNAarray/probewiz.html>

<http://www.nwfsc.noaa.gov/protocols/oligoTmcalc.html>

<http://www.anachem.co.uk/public/new-products/hybsimulator/default.asp>

[http://www-genome.wi.mit.edu/cgi-bin/primer/primer3\\_www.cgi](http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi)

<http://bioweb.pasteur.fr/seqanal/interfaces/melting.html>

- **probe and primer design:**

<http://berry.engin.umich.edu/oligoarray/index.html> (JM Rouillard)

<http://arrayit.com/Services/ArrayDesign/arraydesign.html>

<http://ural.wustl.edu/~lif/probe.pl>

<http://www.labvelocity.com/jellyfish/index.jhtml>

- **Secondary structures:**

<http://bioinfo.math.rpi.edu/~zukerm/>



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