

# Study of *merA* Gene in Gram-negative Bacteria from Brazilian Aquatic Systems as a First Step to Select Microorganisms to Bioremediate Mercury Pollution

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# STATE OF THE ART

- Studies about trace metal pollution is a priority in National Health Programs.
- Mercury is considered the most toxic among trace metals

Neustadt & Pieczenik, 2007



INC 1, 7 - 11 June 2010, Stockholm, Sweden

INC 2, 24 - 28 January 2011, Chiba City, Japan

INC 3, 31 October - 4 November 2011, Nairobi, Kenya,

INC 4, 27 June - 2 July 2012, Punta del Este, Uruguay,

INC 5, 13 - 19 January 2013, Geneva, Switzerland

# MERCURY(Hg)

- It is present in several forms:

metallic Hg

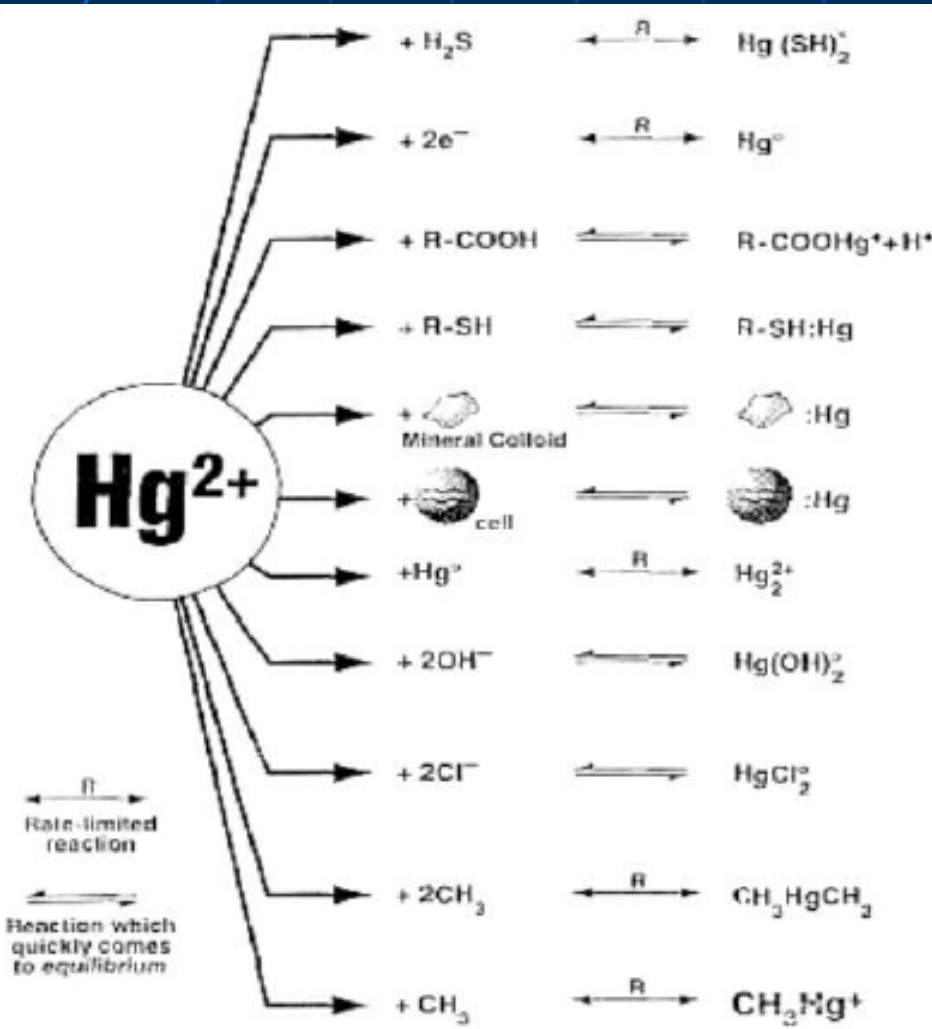
organic Hg

inorganic Hg

- It can be founded in three different oxidation states that are easily interconverted in the environment:

0, +1, +2

# MERCURY(Hg)



≠ chemical species



≠ physical-chemical property



≠ toxicity

Organomercury

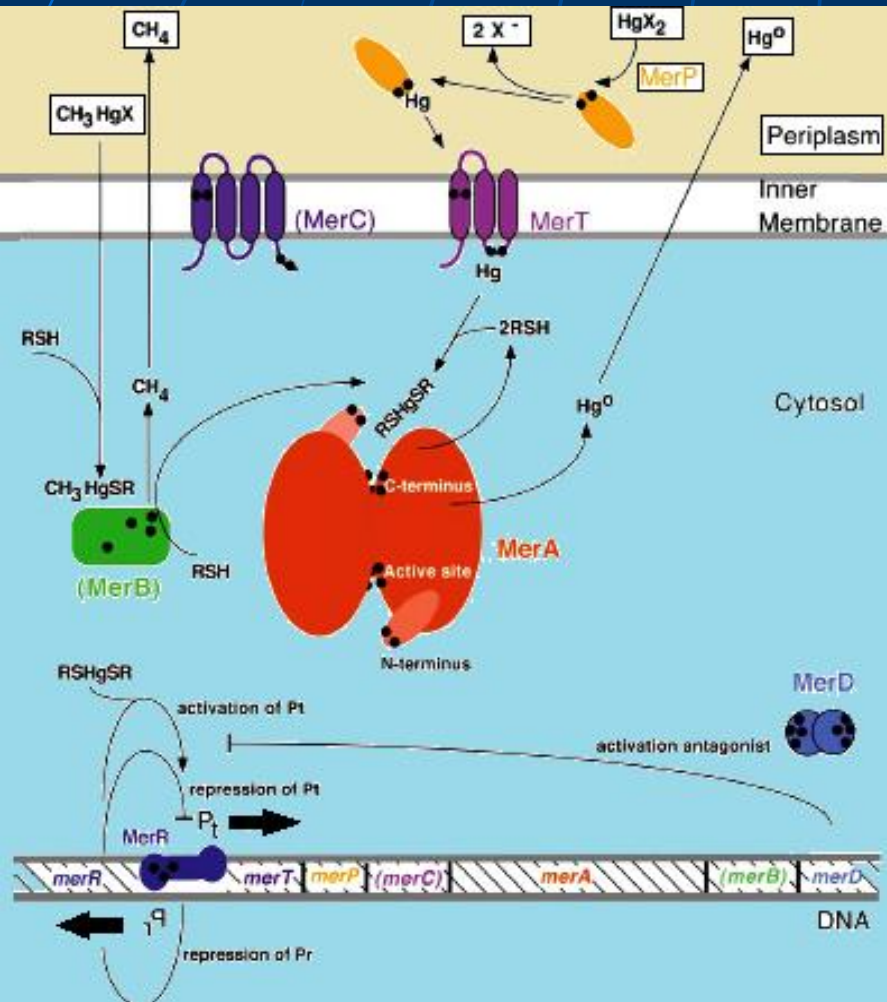


> toxicity

# EXAMPLE: Minamata Disaster



# MERCURY(Hg)



Resistance= Hg conversion



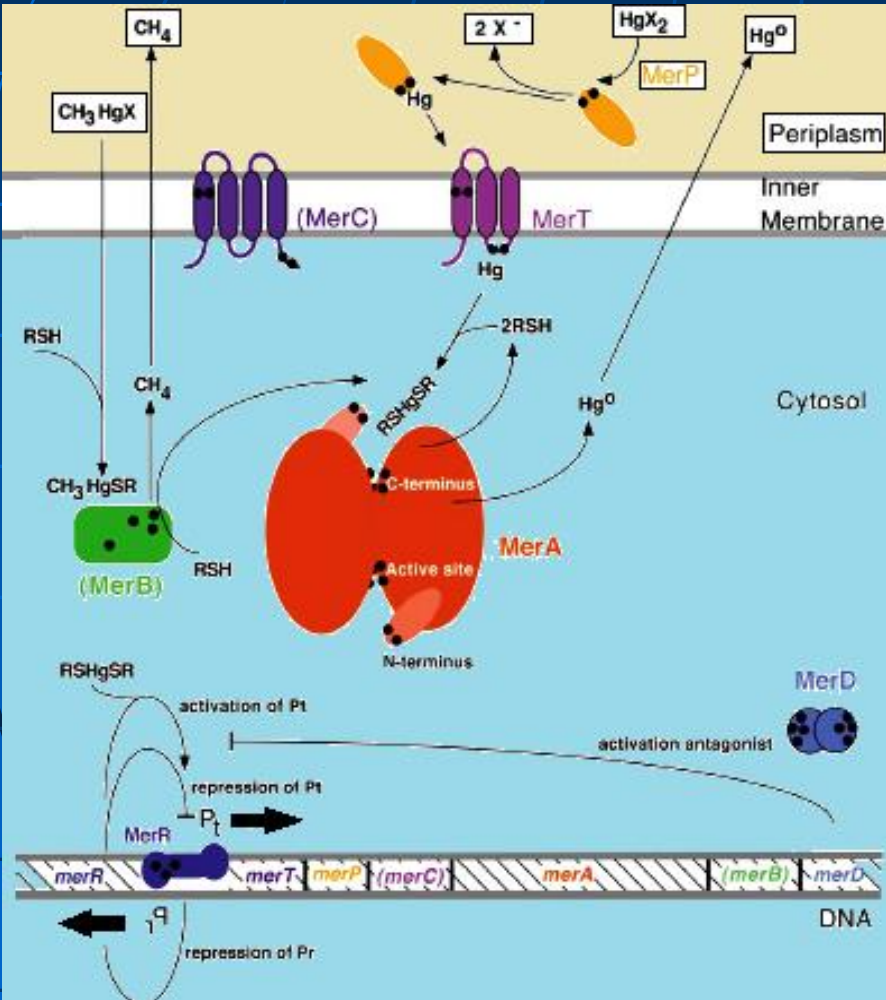
mer operon



Narrow  
(*merA*)

Broad  
(*merA+merB*)

# MERCURY(Hg)



**MerA**  
mercuric ion reductase

**MerR** metal-responsive regulatory protein

**MerT** transport

**MerB** Organomercuriolyase

**MerC** secondary transport

**MerD** activation antagonist

**MerP** periplasmatic protein

# STUDY'S RELEVANCE

- Mercury is an important global pollutant.
- Mercury biogeochemical cycle has been altered by anthropogenic emissions.
- Methylmercury is biomagnified through food chain, reaching high levels at the top.
- Bacteria are capable of converting Hg to less toxic form and can be used to reduce Hg emissions to the environment.



# OBJECTIVE

Identify *merA* gene in phenotypically Hg resistant bacteria from “*Bacteria Collection Resistant to Environmental Pollutants*” from the Environmental Health and Sanitation Department, National School Of Public Health – Oswaldo Cruz Foundation

# METHODOLOGY

**Sampling and Isolation,**

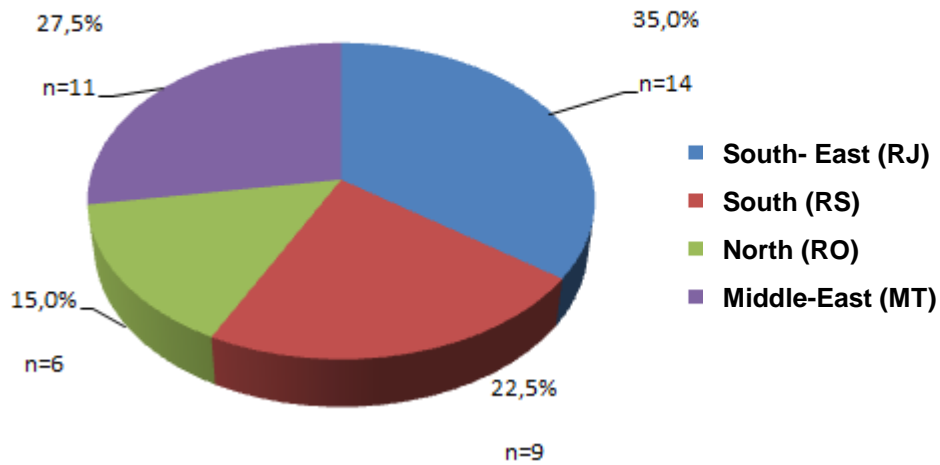
**Determination of Hg's Phenotypic Resistance Level ( $MIC_{Hg}$ ),**

**Biochemical Identification and Strains Storage**

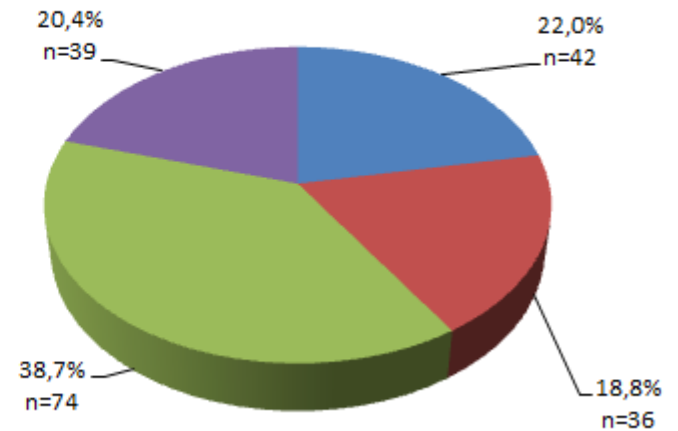
**were previously performed by the research group**



### Water Sampling



### Bacterial Isolates



# METHODOLOGY

## Bacterial Samples' Selection

Highest Hg resistant Gram-negative Strains ( $MIC_{Hg} \geq 4 \text{ mg Hg L}^{-1}$ )

## DNA Extraction

Sambrook *et al.*(1989)

## *merA* Gene's Detection (PCR Method)

Primers:

A1 Forward 5'- ACCATCGGCGGCACCTGCGT-3'

A5 Reverse 5'- TTGGTCCCCTACCTGACGATGGT-3'

Liebert *et al.*,1997

## PCR Mix:

100 ng A1 + 100 ng A5,  
0,05 mM of each dNTP,  
3 mM MgCl<sub>2</sub>,  
1,5 U Taq Pol (Promega<sup>®</sup>, USA),  
final volume 50 µL

## Amplifications Condition:

1 cycle 95°C/5',  
39 cycles  
(94°C/30''+64°C/30''+72°C/1'),  
1 cycle 72°C/10'

# METHODOLOGY

## DNA Sequencing

### Chain Termination Method

Sanger, 1977

### DNA Sequencing Platform PDTIS/FIOCRUZ-RJ

([http://plataformas.cdts.fiocruz.br/subunidade/exibe\\_sub/1](http://plataformas.cdts.fiocruz.br/subunidade/exibe_sub/1))

### Primers:

A1 Forward 5'- ACC ATC GGC GGC ACC TGC GT-3';

A5 Reverse 5'-TTG GTC CCC TAC CTG ACG ATG GT-3';

Liebert *et al.*, 1997

*merABR F* 5'-ACA TTC CCG AAC GCC TTG CAG TAA- 3'

*merABR R* 5'-TTA CTG CAA GGC GTT CGG GAA TGA-3'

De Falco, 2013

1pb

1234pb



A5

1<sup>st</sup> step: Sequencing using A5 primer



false positive screening

A1



*merABR F*



1pb

411pb

1234pb



*merABR R*



A5

2<sup>nd</sup> step: Sequencing using A1, *merABR F* and *merABR R* primers



Efficient complete sequencing

# METHODOLOGY

## Analyses of obtained sequences

BioEdit Program       Analyze Sequences Chromatograms  
BLAST Program       Identify Sequences as *merA*

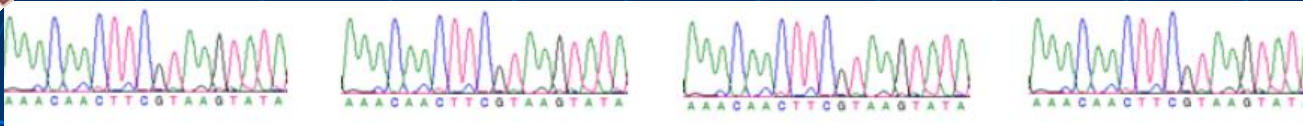
## Multiple Align of Partial *merA* Genes Detected on the Study

T-COFFEE Multiple Alignment Program

## Phylogenetic Tree Design and Analysis

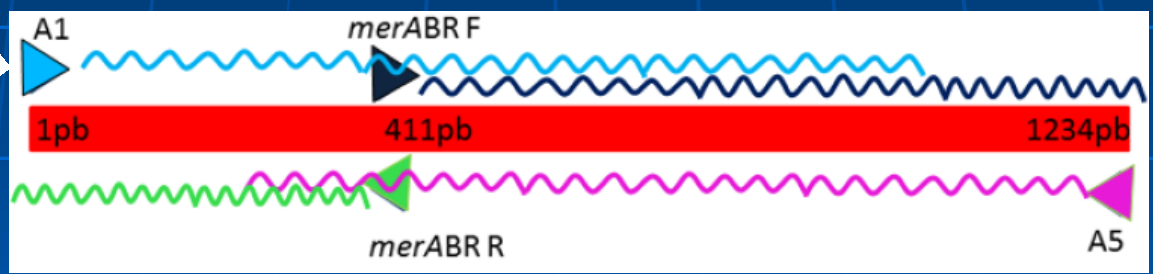
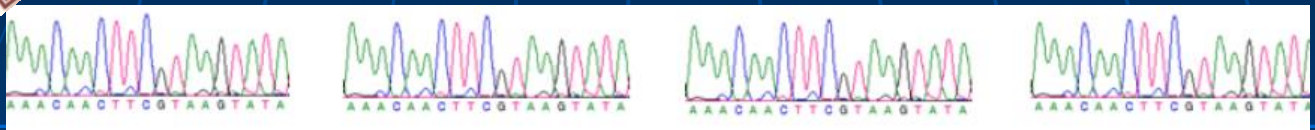
CLUSTAL-W Program

# FRAGMENT CONSTRUCTION

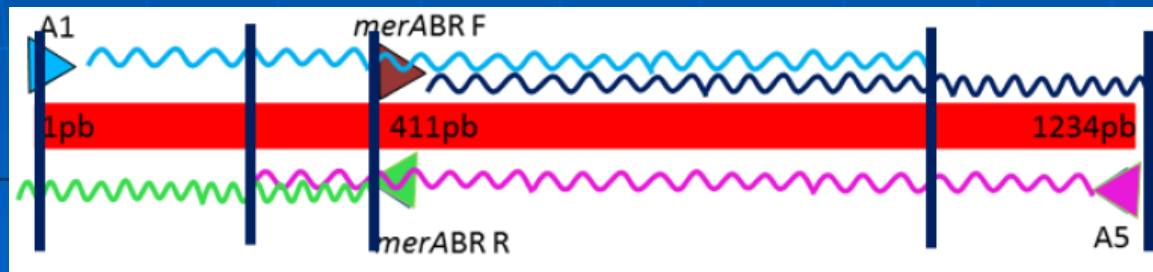
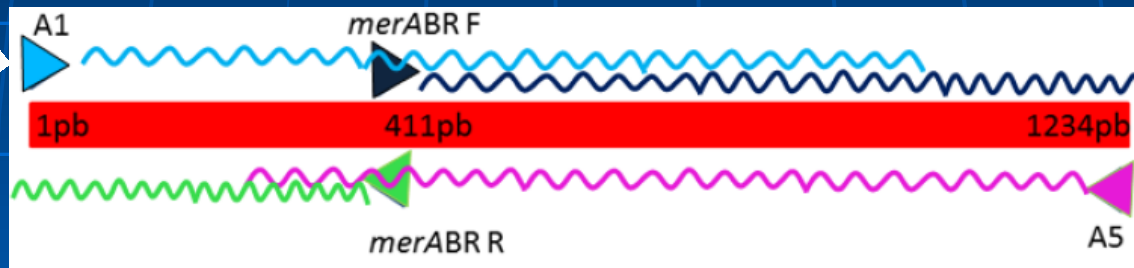
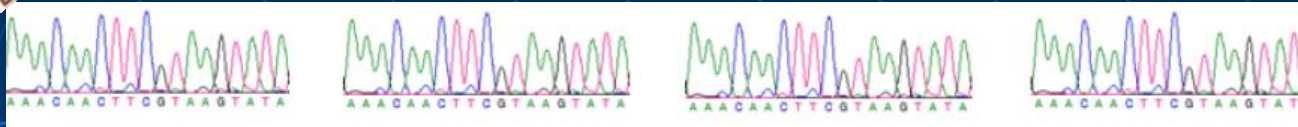
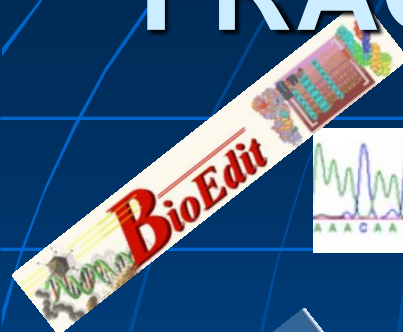




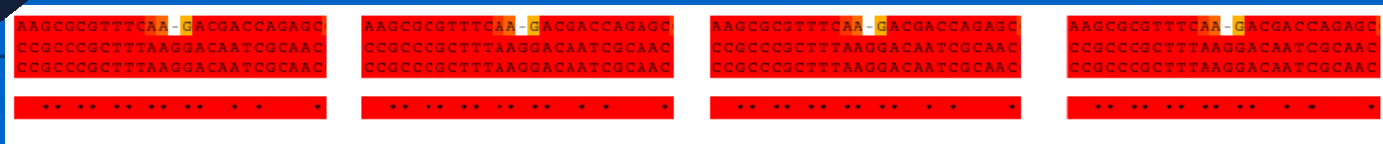
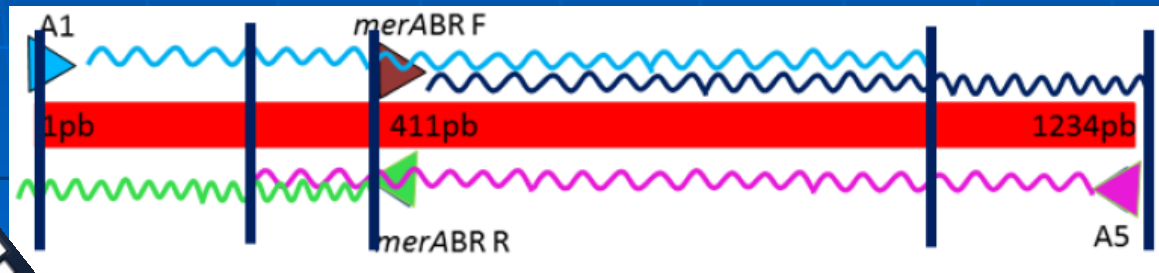
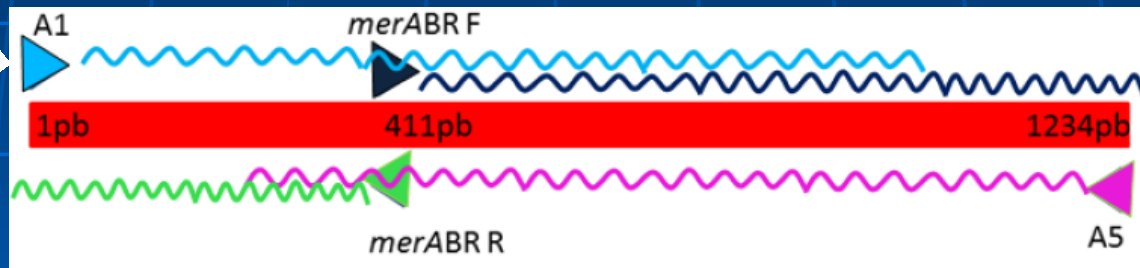
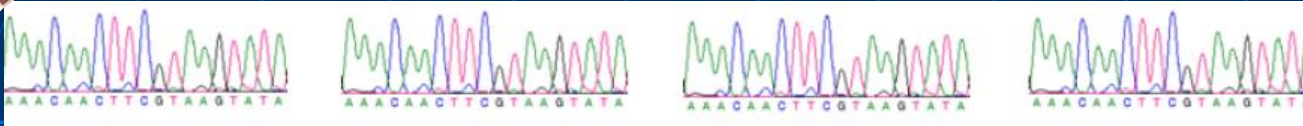
# FRAGMENT CONSTRUCTION



# FRAGMENT CONSTRUCTION



# FRAGMENT CONSTRUCTION



# FRAGMENT CONSTRUCTION

AAGCGCGTTTCAA-GACGACCAGAGC CCGCCCGCTTTAAGGACAATCGCAAC CCGCCCGCTTTAAGGACAATCGCAAC	AAGCGCGTTTCAA-GACGACCAGAGC CCGCCCGCTTTAAGGACAATCGCAAC CCGCCCGCTTTAAGGACAATCGCAAC	AAGCGCGTTTCAA-GACGACCAGAGC CCGCCCGCTTTAAGGACAATCGCAAC CCGCCCGCTTTAAGGACAATCGCAAC	AAGCGCGTTTCAA-GACGACCAGAGC CCGCCCGCTTTAAGGACAATCGCAAC CCGCCCGCTTTAAGGACAATCGCAAC
.. * * * * *	.. * * * * *	.. * * * * *	.. * * * * *



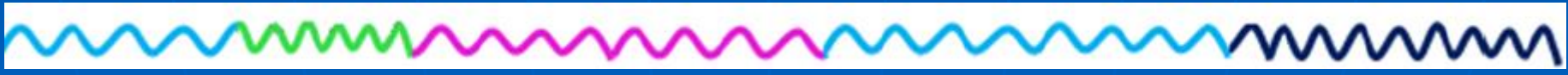
..AAACAAC TTCGTAAGTATA ... + ...AAACAAC TTCGTAAGTATA ... + ...AAACAAC TTCGTAAGTATA ... + ...AAACAAC TTCGTAAGTATA ..

# FRAGMENT CONSTRUCTION

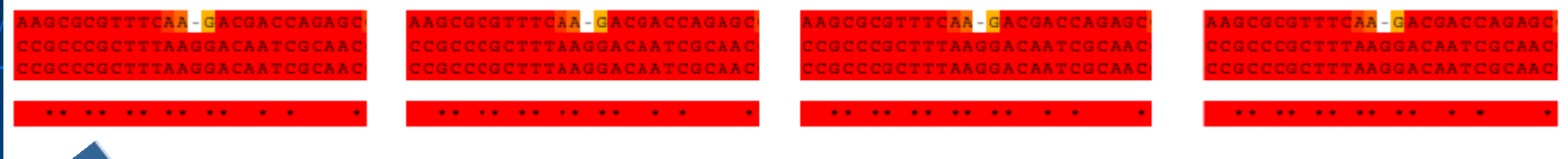
AAGCGCGTTTCAA-GACGACCAGAGC CCGCCCCGCTTTAAGGACAATCGCAAC CCGCCCCGCTTTAAGGACAATCGCAAC	AAGCGCGTTTCAA-GACGACCAGAGC CCGCCCCGCTTTAAGGACAATCGCAAC CCGCCCCGCTTTAAGGACAATCGCAAC	AAGCGCGTTTCAA-GACGACCAGAGC CCGCCCCGCTTTAAGGACAATCGCAAC CCGCCCCGCTTTAAGGACAATCGCAAC	AAGCGCGTTTCAA-GACGACCAGAGC CCGCCCCGCTTTAAGGACAATCGCAAC CCGCCCCGCTTTAAGGACAATCGCAAC
.. * * * * *	.. * * * * *	.. * * * * *	.. * * * * *



...AAACAAC TTCGTAAGTATA ... + ...AAACAAC TTCGTAAGTATA ... + ...AAACAAC TTCGTAAGTATA ... + ...AAACAAC TTCGTAAGTATA ..



# FRAGMENT CONSTRUCTION



# RESULTS & DISCUSSION

Sampling: 191 Bacterial Strains

Selection: 150 Gram-negative Bacterial Strains,  $\text{MIC}_{\text{Hg}} \geq 4 \text{ mg L}^{-1}$

Growth: 125 Bacterial Strains

Pure Isolated Strains: 110 Bacterial Strains in Accordance to Required Conditions

*merA* gene Detection: 69 Bacterial Strains (62,7%)

# RESULTS & DISCUSSION

## *merA* Gene Sequencing Results:

1<sup>st</sup> step: Sequencing using A5 primer



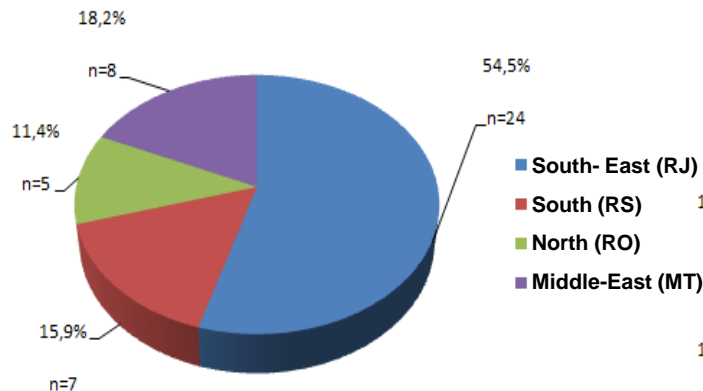
60 sequences

2<sup>nd</sup> step: Sequencing using A1, *merABR* F and *merABR* R primers

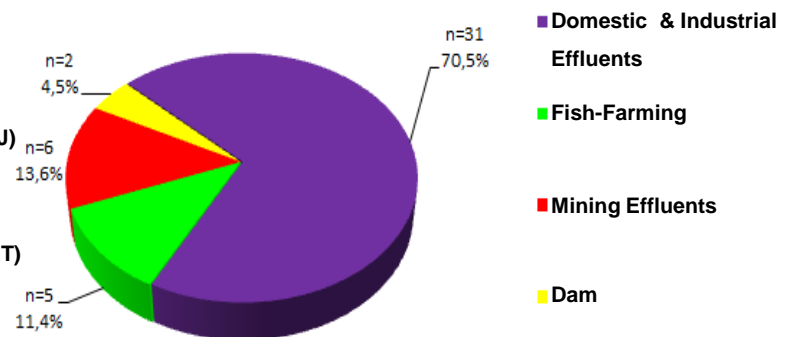


44 sequences (1115 bp, compared with 1234 bp target)

Sequences Classified by Geographic Origin



Sequences Classified by Sampling Point





# RESULTS & DISCUSSION

## *merA* Multiple Alignment Results

### All Studied Sequences' Multiple Alignment

Identity (Sequence vs Target) = 91 to 99%

Global Alignment Identity = 84%

Individual Identity on Global Alignment = 78 to 86%

### All Deposited Sequences' Multiple Alignment

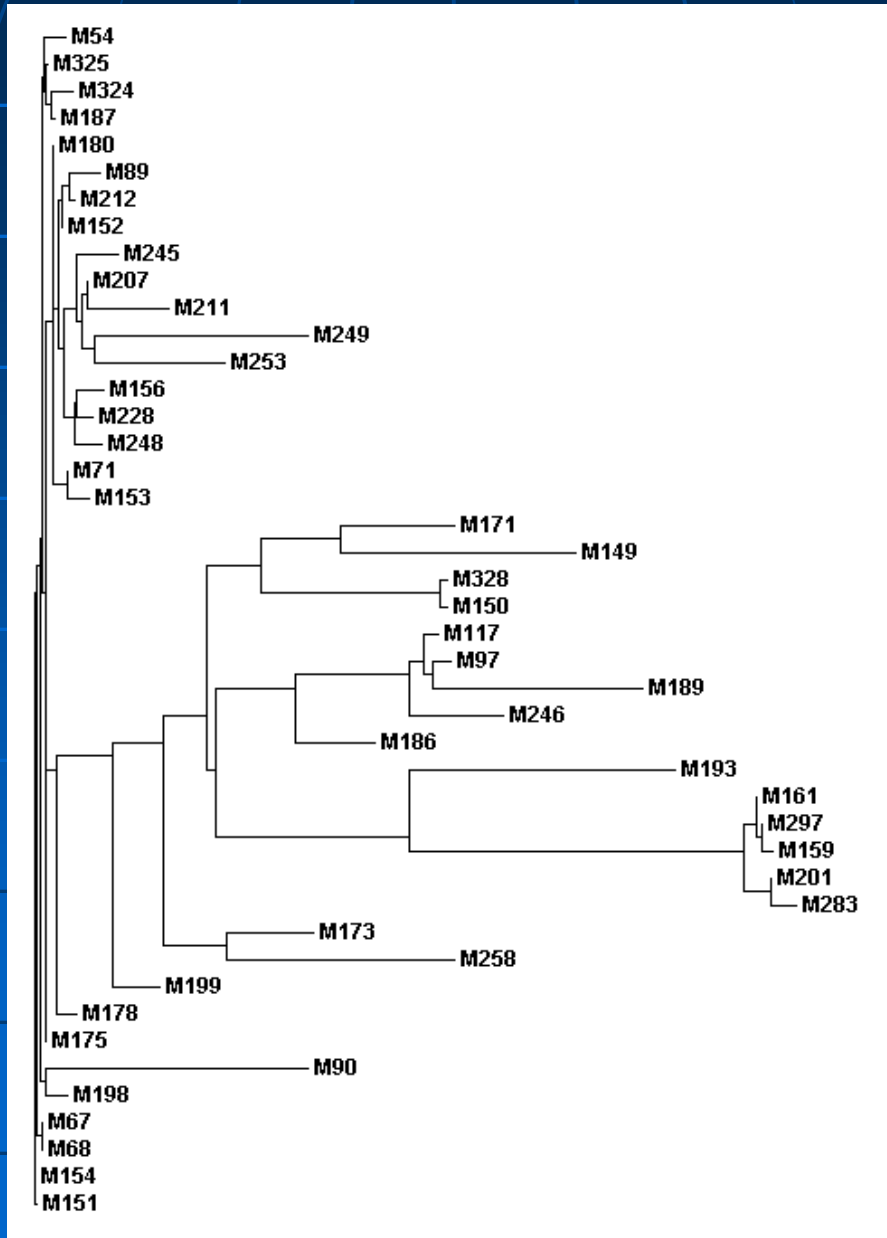
(Limits: UNTIL 06/03/2013; NOT *Uncultured bacteria*; 50 seq > Identity)

Identity (Sequence vs Target) = 84 to 96%

Global Alignment Identity = 62%

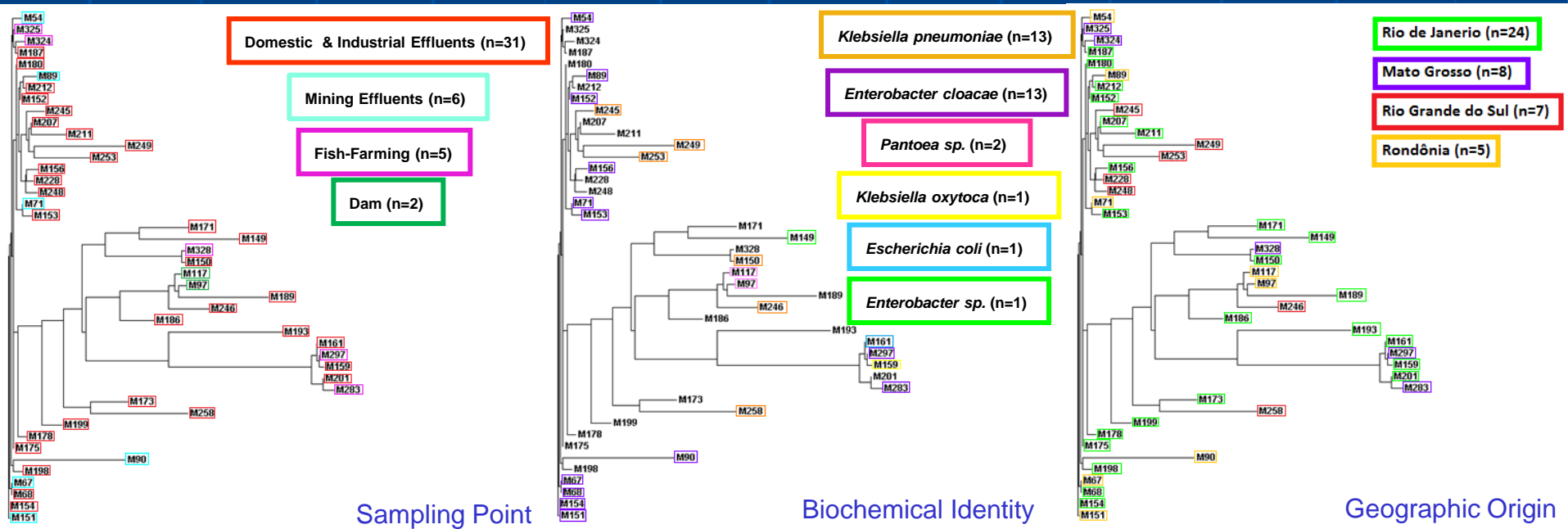
Individual Identity on Global Alignment = 33 to 66%

# *merA* Multiple Alignment Results (Phylogenetic Tree)



# RESULTS & DISCUSSION

## merA Gene's Multiple Align Result (Phylogenetic Tree)



# Presence of *merA* Gene Polymorphism

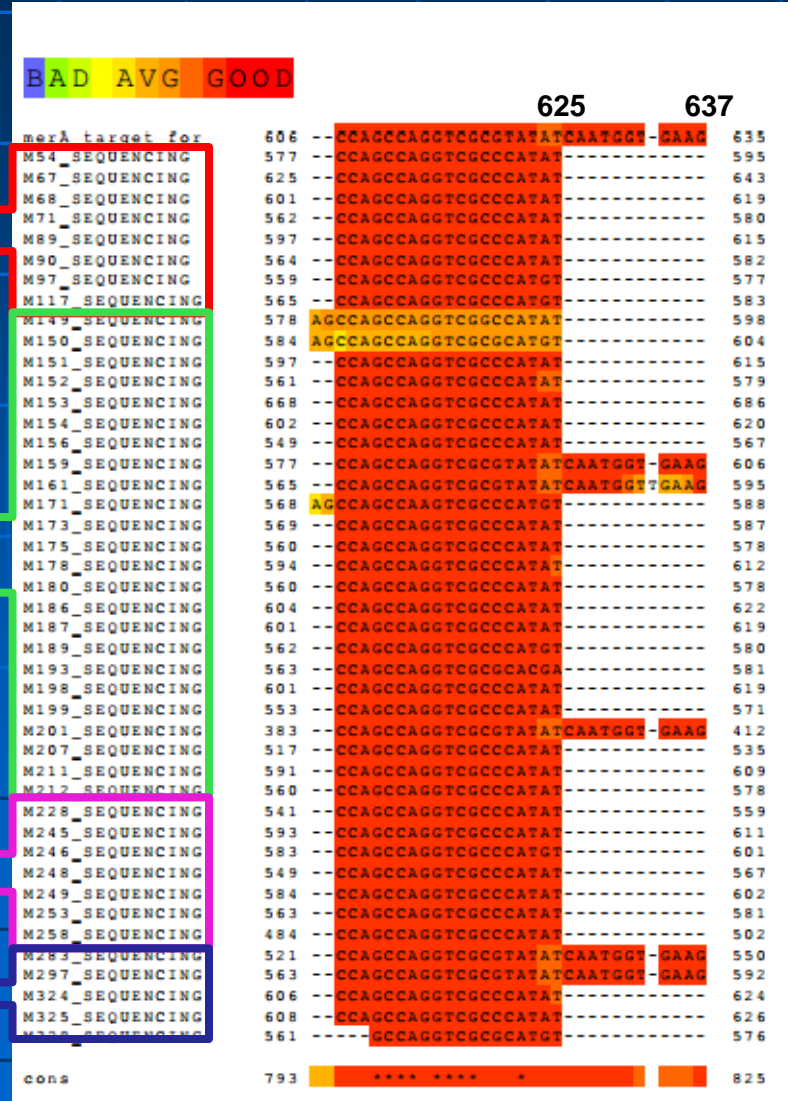
88,6% 87,5%

RO 100%

RJ

RS 100%

MT 60,0%



Individual Identity  
on Global  
Alignment



78 to 86%

# CONCLUSIONS

- ❑ The *merA* gene was found in the majority of the isolates;
- ❑ The use of two new primers - *merABR R* and *merABR F* - has increased the sequencing efficiency;
- ❑ The bioinformatics analysis of multiple alignments showed high identity between the sequences;
- ❑ The identity increased if the data was grouped based on the collection points, supporting the hypothesis of horizontal communication among bacteria that belonged to the same aquatic environment;
- ❑ A sequence deletion was detected in 86,4% of the studied strains.

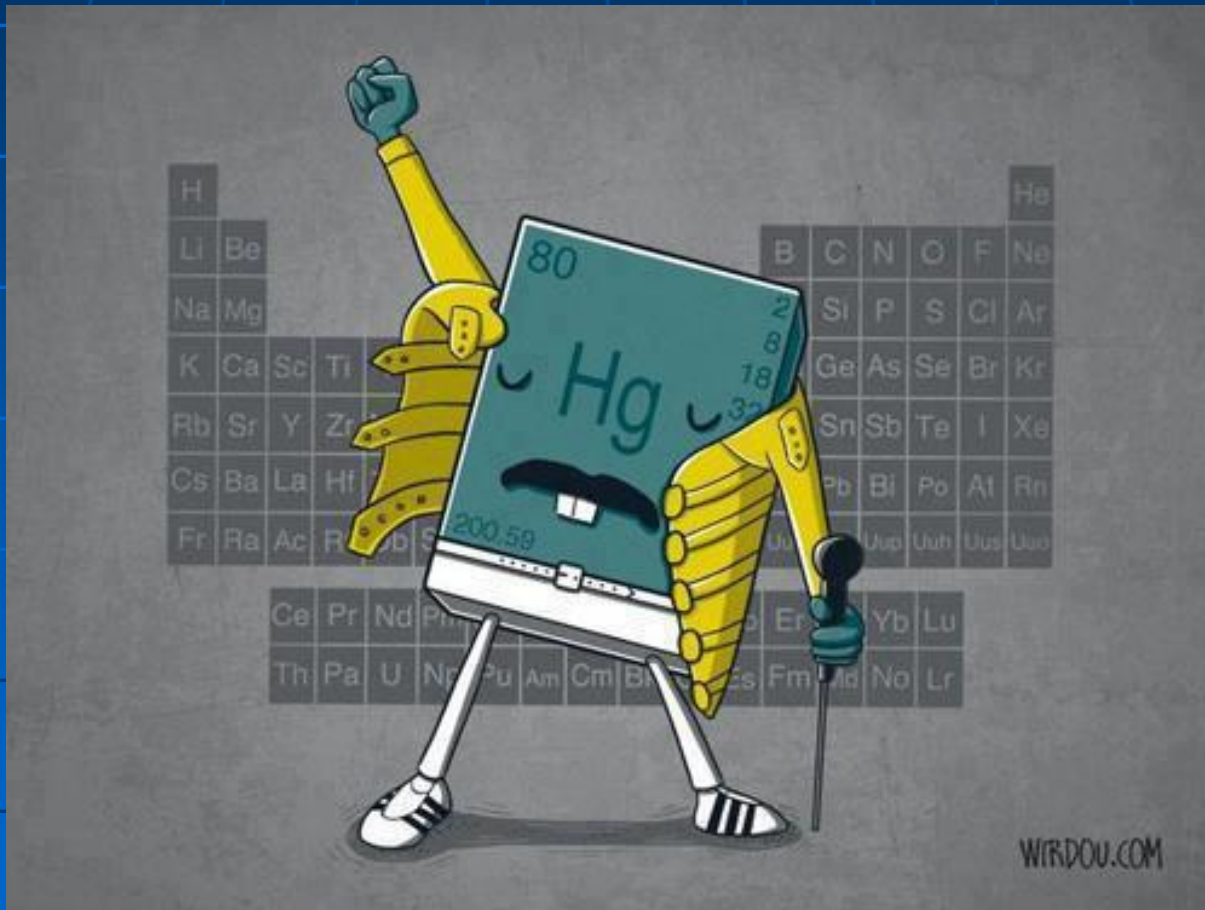
# FUTURES GOALS

- Identify all the studied strains;
- Detect *merB* gene on the strains;
- Analyze the 12pb deletion with proteomic study, including 3D modeling, coupled with activity assays of the MerA enzyme;
- Analyze the ability of the most interesting strains to grow in bioreactor condition.

# ACKNOWLEDGMENTS

- ❑ **Oswaldo Cruz Foundation – Fiocruz , Ministry of Health, Brazil**
- ❑ **National Research Council – CNPq**
- ❑ **Research Support Foundation of Rio de Janeiro State – FAPERJ**
- ❑ **Genomic Platform - DNA Sequencing of Oswaldo Cruz Foundation – PDTIS/FIOCRUZ**

# ...THANK YOU FOR YOUR ATTENTION

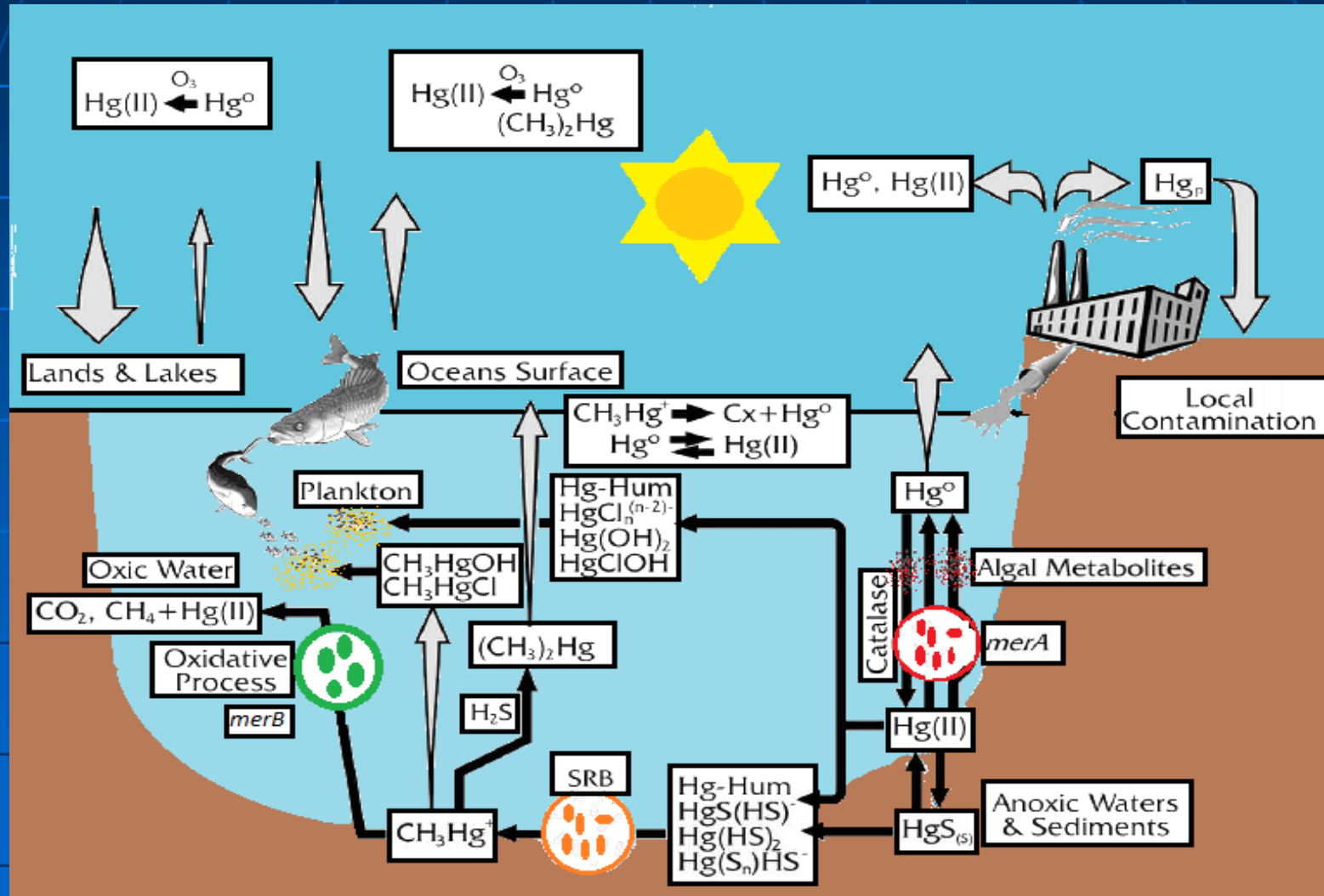








# MERCURY(Hg)



# Summary of all Multiple Alignments Results

Region	Aquatic System Sampled	N° samples	Consensus (%)	Max (%)	Min (%)
South-East, Rio de Janeiro, RJ	A	n=9	96	97	95
	B	n=2	99	99	99
	C	n=2	97	97	97
	D	n=3	96	96	94
	E	n=3	98	98	98
	<b>All</b>	<b>n=24</b>	<b>86</b>	<b>88</b>	<b>83</b>
South, Rio Grande do Sul, RS	F	n=2	93	93	93
	G	n=2	92	92	92
	<b>All</b>	<b>n=7</b>	<b>90</b>	<b>92</b>	<b>87</b>
North, Rondônia, RO	H	n=6	98	98	97
	I	n=2	99	99	99
	<b>All</b>	<b>n=8</b>	<b>98</b>	<b>98</b>	<b>98</b>
Middle-East, Mato Grosso, MT	J	n=3	99	99	98
	L	n=2	99	99	99
	M	n=2	96	96	96
	<b>All</b>	<b>n=5</b>	<b>97</b>	<b>97</b>	<b>95</b>
	<b>MIC<sub>Hg</sub> = 4 mg L<sup>-1</sup></b>	n=40	84	87	79
	<b>MIC<sub>Hg</sub> = 6 mg L<sup>-1</sup></b>	n=4	99	99	98
	<b>Domestic Effluents (RJ and RS)</b>	n=31	83	86	78
	<b>All</b>	<b>n=44</b>	<b>84</b>	<b>86</b>	<b>78</b>

