

Genetic variability and selection of abalone
(*Haliotis tuberculata*) strains for a better growth
rate and resistance to their specific pathogen
(WP 4 +5)

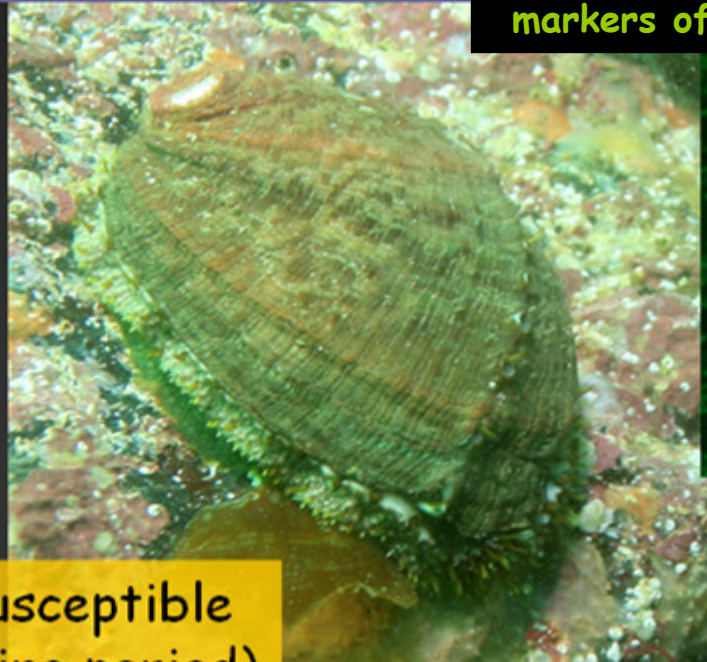
Anne-Leila Meistertzheim, Marie-Agnès Travers, Marion Cardinaud David Schikorski, Carolyn S. Friedman, Alain Van Wormhoudt, Tristan Renault Sylvain Huchette, Dario Moraga and Christine Paillard.



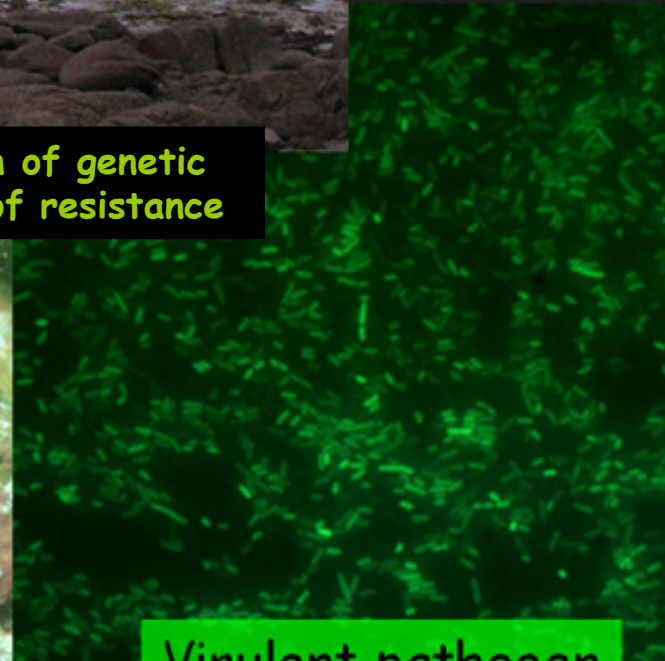


Favorable environment
(temp > 17°C)

Research of genetic
markers of resistance



Host susceptible
(Spawning period)



Virulent pathogen

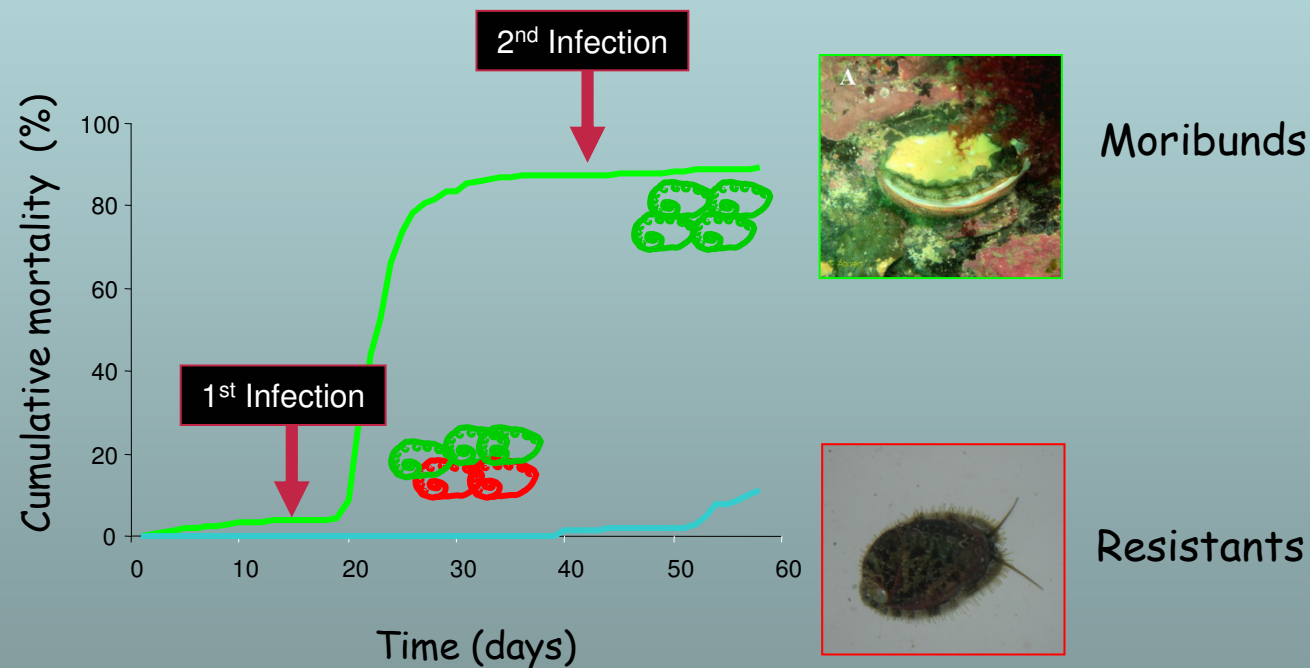
Research of genetic makers of resistance (WP5)

- Identification of physiological markers
(= gene sequences)
- Genotype diagnostic tools for identification
of phenotypes
[vibriosis resistance]
[resistance and better growth]
- New generation of high level genotyping tools
(High Resolution Melting Analysis)

Experimental design :

Conditioning fast for several days at 15°C then 19°C of individuals of 2,5 years old from different families

Two successive infections using GFP ORM4 *V. harveyi* at 19°C

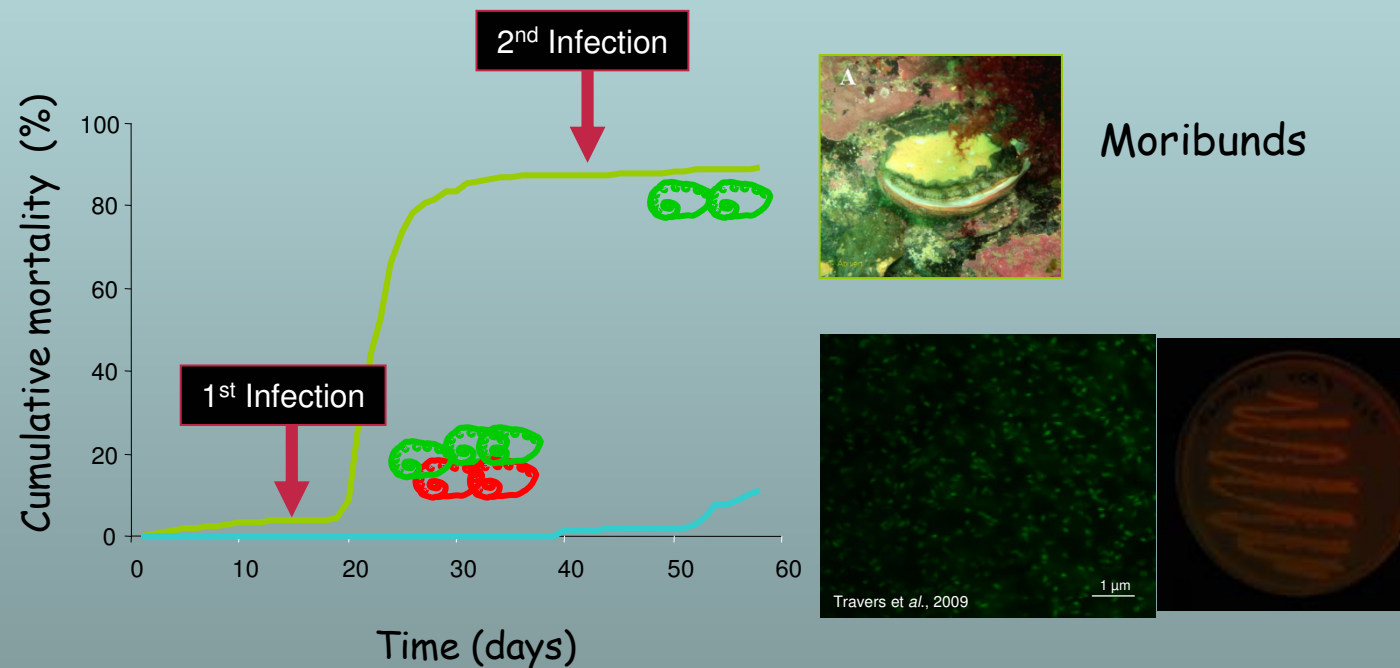


- Number of died ormers per day
- Checking presence of pathogen on moribunds (ORM4 - *GFP V. harveyi*)
- Sex and size identification

Experimental design :

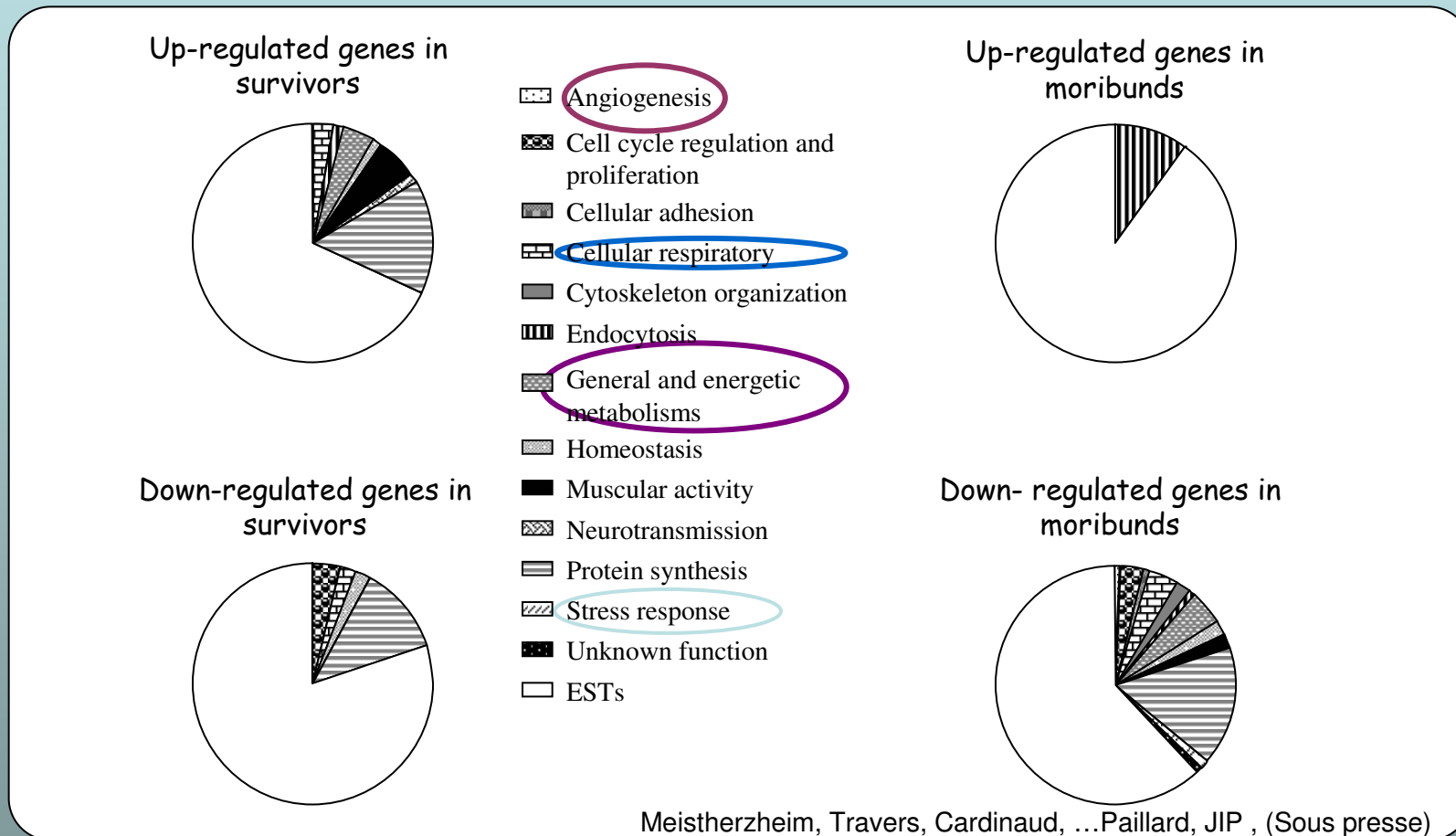
Conditioning fast for several days at 15°C then 19°C of individuals of 2,5 years old from different families

Two successive infections using GFP ORM4 *V. harveyi* at 19°C



Generation of suppressive subtraction hybridization libraries from resistants vs controls and moribunds vs controls

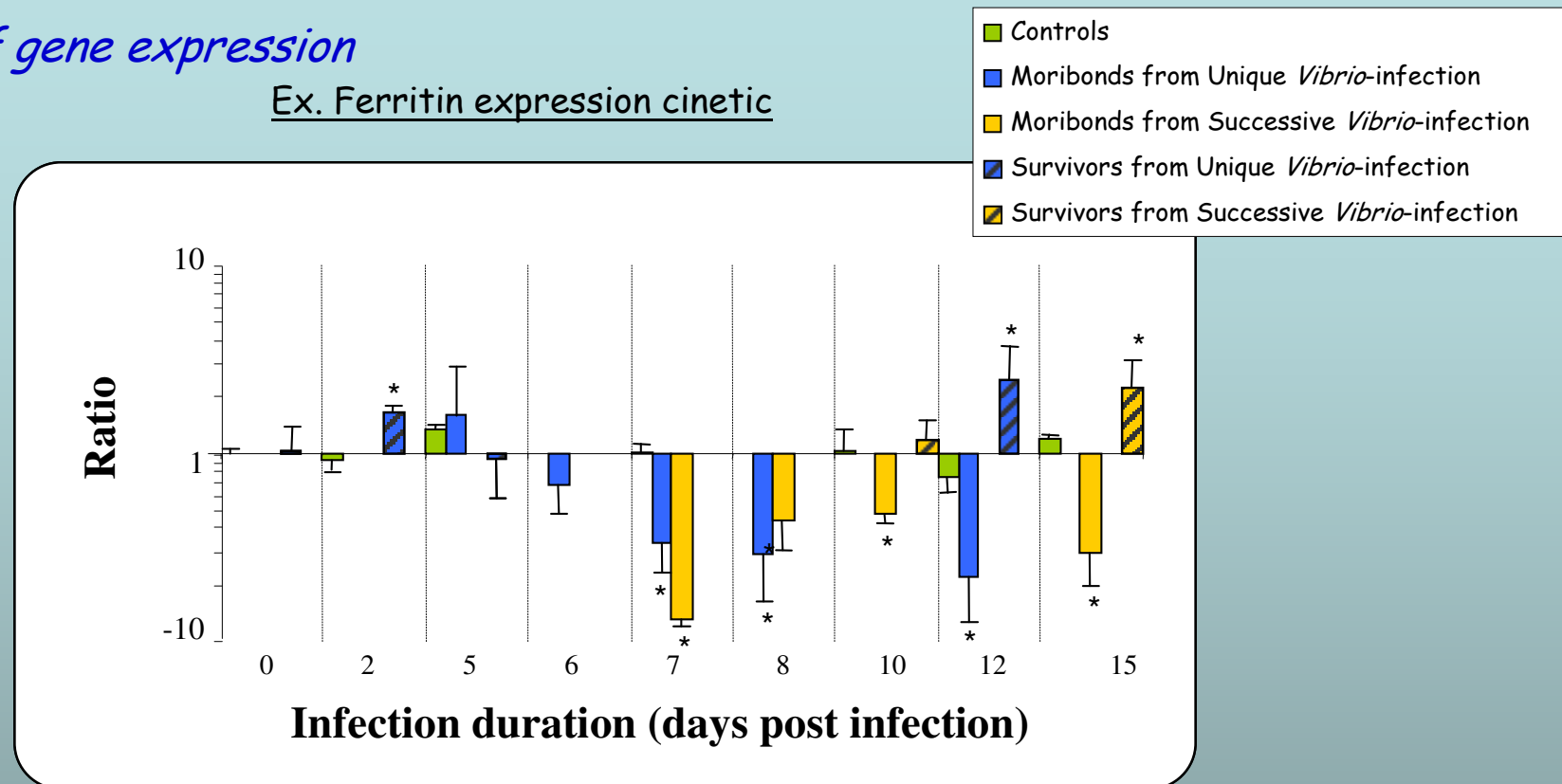
221 sequences potentially regulated by two bacterial challenges in *H. tuberculata* with among them 67% unknown (EST)



Several physiological processes
High number of ESTs

Cinetic of gene expression

Ex. Ferritin expression cinetic

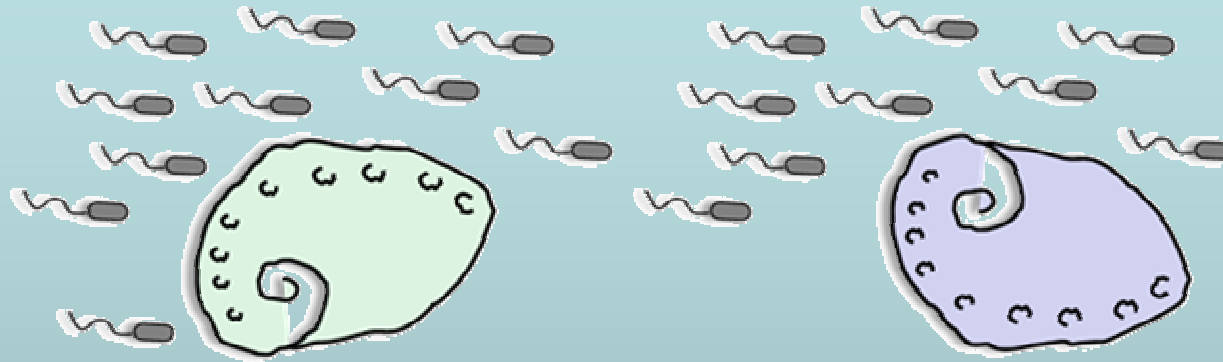


Meistherzheim, Travers, Cardinaud, ...Paillard, JIP , (Sous presse)

- ✓ Surexpression at the end of the 1rst and 2nde infection in survivors
- ✓ Inhibition at the end of the 1rst and 2nde infection in moribonds

- ✓ Gene involved in infection : Role in the limitation of iron disponibility for pathogen?
- ✓ Gene involved in limitation of ROS production in the survivors

Two successive *Vibrio harveyi* infections



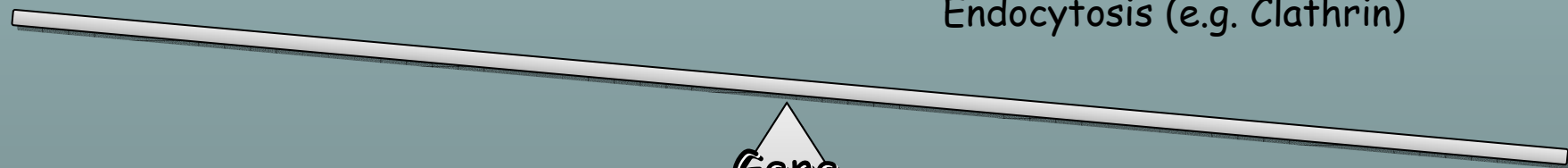
Surviving abalone



Susceptible abalone

↑ Indirect immune system (e.g. Ferritin)
 ↑ Stress (e.g. HSP84)
 ↑ Metabolism (e.g. FABP, GLS)
 ...

↓ Muscular activity (e.g. Calponin 2)
 ↓ Cytoskeleton (e.g. Troponin)
 ↓ Endocytosis (e.g. Clathrin)



Gene
 expression

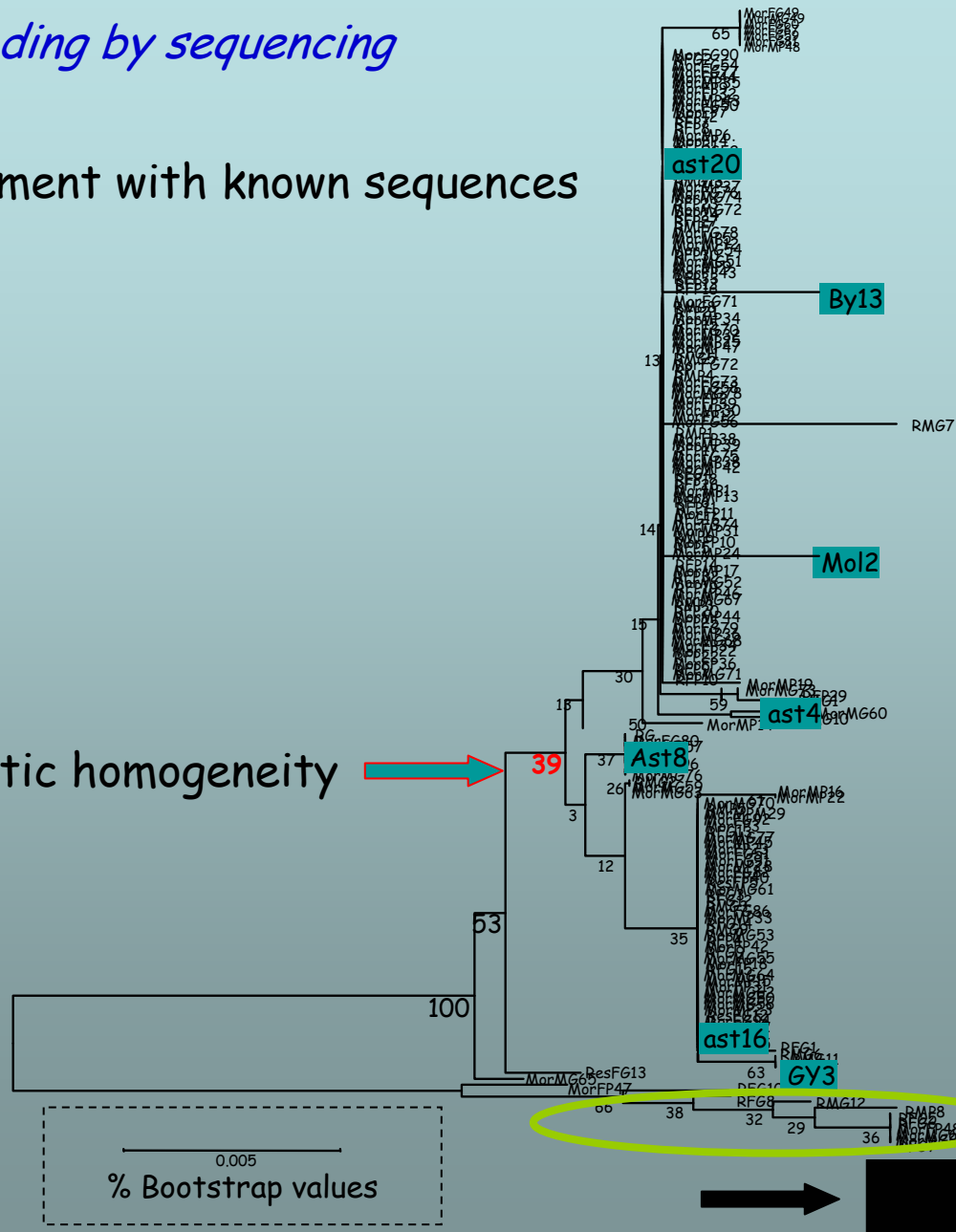
Meistherzheim, Travers, Cardinaud, ...Paillard, JIP , (in press

Identification of 69 regulated genes

COI Barcoding by sequencing

Alignment with known sequences

Genetic homogeneity →

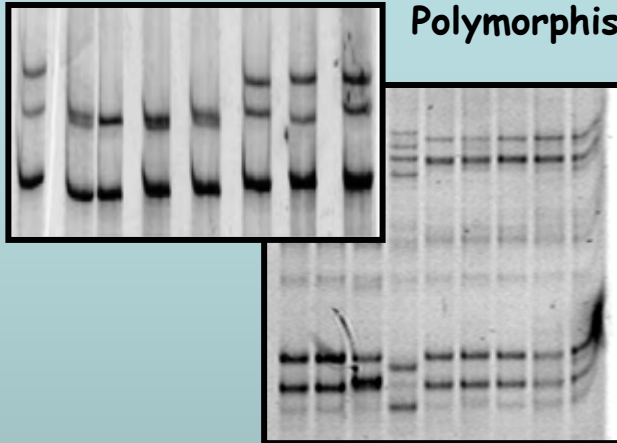


Identification of *H. tuberculata coccinea*

Samples removed of the data

Actual genotyping techniques ...

Single Strand Conformation Polymorphism



Sequencer



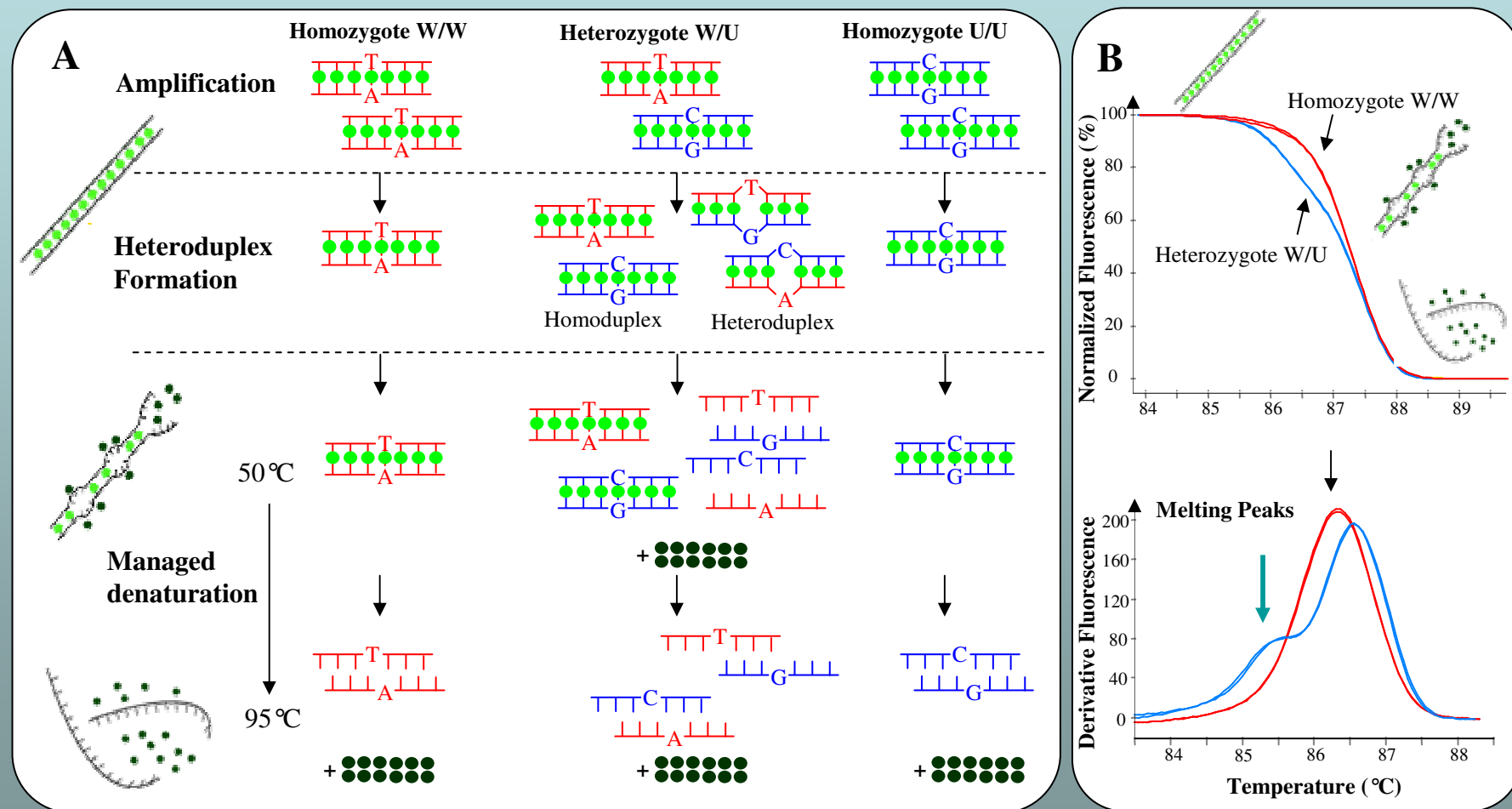
qPCR- High Resolution Melting



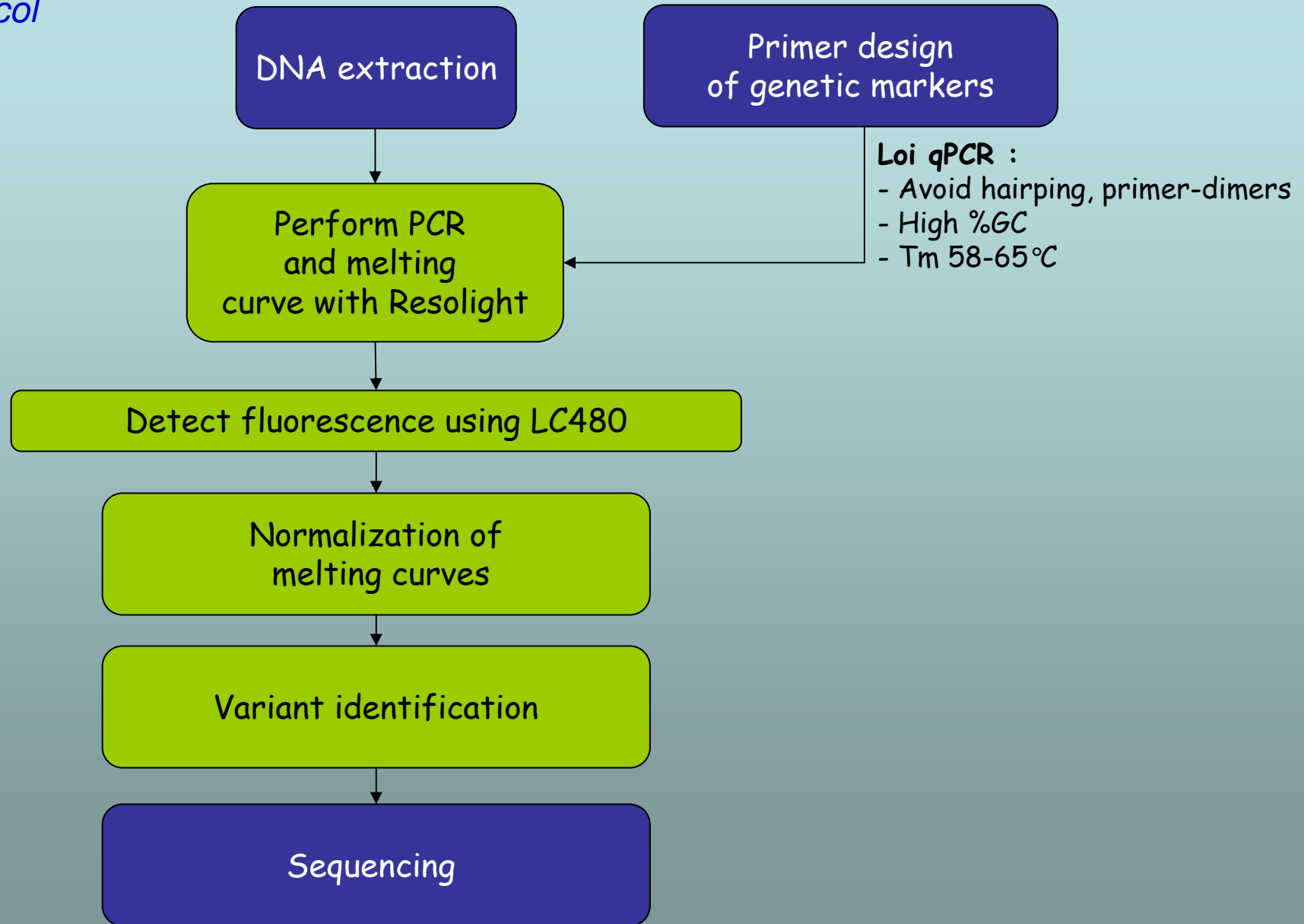
Techniques	Consumable cost / sample	Time /assay	Machine time	Technician time	Max number sample/assay	Reproducibility	Extra
SSCP	1.50	24h30	18h30	6h	228	bad	séquençage par bande
Sequencer	4 (2)	24h	18h	5h30	96	middle	} séquençage des variants
HRM LightScanner	1.94	2h25	1h45	40min	96	middle	
HRM LightCycler 480	0.91	1h15	1h00	15min	96	high	

High level genotyping by High Resolution Melt (HRM)

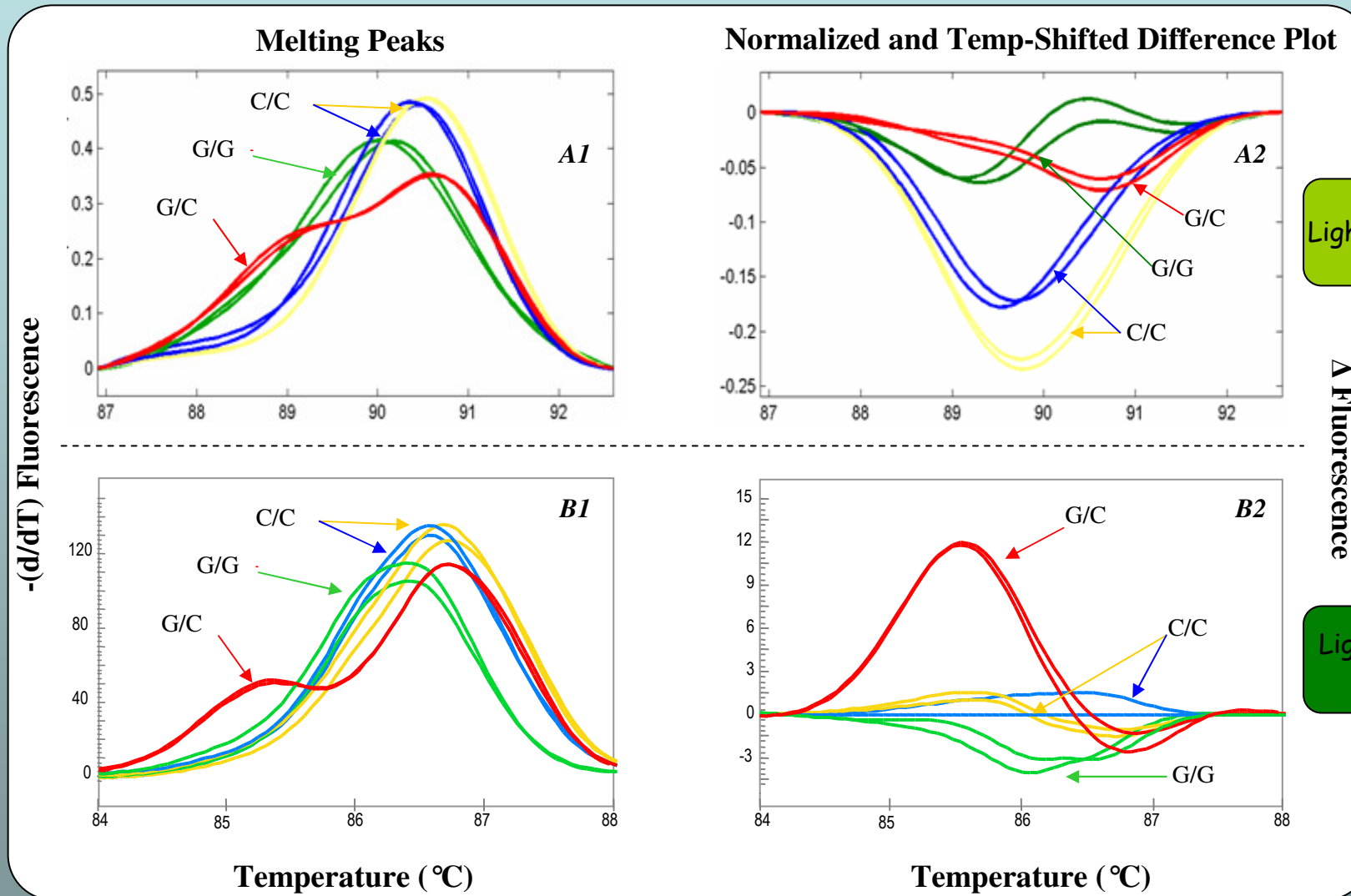
- Description = Melting curve with inserting fluorochrome of the double strand



Protocol



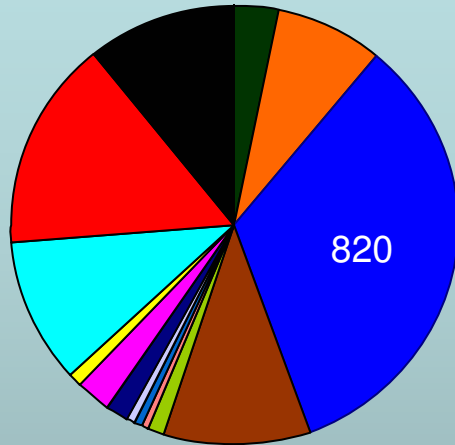
Ferritin polymorphism of H. tuberculata



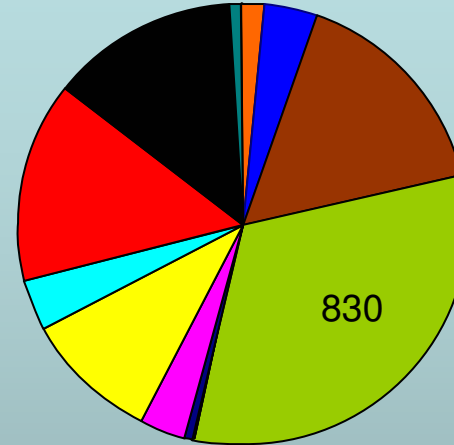
Genotyping by High Resolution Melt (HRM)

ATP F0

Moribunds



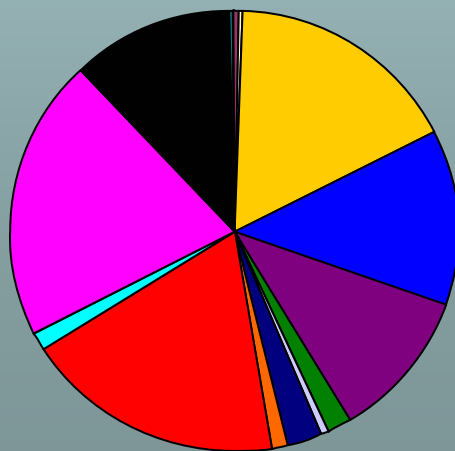
Resistants



Alleles

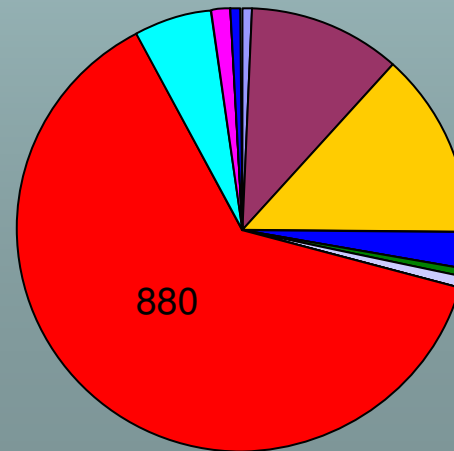
- 810
- 815
- 820
- 825
- 830
- 835
- 840
- 850
- 855
- 860
- 865
- 870
- 875
- 880
- 885

Ferritin



Alleles

- 830
- 840
- 844
- 845
- 850
- 855
- 860
- 865
- 870
- 875
- 880
- 885
- 890
- 895
- 900
- 910



Validated by sequencing in process

Genetic markers developed for HRM genotyping for H. tuberculata



Genetic markers selected:
 Ferritine
 ATPFO
 Glutamine synthétase
 Reptin
 Calponine 3
 Nascent polypeptide-associated complex (NAC)
 SAR 1

Final step

Ex. Genetic differentiation index for ATPFO and Ferritine

Fst (* $p < 0.05$; ** $p < 0.01$; ***
 $p < 0.001$)

Mor vs Resistant	
Multilocus	0.121**
ATPFO	0.127***
Ferritine	0.175***

- ✓ No genetic structure defined by sex
- ✓ Resistant haplotypes identified
- ✓ Allelic and genotype associated? *In progress*
- ✓ Groups defined by « neutral » markers: COI

→ **Use of single genetic pool**

- ✓ Validation of markers on biparental families?
- ✓ Genetic structure with 7 genes on cultivated populations: identification of potential resistant genitors
- ✓ Application of these tools on field populations : percentage of resistant on stock populations

Institut Universitaire Européen de la Mer (IUEM)



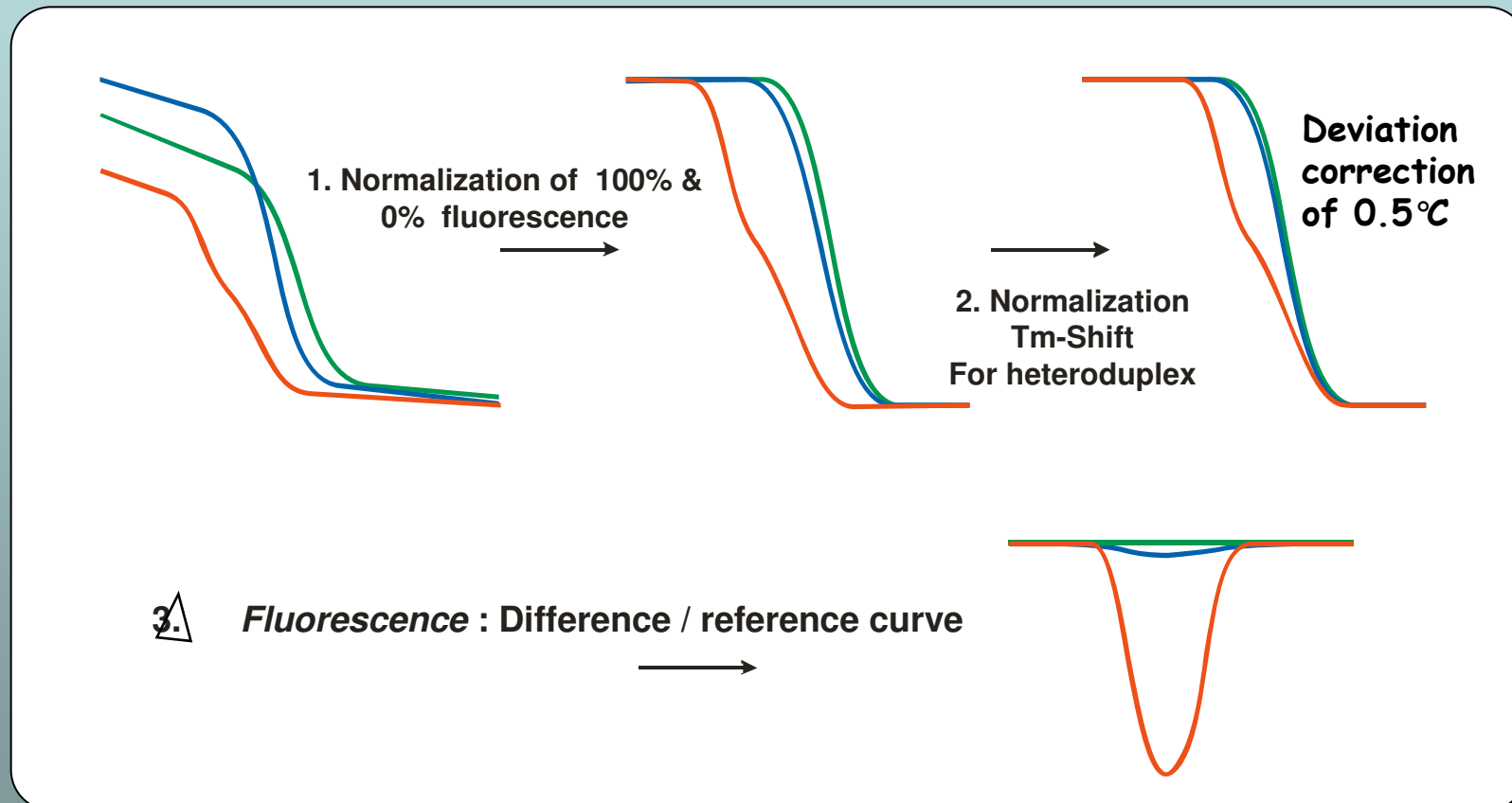
Thanks to the team

Stéphanie Bordenave
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Jean-Louis Nicolas
Claude Ferec

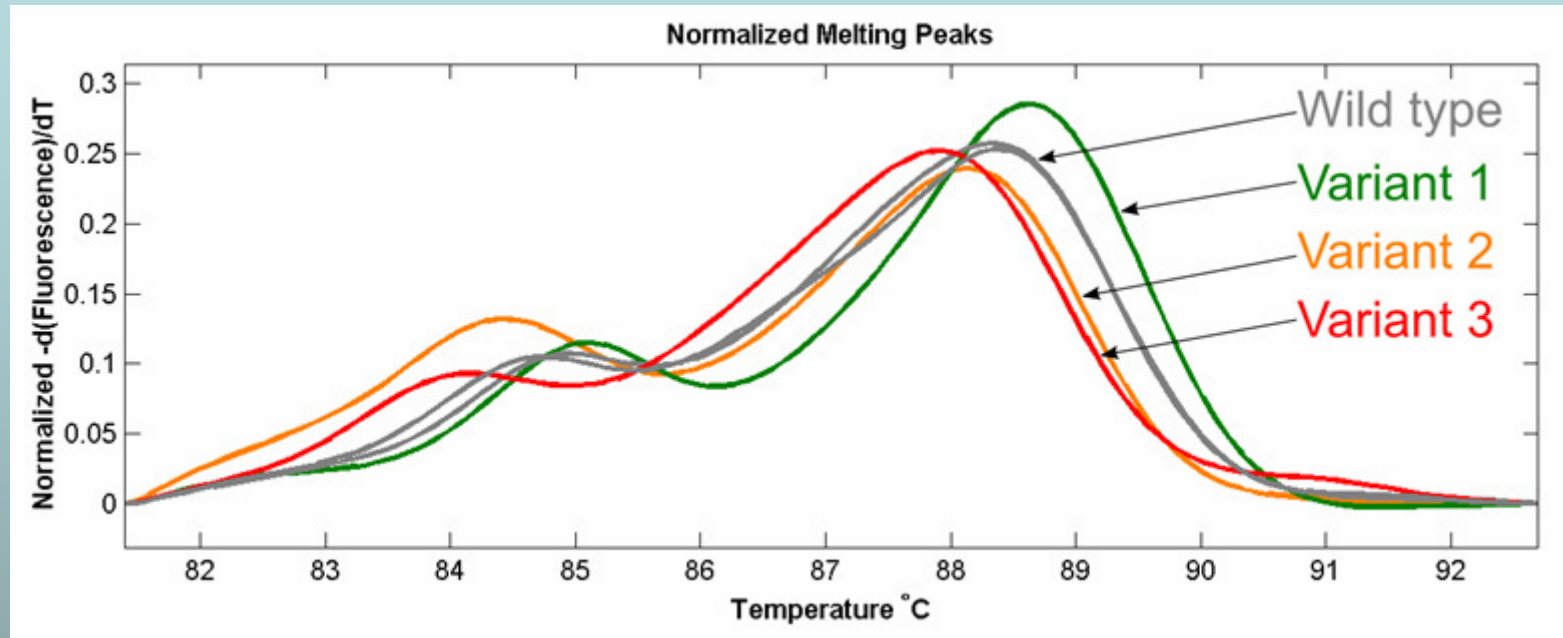


Fluorescence normalization

The software realize a specific analysis by curve differences



Results observed



Identification of variants