



# GENIALG

e-Learning course  
SUSTAINABLE SEAWEED FARMING PRACTICES

## Module 3 - Breeding Seaweed

### LESSON 2

*Advanced methods for seaweed breeding*

©Atlantic Sea Farms



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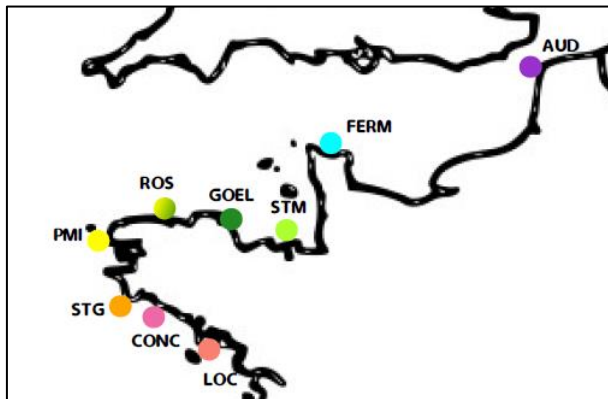
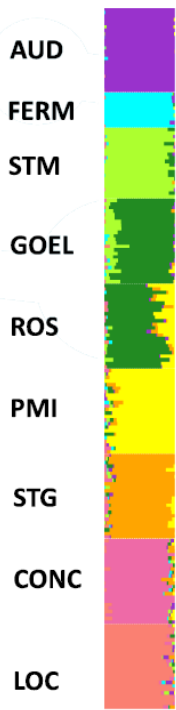
# Module 3 - Breeding Seaweed

## LESSON 2 – Advanced methods for seaweed breeding

### Seaweed breeding process



### GENETIC DIFFERENTIATION ALONG THE BRETON COAST - EXAMPLE



- 198 individuals total
- 10 to 24 individuals per locality
- 4,104 SNPs with one randomly selected SNP per locus to minimize linkage disequilibrium



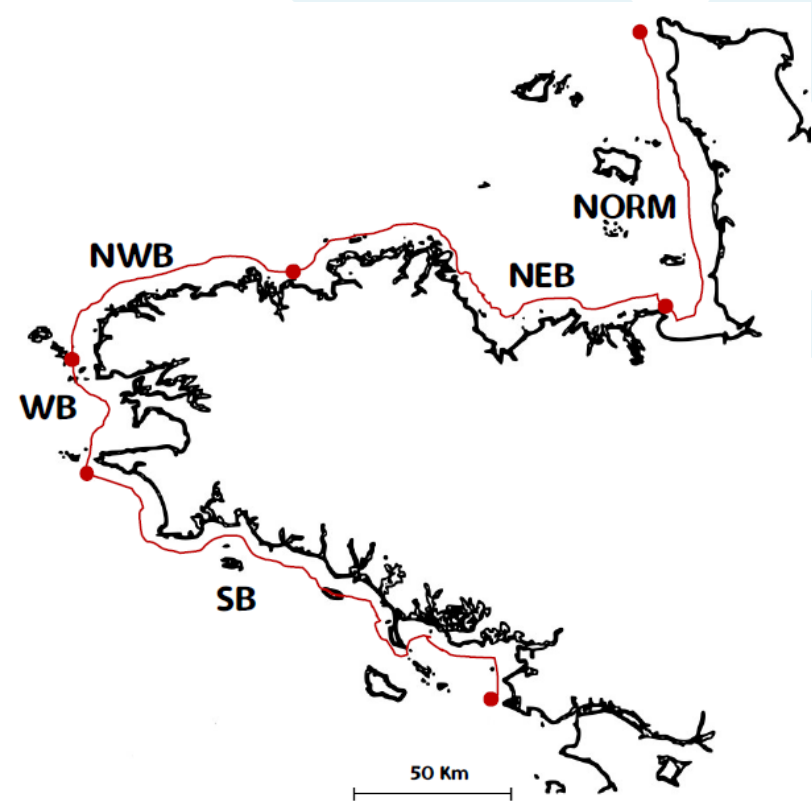
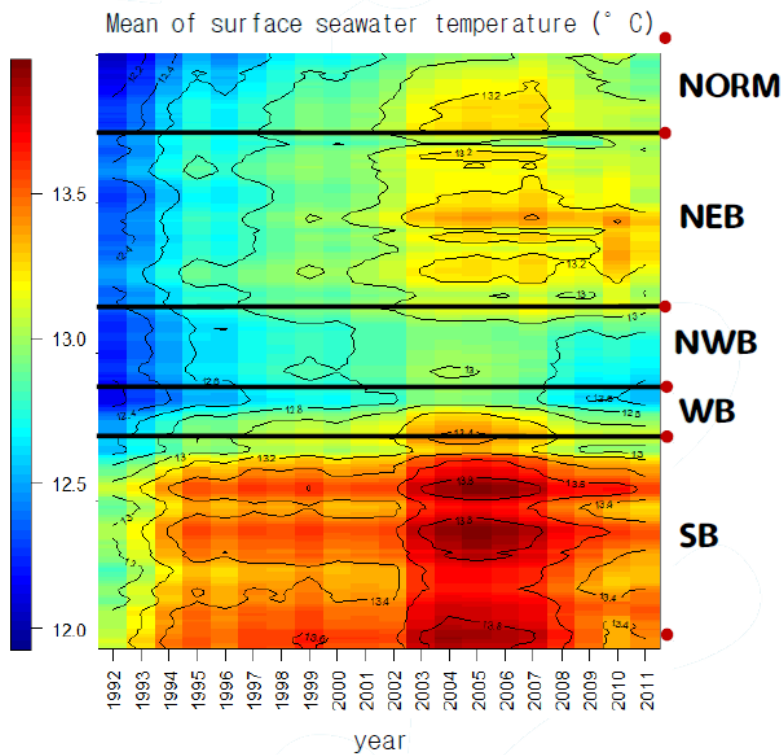
# Module 3 - Breeding Seaweed

## LESSON 2 – Advanced methods for seaweed breeding

### Seaweed breeding process



### CONTRASTED SEAWATER TEMPERATURES ALONG THE BRETON COAST- EXAMPLE



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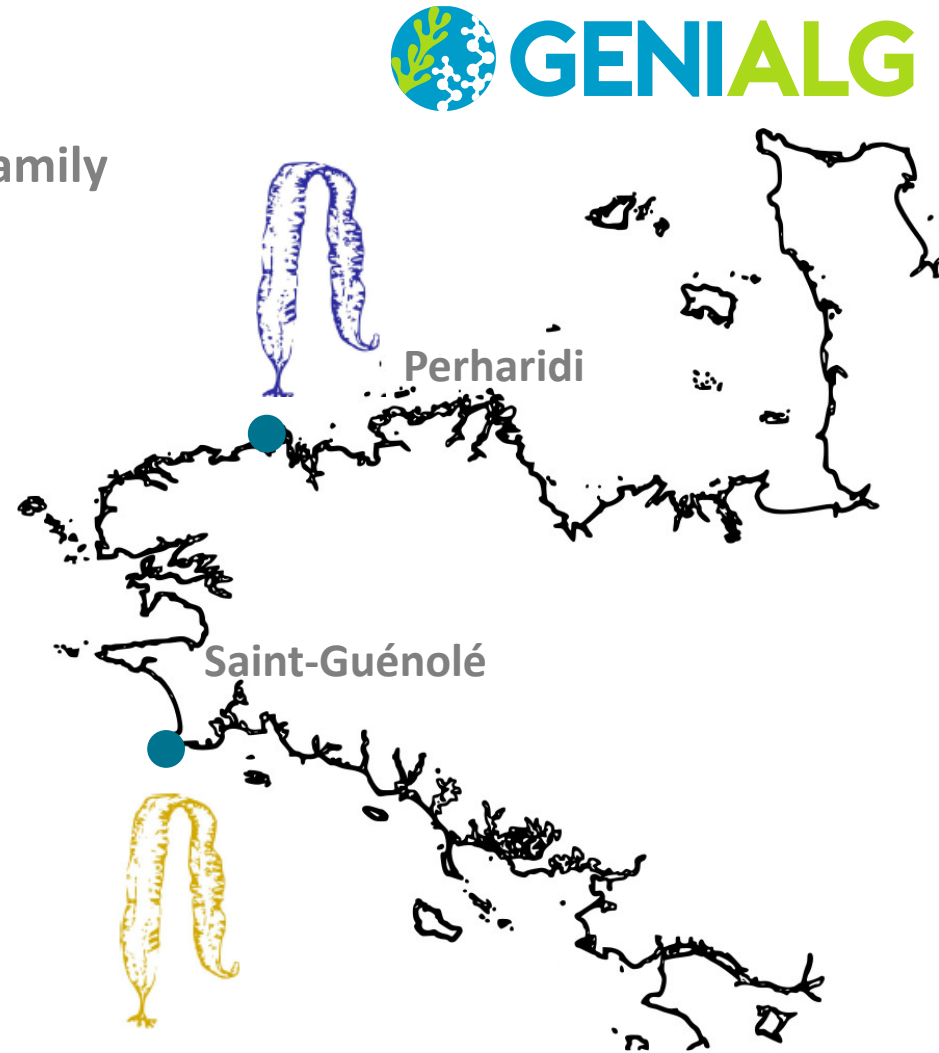
# Module 3 - Breeding Seaweed

## LESSON 2 – Advanced methods for seaweed breeding

### Seaweed breeding process

#### 1 LINKAGE MAPPING – The mapping family

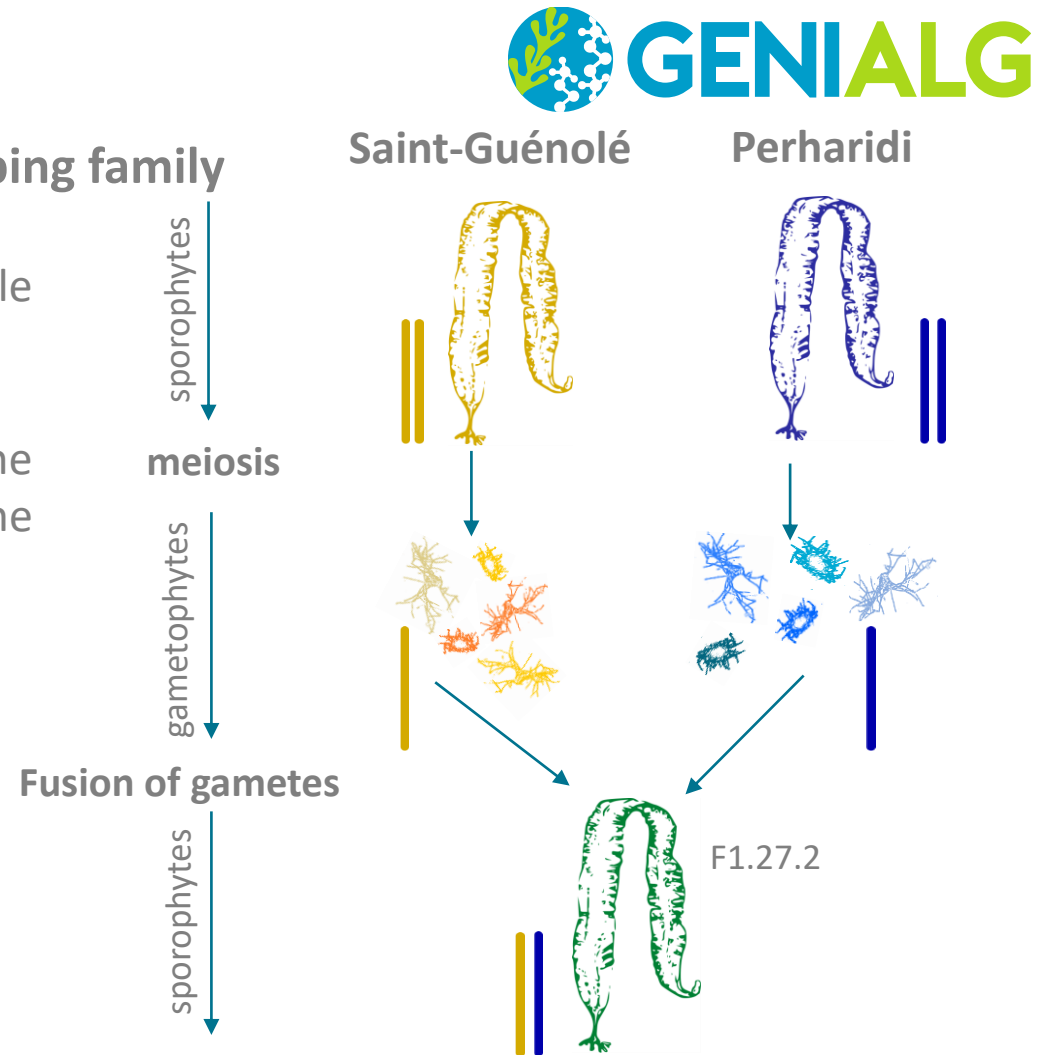
1. Diploid parental sporophytes from North and South Brittany
2. Spores release in the lab and gametophyte culture



### Seaweed breeding process

#### 1 LINKAGE MAPPING – The mapping family

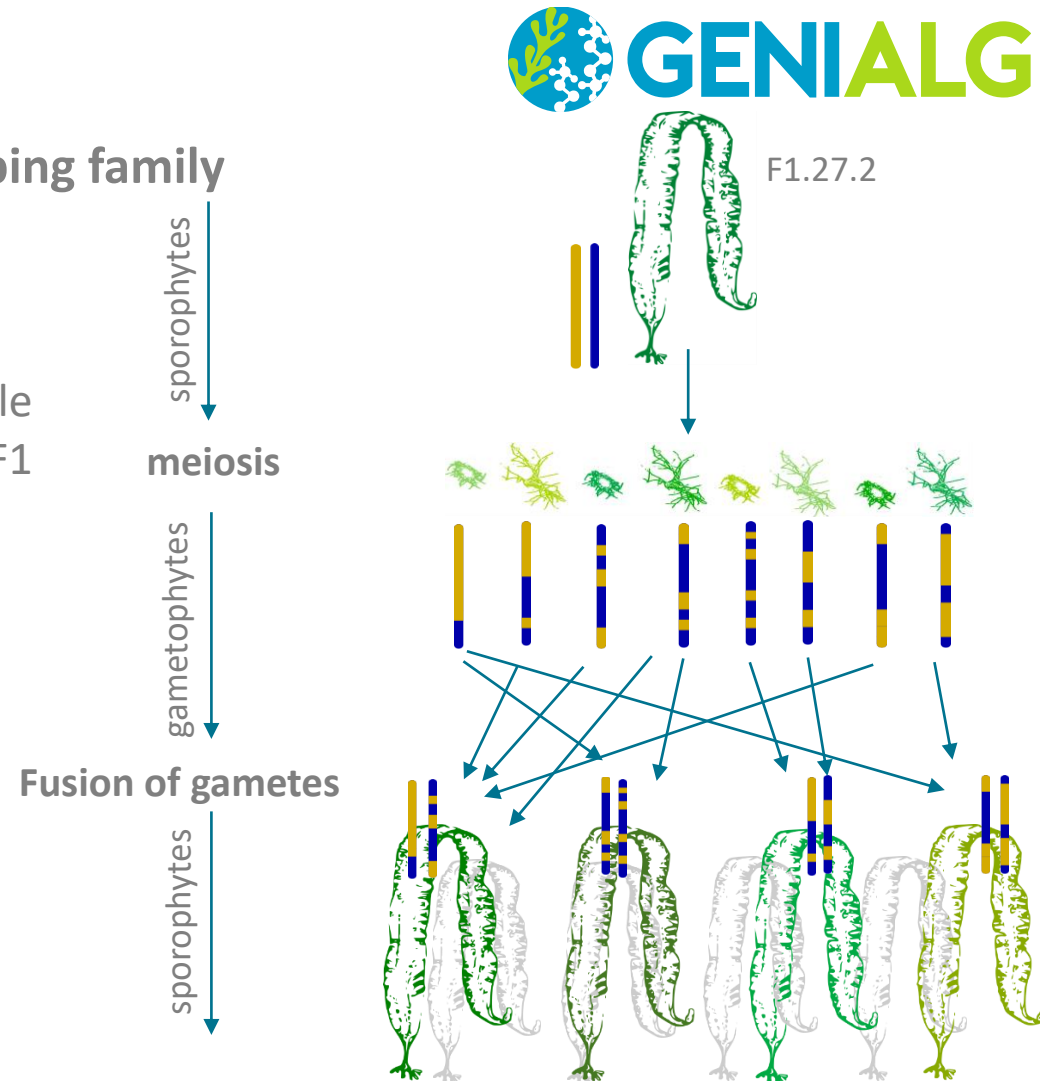
3. Bulk cross of male and female gametophytes
4. Identification and selection of one F1 hybrid to establish the mapping population



### Seaweed breeding process

#### 1 LINKAGE MAPPING – The mapping family

5. Diploid F1 sporophyte
6. Bulked cross of male and female gametophytes (from the F1 individual)
7. F2 diploid mapping progeny
8. 129 individuals



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#### 1 LINKAGE MAPPING – The mapping family

Few months later....



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# Module 3 - Breeding Seaweed

## LESSON 2 – Advanced methods for seaweed breeding



### Seaweed breeding process

#### 1 LINKAGE MAPPING – Phenotyping or Algal slaughtering?

Sporogenesis explants

Back-up explants

Stress explants

Growth explants



*Saccharides*  
explants

DNA sample



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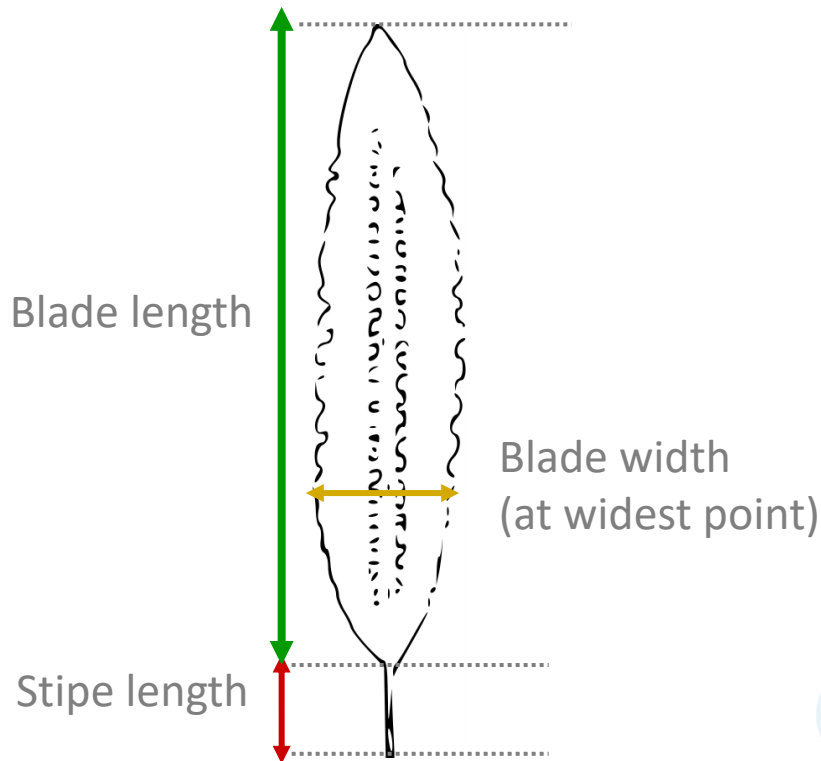
## LESSON 2 – Advanced methods for seaweed breeding

### Seaweed breeding process



#### 1 LINKAGE MAPPING – Phenotyping or Algal slaughtering?

##### 1. Morphometry



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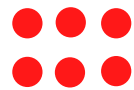
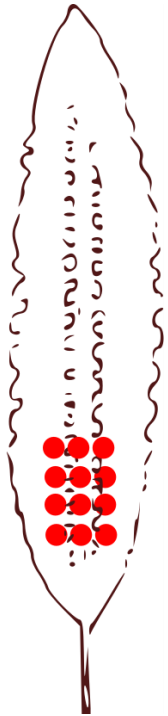
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### Seaweed breeding process

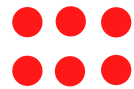


#### 1 LINKAGE MAPPING – Phenotyping

#### 2. Heat stress resistance/tolerance



for the control temperature (13°C)



for heat stress (21°C)

3 discs for Fv/Fm  
measurements

3 discs for futur  
transcriptomic analysis



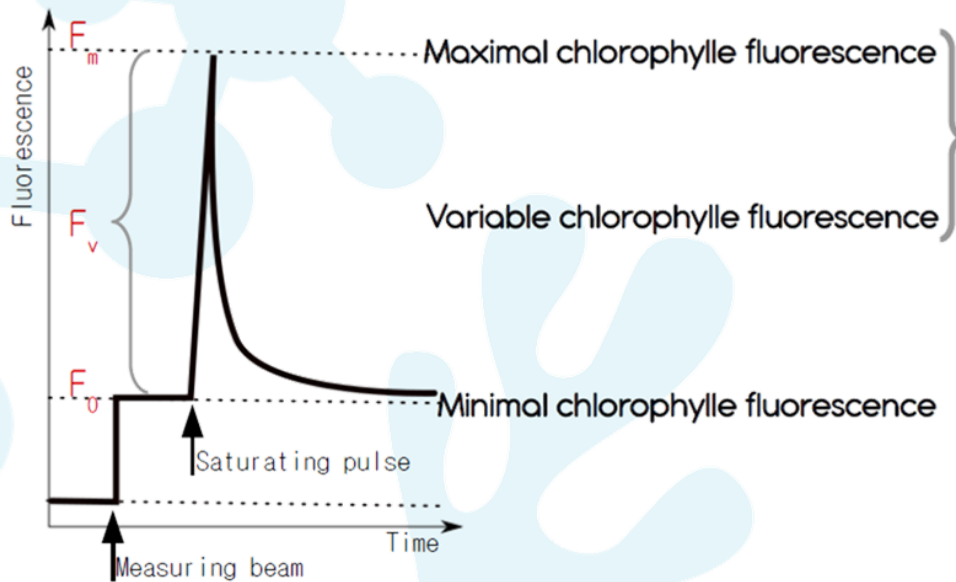
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## LESSON 2 – Advanced methods for seaweed breeding

### Seaweed breeding process

#### 1 LINKAGE MAPPING – Phenotyping

#### 2. Heat stress resistance/tolerance



$F_v/F_m$  represents  
the photosynthetic activity



Proxy for physiological state  
and tolerance to heat stress

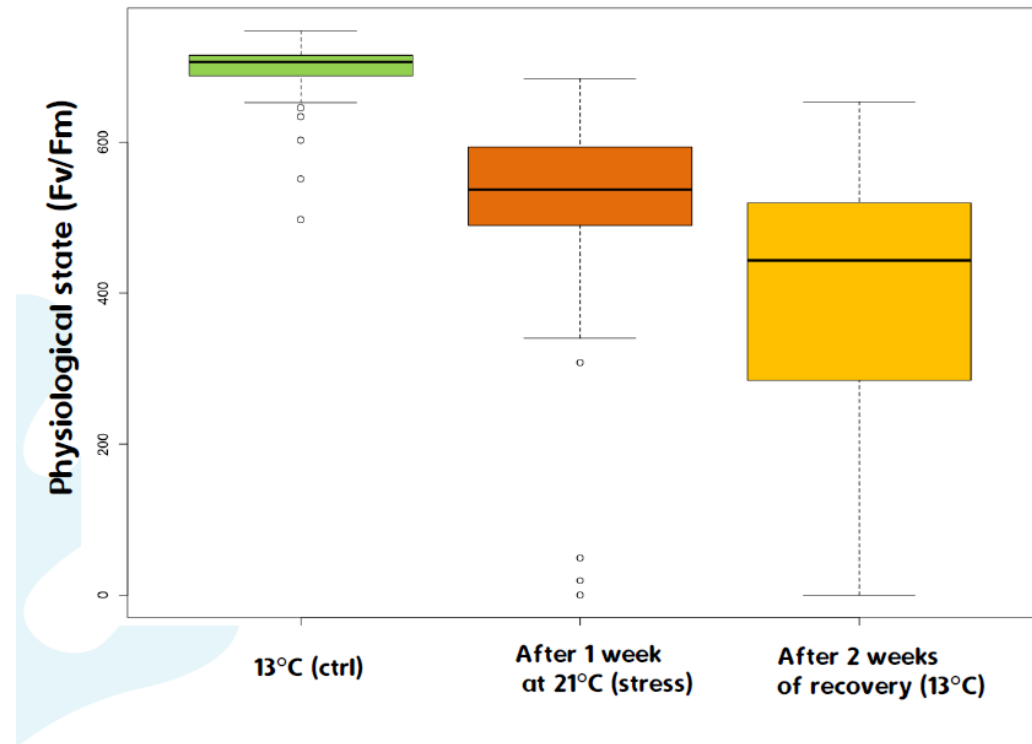


### Seaweed breeding process



- 1 LINKAGE MAPPING – Phenotyping
2. Heat stress resistance/tolerance - results

Various response to stress:  
from tolerance to death



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### Seaweed breeding process

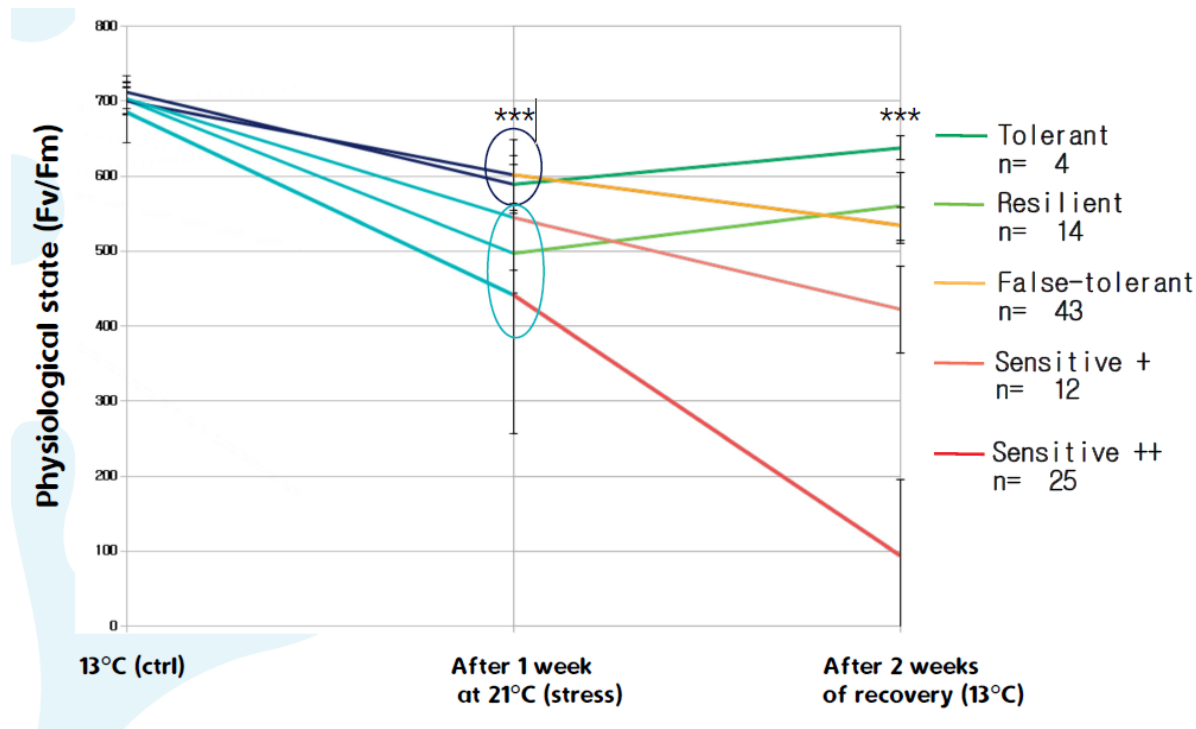


#### 1 LINKAGE MAPPING – Phenotyping

#### 2. Heat stress resistance/tolerance - results

#### Different types of stress tolerance:

- After 1 week of stress: two groups
- Different stress responses observed after recovery



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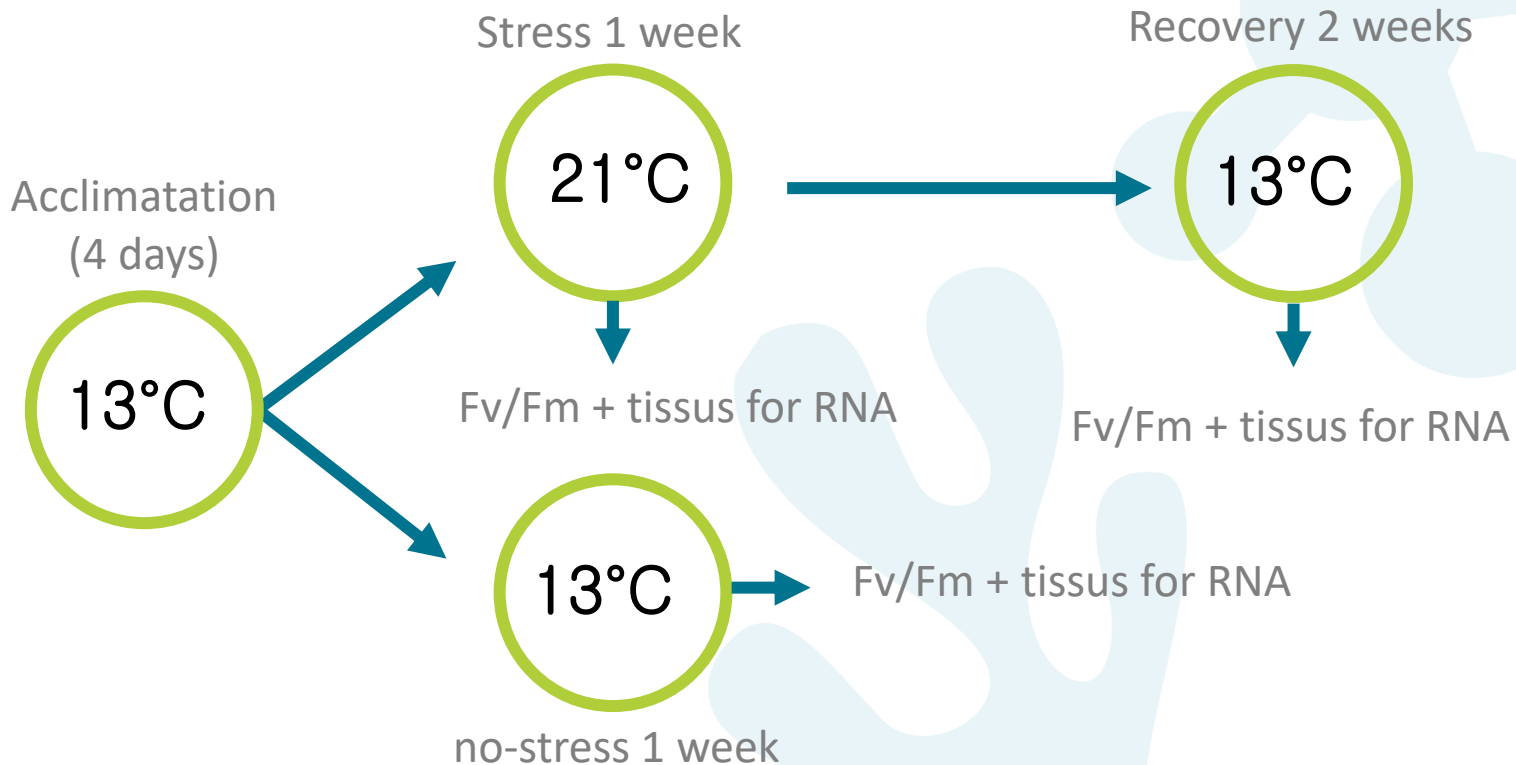
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### Seaweed breeding process

#### 1 LINKAGE MAPPING – Phenotyping

#### 3. Heat stress transcriptomic response

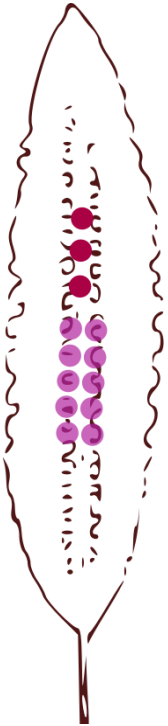


### Seaweed breeding process



#### 1 LINKAGE MAPPING – Phenotyping

#### 4. Saccharides and phenolic content



● ● ● Polysaccharides content:

- Fucans
- Alginates
- Laminarin
- Mannitol
- Phenolics



For later biochemical analyses, back-up (RNA, DNA)

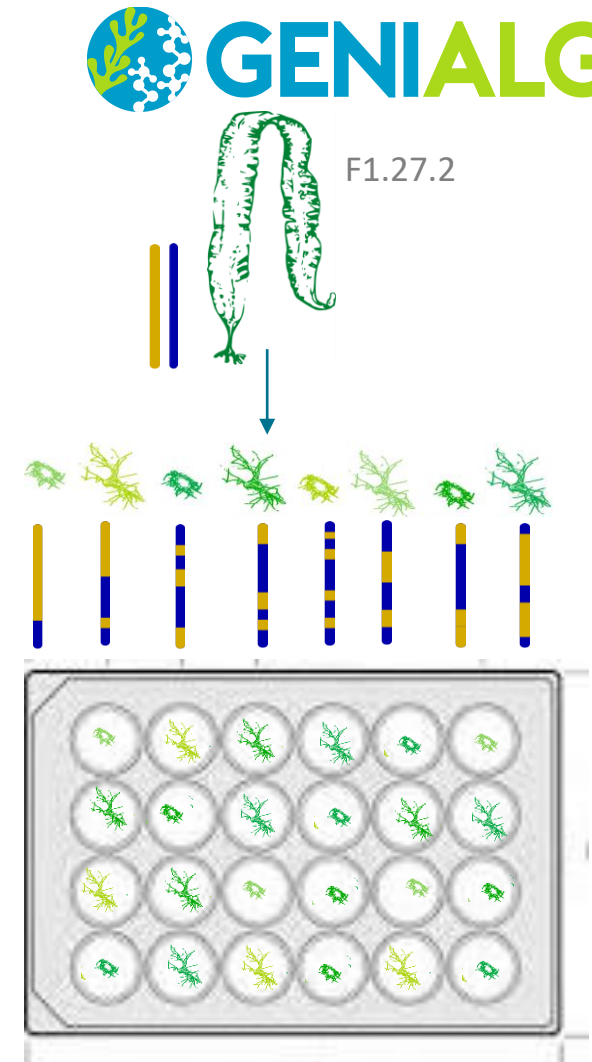


### Seaweed breeding process

#### 1 LINKAGE MAPPING – The other mapping family

- Diploid F1 sporophyte
- Gametophytes isolation and clonal vegetative culture
- Haploid mapping progeny: 96 males and 112 females

sporophytes  
↓  
meiosis  
↓  
gametophytes





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## LESSON 2 – Advanced methods for seaweed breeding

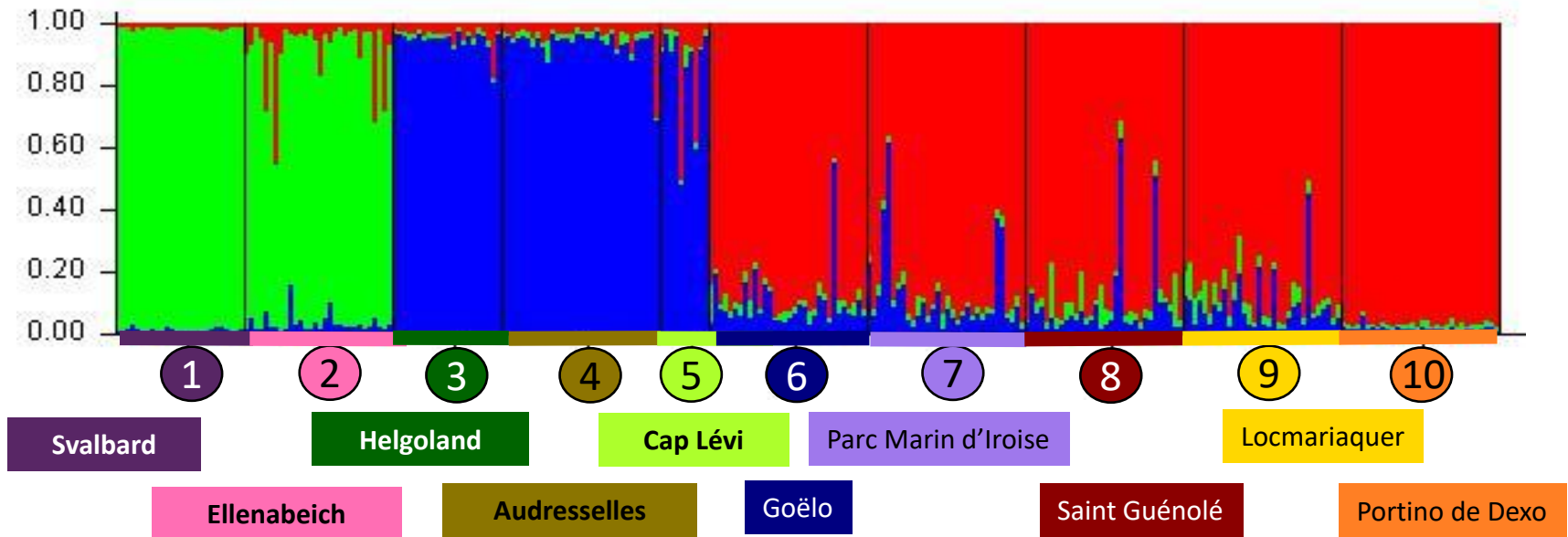
### Seaweed breeding process



## 2 GENOME WIDE ASSOCIATION STUDY (GWAS)

### 1. Sampling

- The goal is to sample the largest diversity
- *Saccharina latissima*: Genetic diversity at European scale – 18 Microsatellites loci



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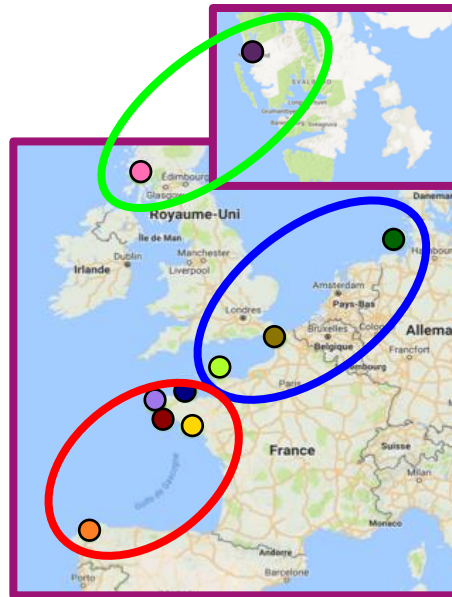
## LESSON 2 – Advanced methods for seaweed breeding



### Seaweed breeding process

## 2 GENOME WIDE ASSOCIATION STUDY (GWAS)

### 1. Sampling



- 1**  
Svalbard
- 2**  
Helgoland
- 3**  
Cap Lévi
- 4**  
Parc Marin d'Iroise
- 5**  
Ellenabeich
- 6**  
Audresselles
- 7**  
Goëlo
- 8**  
Saint Guénolé
- 9**  
Locmariaquer
- 10**  
Portino de Dexo



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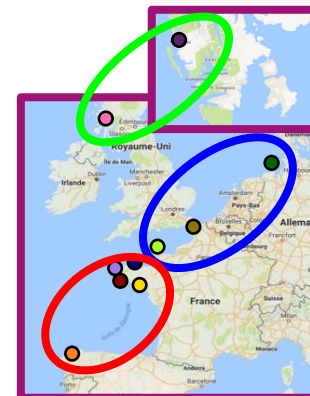
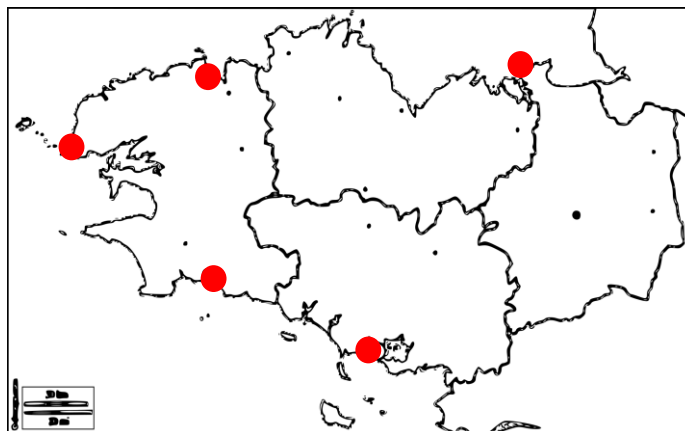
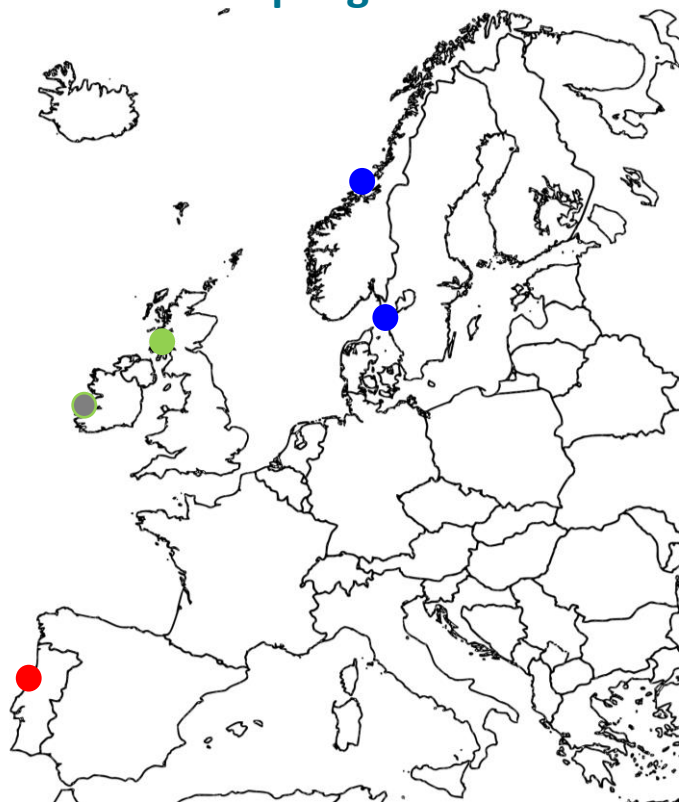
## LESSON 2 – Advanced methods for seaweed breeding

### Seaweed breeding process



## 2 GENOME WIDE ASSOCIATION STUDY (GWAS)

### 1. Sampling



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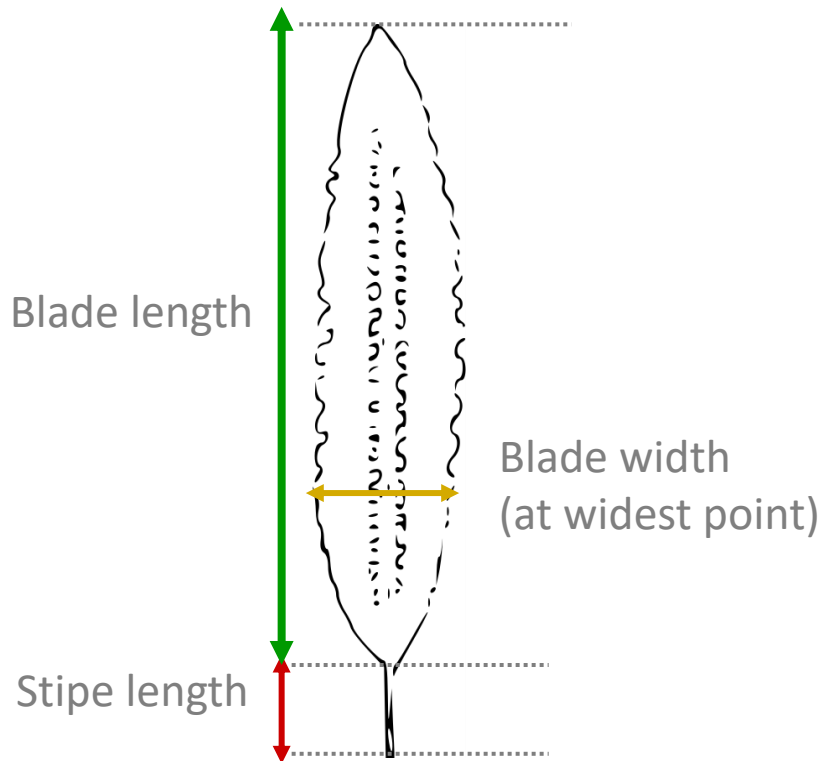
## LESSON 2 – Advanced methods for seaweed breeding

### Seaweed breeding process



## 2 GENOME WIDE ASSOCIATION STUDY (GWAS)

### 1. Sampling



- 10 populations sampled
- 30 « parent » sporophytes each (total 300 individuals)

+ DNA sample for genotyping



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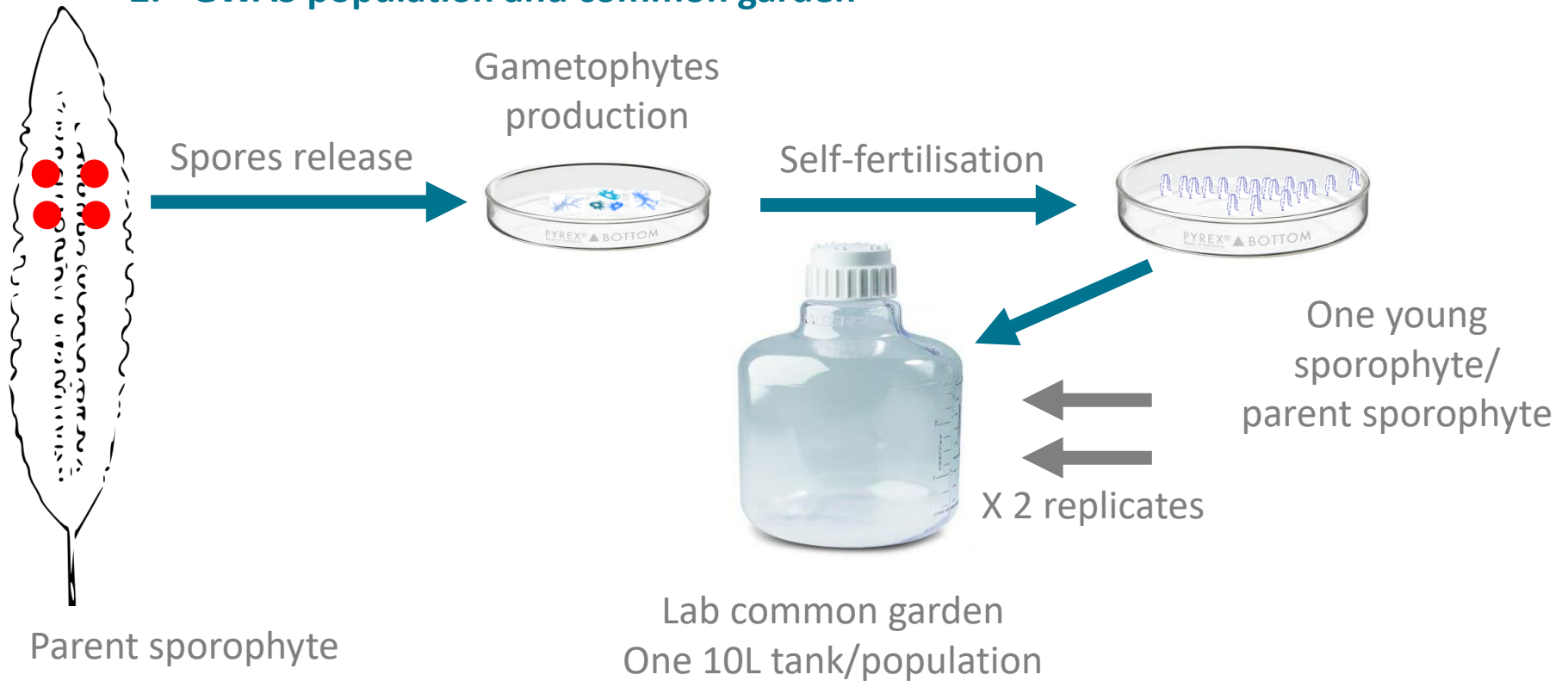
## LESSON 2 – Advanced methods for seaweed breeding



### Seaweed breeding process

#### 2 GENOME WIDE ASSOCIATION STUDY (GWAS)

##### 2. GWAS population and common garden



### Seaweed breeding process



#### 2 GENOME WIDE ASSOCIATION STUDY (GWAS)

##### 3. Traits to be phenotype

- Dry weight / Wet weight ratio
- Alginates content
- Iodine content
- Stress tolerance - Depending of growth of the sporophytes in the lab



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## LESSON 2 – Advanced methods for seaweed breeding

### Seaweed breeding process



#### GENETIC LINKAGE

- Two loci physically linked tend to remain together after the meiosis;
  - They do not recombine.
- If we know the parental alleles, we can calculate the distance between two loci by calculating the recombination rate;
- We can now map specific traits on the genome: major genes or Quantitative trait loci.



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#### GENOTYPING

### Double Digested Restriction Associated DNA sequencing (ddRAD-Seq)

Genomic DNA

Digestion with two different restriction enzymes

GNNNNNNNGCG  
ACGTCNNNNNNNC

Ligation of adaptor that adds barcode to each fragment

Pooling

PCR amplification and addition of index (each individual has a different combination of barcode and index)





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## LESSON 2 – Advanced methods for seaweed breeding

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### GENOTYPING - Library preparation and sequencing

#### Double Digested Restriction Associated DNA sequencing (ddRAD-Seq)



Illumina sequencing



125 pb sequences identified by index+Barcode



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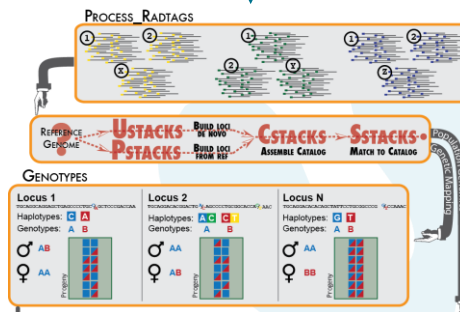
## LESSON 2 – Advanced methods for seaweed breeding

### Seaweed breeding process



### Double Digested Restriction Associated DNA sequencing (ddRAD-Seq)

125 pb sequences identified by index+Barcode



Genotypes table for mapping softwares



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### Seaweed breeding process



Map, select and follow specific traits

Select parents on the field to create specific culture population

Create, select and improve culture populations (size, stress, tolerance...)

Understand the genetic basis of the traits to better follow them

Produce better crops adapted to specific purposes

Manipulate biomolecule content to suit specific downstream applications





**LESSON 2 OF  
MODULE 3  
FINISHED**

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