

DNA profiling workstream outcome

Masami Inaba, Ronan Sulpice
NUI Galway

Topics

1. DNA markers for geographic provenance as a tool for branding of high quality seaweeds from the NPA regions
2. Metabarcoding assay for identification of commercial seaweed species worldwide

DNA markers for geographic provenance

Aim: Branding seaweeds via creating a seaweed label based on geographical origin, and ensure traceability

What we need: A tool to determine seaweed geographic provenance and species identity

Such tool could also be used to monitor invasion of alien strains / effects of climate change on the diversity of seaweed populations.

Strategy

- (1) Assembly of reference genomes
- (2) Next generation sequencing (NGS) of individuals collected from various sites in the NPA regions
- (3) Identification of country-specific single nucleotide polymorphisms (SNPs)
- (4) Design of assays to detect the SNPs

Alaria esculenta



Individuals collected from 12 sites

An individual from Oyrarjógv, FRO, was used to assemble reference genomes

Palmaria palmata

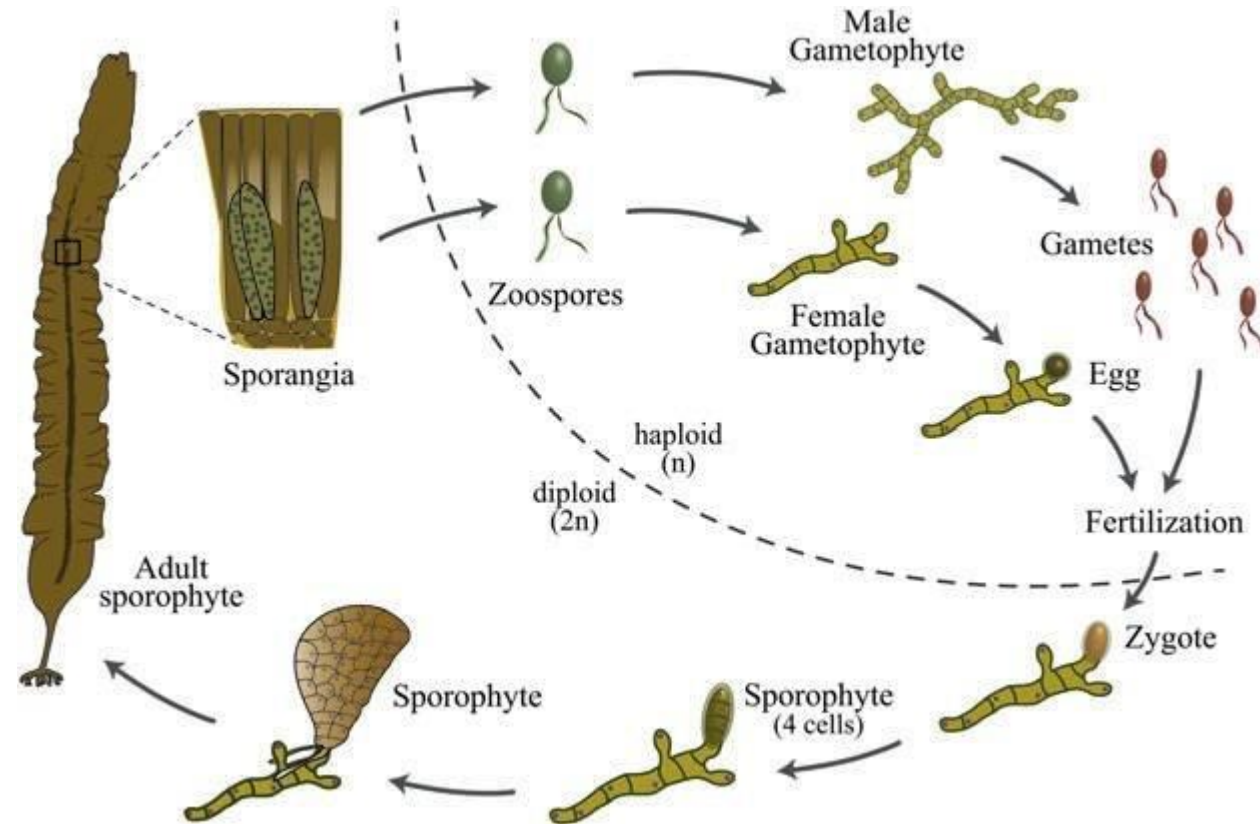


Individuals collected from 12 sites

Attempts to assemble reference genomes unsuccessful so far due to heterozygosity

Life cycle of kelp

Sporophyte was used for reference genome assembly

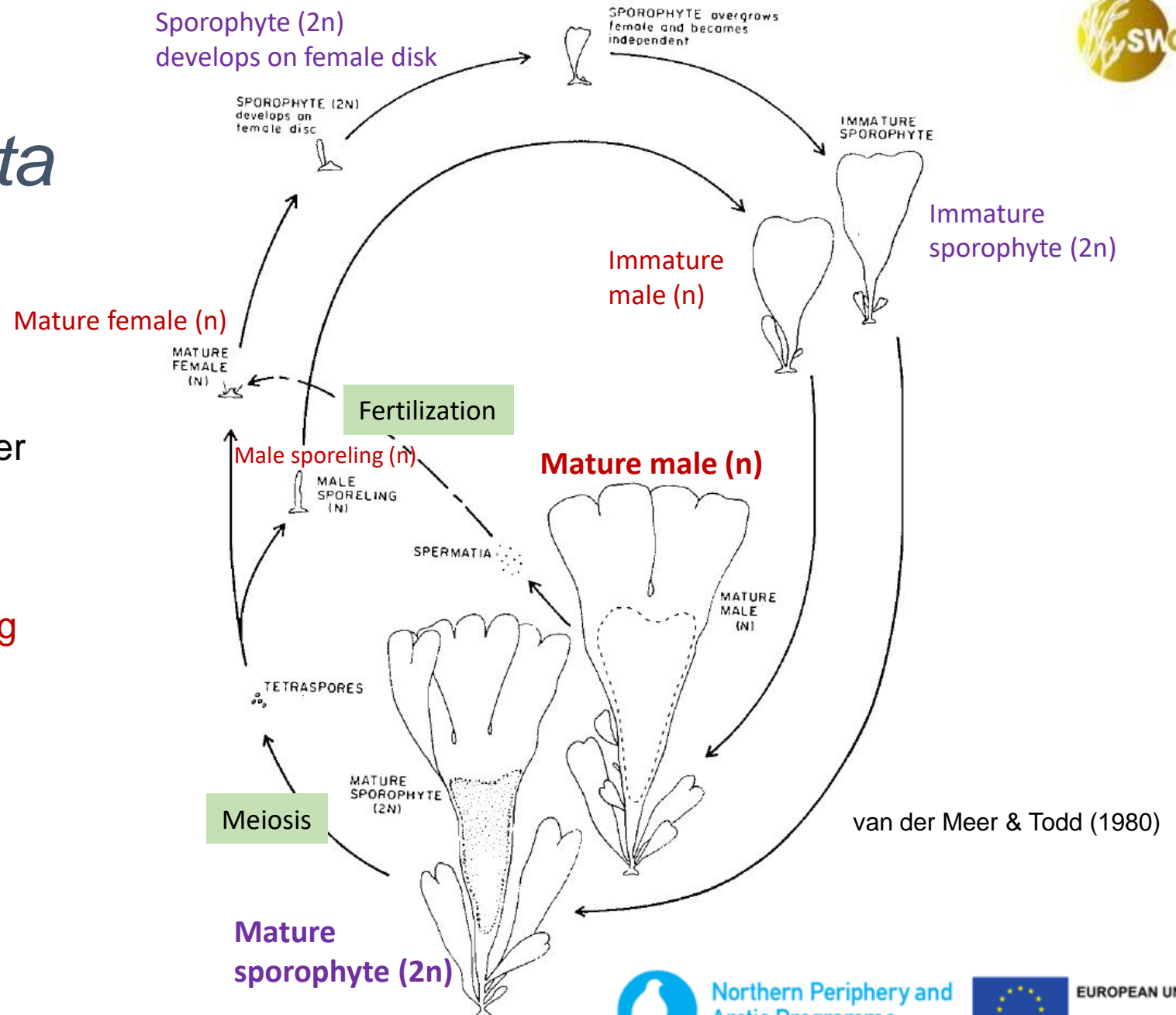


Visch et al. (2019)

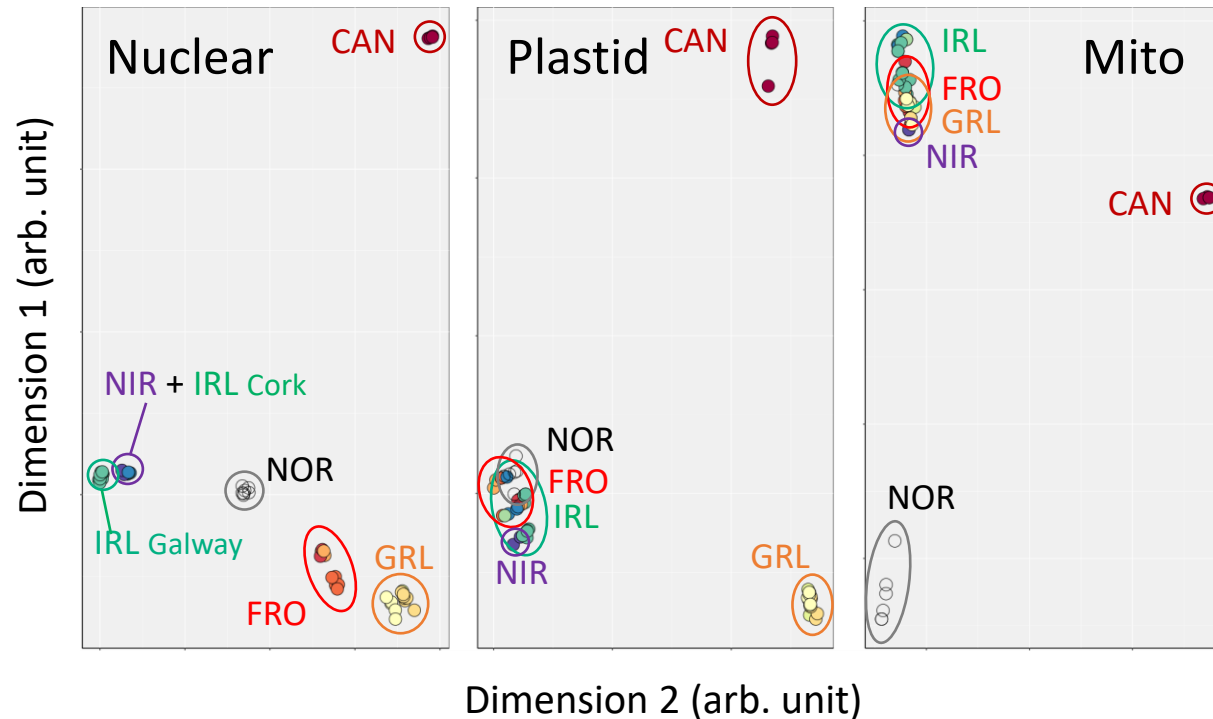
Life cycle of *Palmaria palmata*

Mature fronds are either male haploid or sporophytes, the latter of which can be homo or heterozygous.

→ Find haploid individuals using flow cytometry



Multidimensional scaling (MDS) plots of SNPs on *Alaria* genomes



- CAN, Wallace Cove, Bay of Fundy
- FRO, Famjin
- FRO, Hvitanes
- FRO, Oyragjogv
- GRL, Kobbefjord, Nuuk
- GRL, Kodoen, Qeqertarsuaq, Disko Bay
- GRL, Maniitsoq, Qeqqata
- IRL, Gearhies, Cork
- IRL, Inis Mor, Galway
- IRL, Long Strand, Cork
- NIR, Ballycastle, Antrim
- NOR, Malangenfjord

MDS plot of SNPs on the nuclear genome, but not those on the organellar genomes, shows a clear geographic separation of individual samples

Also part of Bringloe, Trevor; Fort, Antoine; **Inaba, Masami; Sulpice, Ronan; Ní Ghríofa, Clíodhna; Mols-Mortensen, Agnes;** Filbee-Dexter, Karen; Vieira, Christophe; Kawaii, Hiroshi; Hanyuda, Takeaki; Krause-Jensen, Dorte; Olesen, Birgit; Verbruggen, Heroen, **Molecular Ecology**, in review

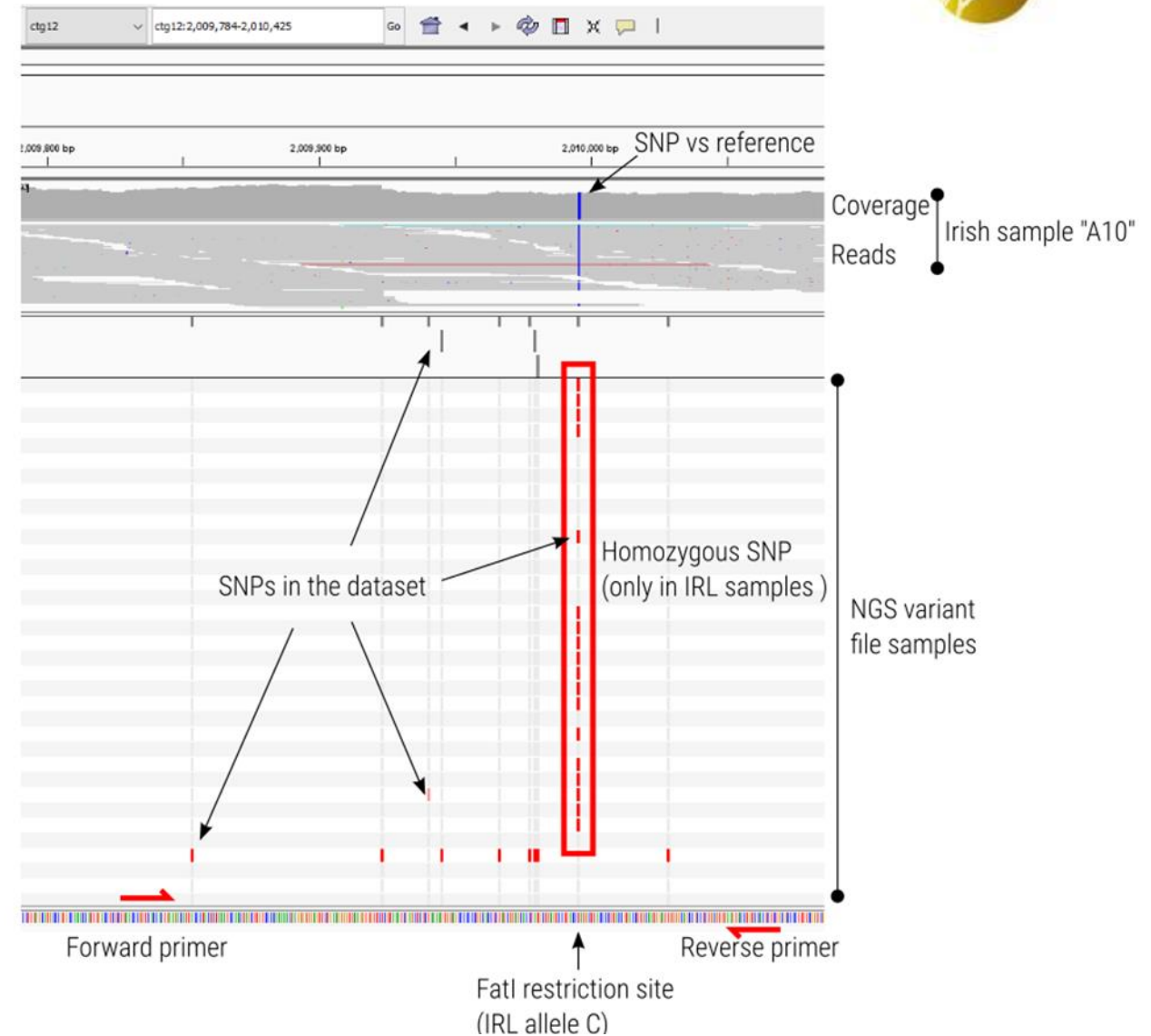
Design of assay to detect country-specific SNPs

Cleaved amplified polymorphic sequences (CAPS) assays

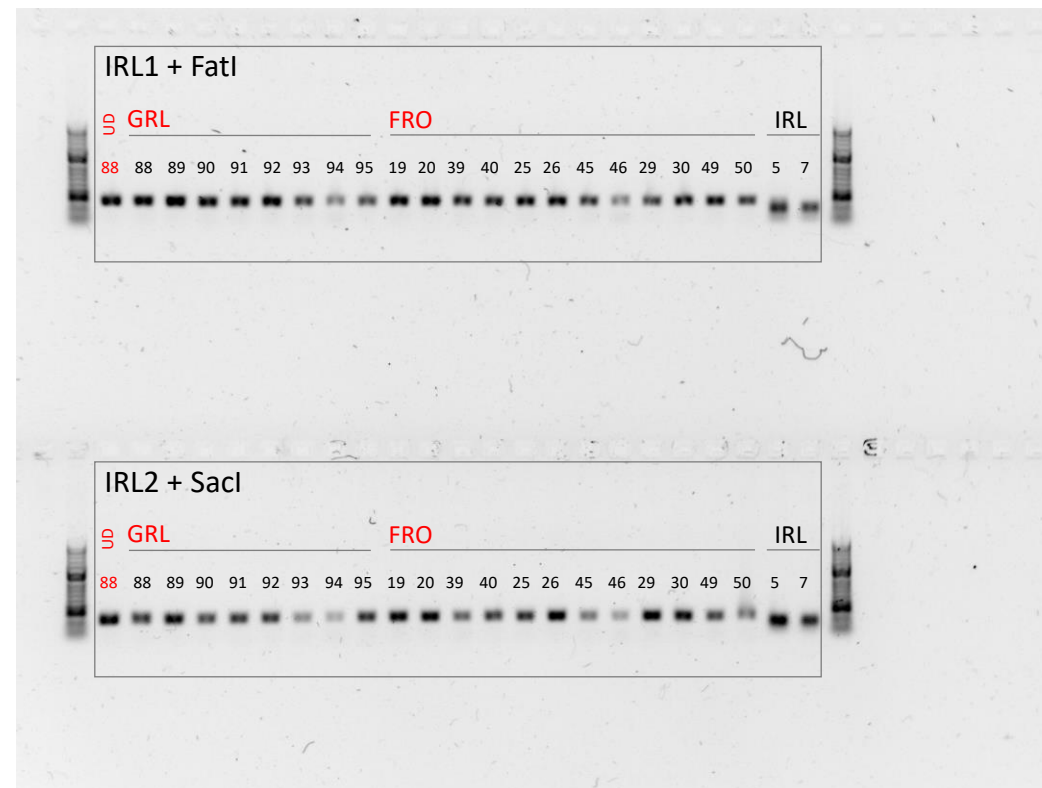
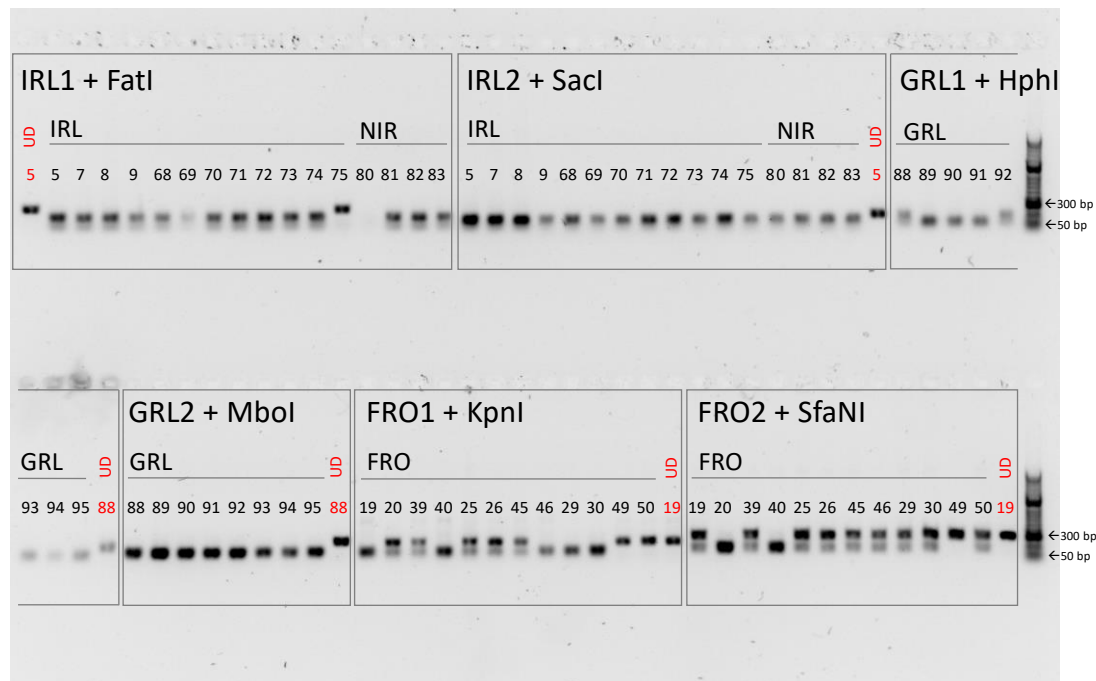
Example: IRL1_Fat1

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TTATGTAGCCTTTAGCATGCCCGTGCCAAACCATTTGACCTC
TCTTAACCCGTTCAACCACTGAAGACATTACCCTACTCTAAAT
CCAACCTAGCTTGTCCCCAAAACGAGGGTGGAGTTCTGAAGG
GGTTAAGGTGTTTCTCTTAAATCGAGCTCTCTAAAAGTGTAG
CCCCCCTGCACCTCTATCGTAGCACCTATGCTTAGTCCTTA
CCCATGCTTACCCCTTACCGTACCTTGCAATGGATTGTGGCA
GCCGCCCTGCGTTGAAGTACCTGTCCACTCTCTGCGCCCCCT
TTTCGGT
    
```



CAPS assay detects individuals from the country



Inaba, Masami; Fort, Antoine; Bringloe, Trevor; Mols-Mortensen, Agnes; Ni Ghriofa, Clíodhna; Sulpice, Ronan, Algal Research, in review

Metabarcoding assay for identification of commercial seaweed species

Quality characteristics of seaweeds are species-dependent. But we don't have reliable methods to identify species in commercial seaweed products.

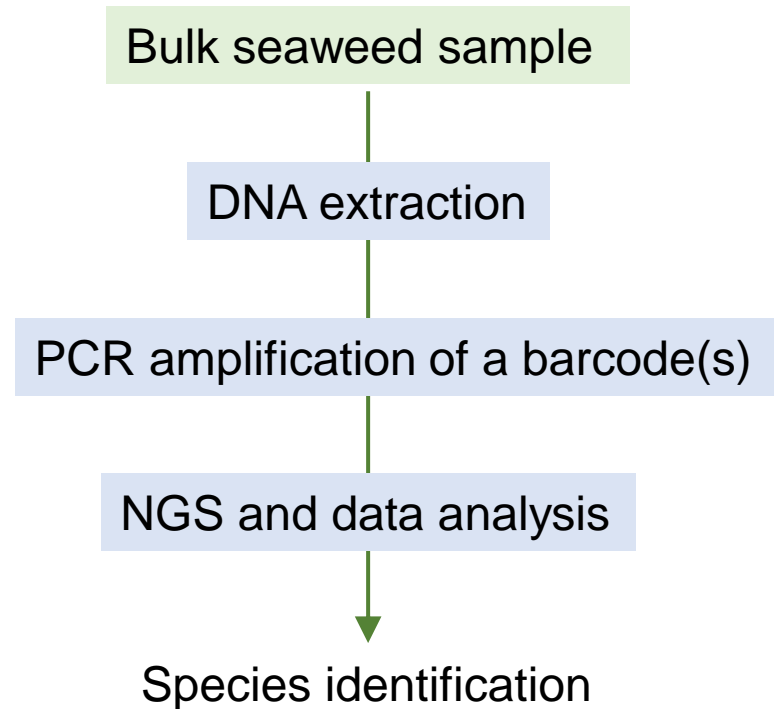
What we need: Establish a robust and reliable tool to identify species in seaweeds sold worldwide

→ Metabarcoding

Masami Inaba and Sandra Garcia

Metabarcoding

DNA barcoding + NGS = Simultaneous identification of multiple species in a sample



Steps

- (1) Design universal primers for brown, red and green algae
- (2) Collect seaweed samples to test the primers
- (3) Find suitable NGS platform and protocols
- (4) Test on commercial seaweed products

Universal primers in traditional barcode regions

- *rbcL*, *tufA*, *cox1*, ITS – sequences collected from NCBI
 - Most of published *rbcL* and *cox1* primers cannot be used
 - Designed 11 pairs of primers on *rbcL* and *cox1* regions
 - Tested the primers on seaweeds collected in Galway and elsewhere
 - PCR & Sanger sequencing successful
- ❖ Search for new barcodes by comparing organellar genome sequences
going on

NGS platforms

Various platforms using different sequencing chemistry and technologies

Illumina

Currently most widely used system for e-metabarcoding / short reads

Roche 454

Long reads / high costs

Oxford Nanopore

Long reads, less expensive / high error rates

We are collecting protocols and identifying service suppliers to find which methods work best

Also, in discussions to share expertise with Olivier De Clerck, who is developing same BUT only for greens.

Summary outcomes

DNA markers for geographic provenance

For *Alaria esculenta*, we identified DNA markers (country-specific SNPs) and established a method to detect. These results were submitted to Algal Res. For *Palmaria palmata*, reference genome assembly has been unsuccessful so far.

Metabarcoding for identification of commercial seaweed species

We designed universal primers for red, brown and green algae, which were successfully used for barcoding several species. Establishing protocols and search for new barcodes are on-going.