A SNP map of the European sea bass genome based on a meiotic gynogenetic family

Münnever Oral, Julie Colléter, Michaël Bekaert, John B. Taggart, Christos Palaiokostas, Brendan J. Mcandrew, Marc Vandeputte, Béatrice Chatain, David J. Penman

A SNP map of the European sea bass (*Dicentrarchus labrax*) genome based on a meiotic gynogenetic family

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AQUAEXCEL Workpackage 9: Isogenic Fish Lines

Overview

I. Background

-Importance of Clonal Lines

-Production of such lines

-Why Meiotic Gynogenesis

-Aim of the present study

II. Material & Methods

-Production of Meiotic Gynogenetics

-ddRAD lib construction

-Genetic Linkage Map (R/OneMAP) & Physical Map

III. Results & Discussion

-ddRAD reads summary

-Genetic Linkage Map

-Physical Map

-Crossover hotspots

IV. Conclusion & Suggestions

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I.Background: Importance of Clonal Lines to research

Clonal Lines = Genetically Identical Individuals

- Homogenity (powerful for the investigation of complex genetic traits, decreases the variation in experiments)
- Standardisation to the research –refined experimental design (Aquaculture related research point of view)
- Speed of generation (2 subsequent production cycles via Gynogenesis-G1 or Androgenesis-A1)
- Eliminates deleterious recessive alleles, reveals genetic variation for many traits
- QTL (Quantitative Trait Loci) identification and whole genome sequencing projects

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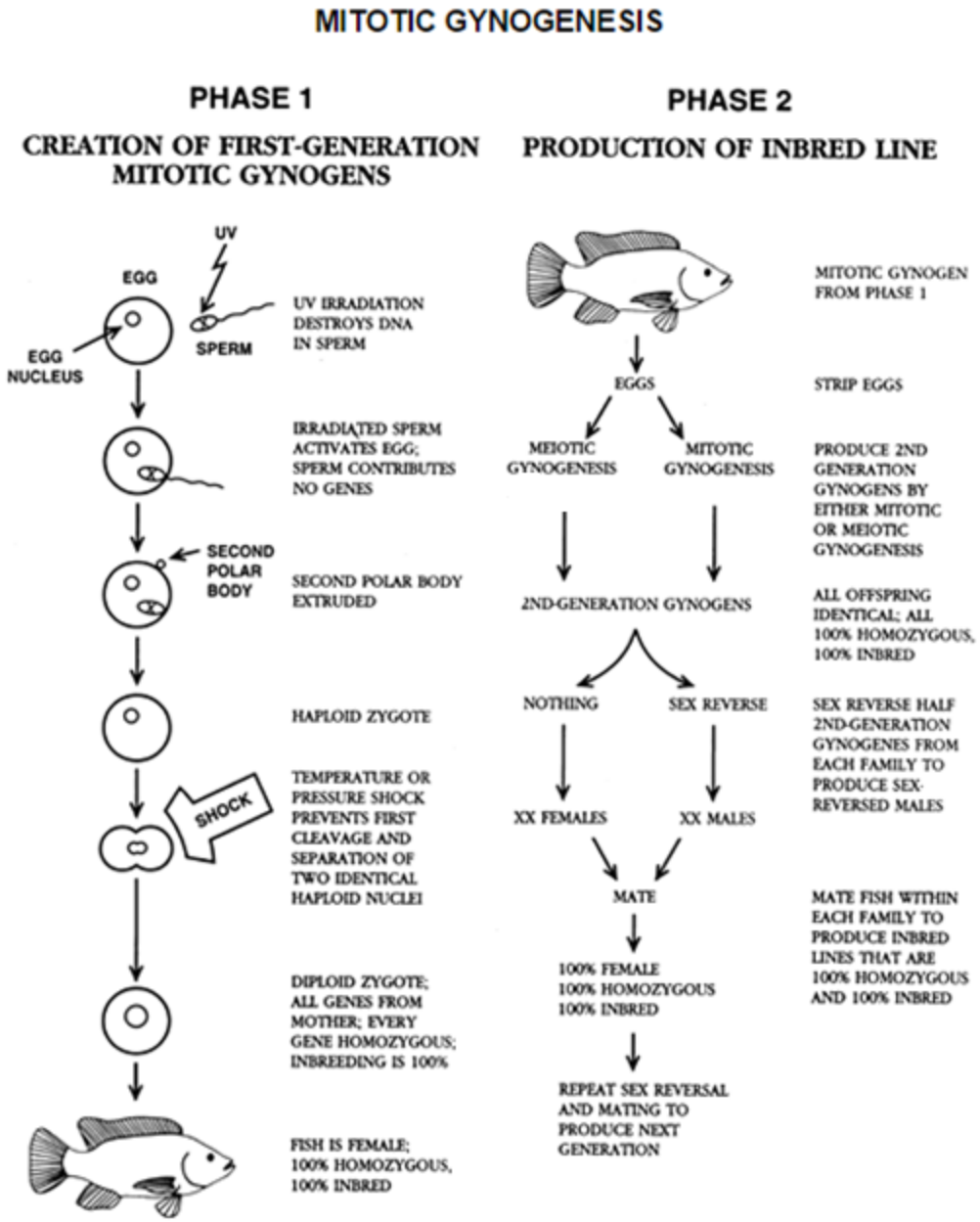
I.Background: Production of Clonal Lines



Gynogenesis (G): All maternal inheritance
Androgenesis (A): All paternal inheritance

Spontaneous meiotic gynogenetics can be a problem when producing isogenic clonal lines via mitotic gynogenesis.

FAO, *Inbreeding and Broodstock Management* Chapter 6, Chromosome Set Manipulations.

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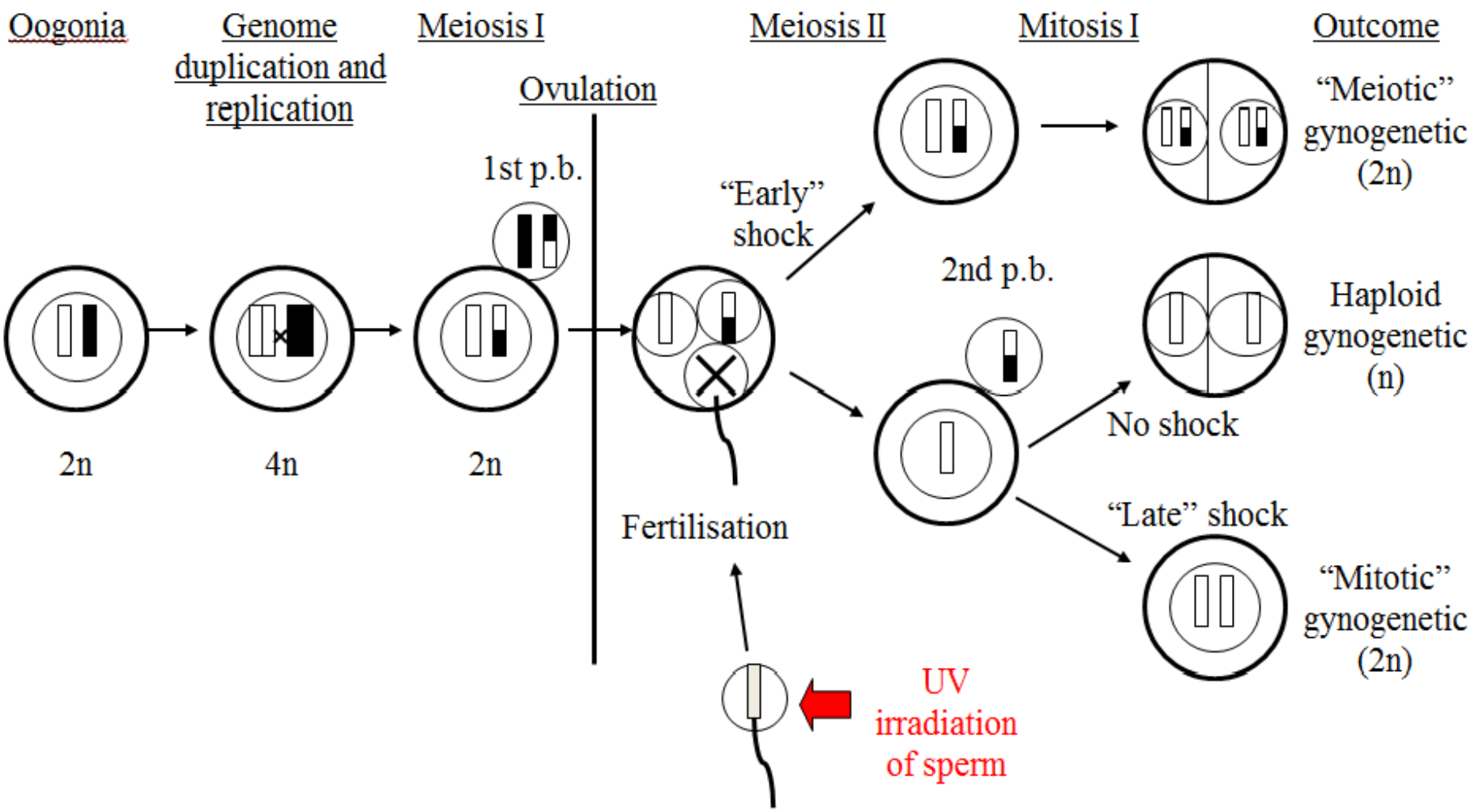
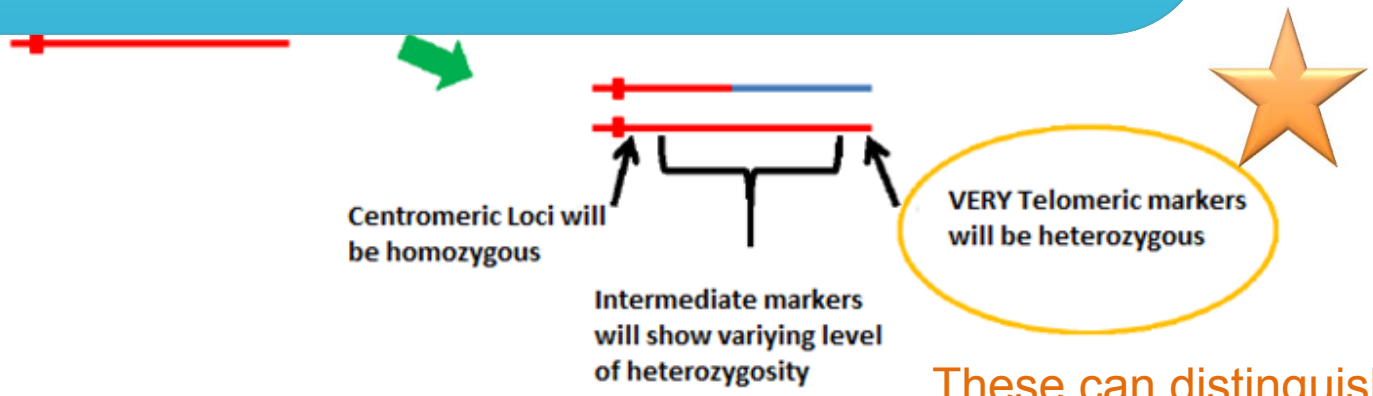
I.Background: Meiotic Gynogenetics



Reliable markers are needed to distinguish meiotic and mitotic gynogenetics

Aim of the study

was to define **telomeric markers** by constructing a SNP based genetic linkage map in *D. labrax* by facilitating the power of high-throughput sequencing to differentiate between meiotic to mitotic gynogenetics.



These can distinguish between meiotic and mitotic gynogenetics

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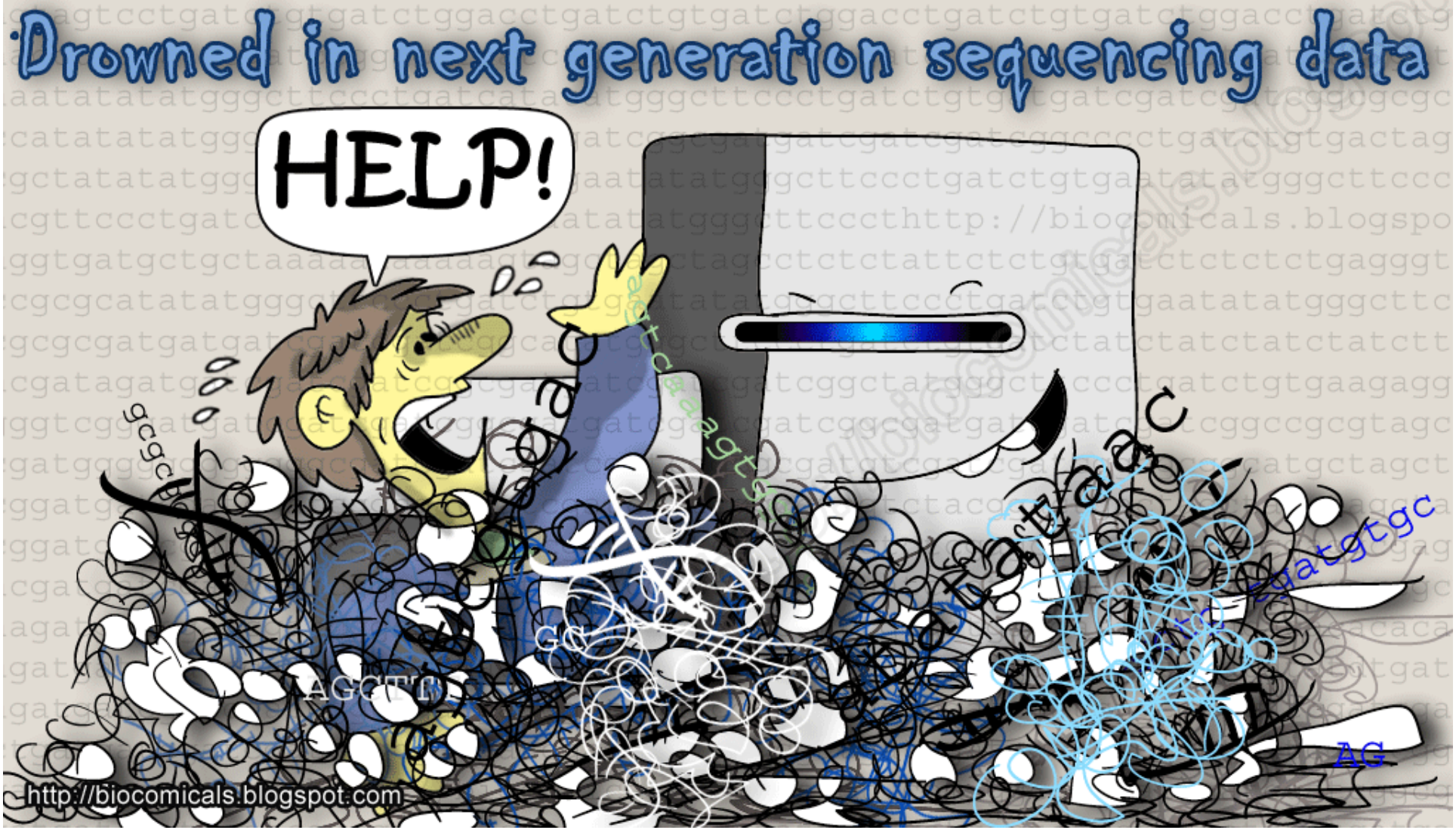
Next Generation Sequencing (NGS)



www.biocomicals.com, Alper Uzun, PhD

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II.Material & Methods:Production of Meiogynogenetics

Ifremer Experimental Aquaculture Station (Palavas-les-Flots, France).

UV irradiation device:

- Four lamps above
- Four lamps below (12 W, 254 nm, Vilber-Lourmat, Marne-la-Vallée, France)

Sperm irradiation (1:20, v/v in SGSS) for 8 minutes was applied for total dose of 326mJ/cm² which corresponds to the dose of 32000erg/mm² described by (Peruzzi & Chatain, 2000).

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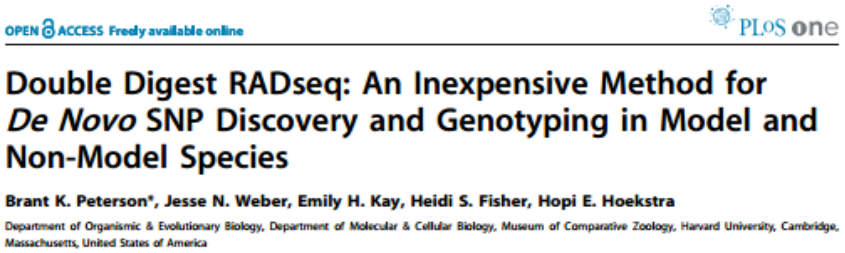
II.Material & Methods

DNA extraction

- One Meiotic Gynogenetic *D. labrax* family:
  - Sire (♂) and dam (♀):Fin Clips
  - 80 offspring: Whole larvae
- REALpure kit (REAL Laboratories Spain)
- Nanodrop Spectrophotometry
- Agarose Gel Electrophoresis
- Final DNA concentration assessment: Quantica qPCR thermal cycler by using dsDNA fluorescent dye.

Double digest-ddRAD library preparation and sequencing


OPEN ACCESS Freely available online

PLoS one

Double Digest RADseq: An Inexpensive Method for *De Novo* SNP Discovery and Genotyping in Model and Non-Model Species

Brant K. Peterson*, Jesse N. Weber, Emily H. Kay, Heidi S. Fisher, Hopi E. Hoekstra

Department of Organismic & Evolutionary Biology, Department of Molecular & Cellular Biology, Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, United States of America

- Peterson et al., 2012

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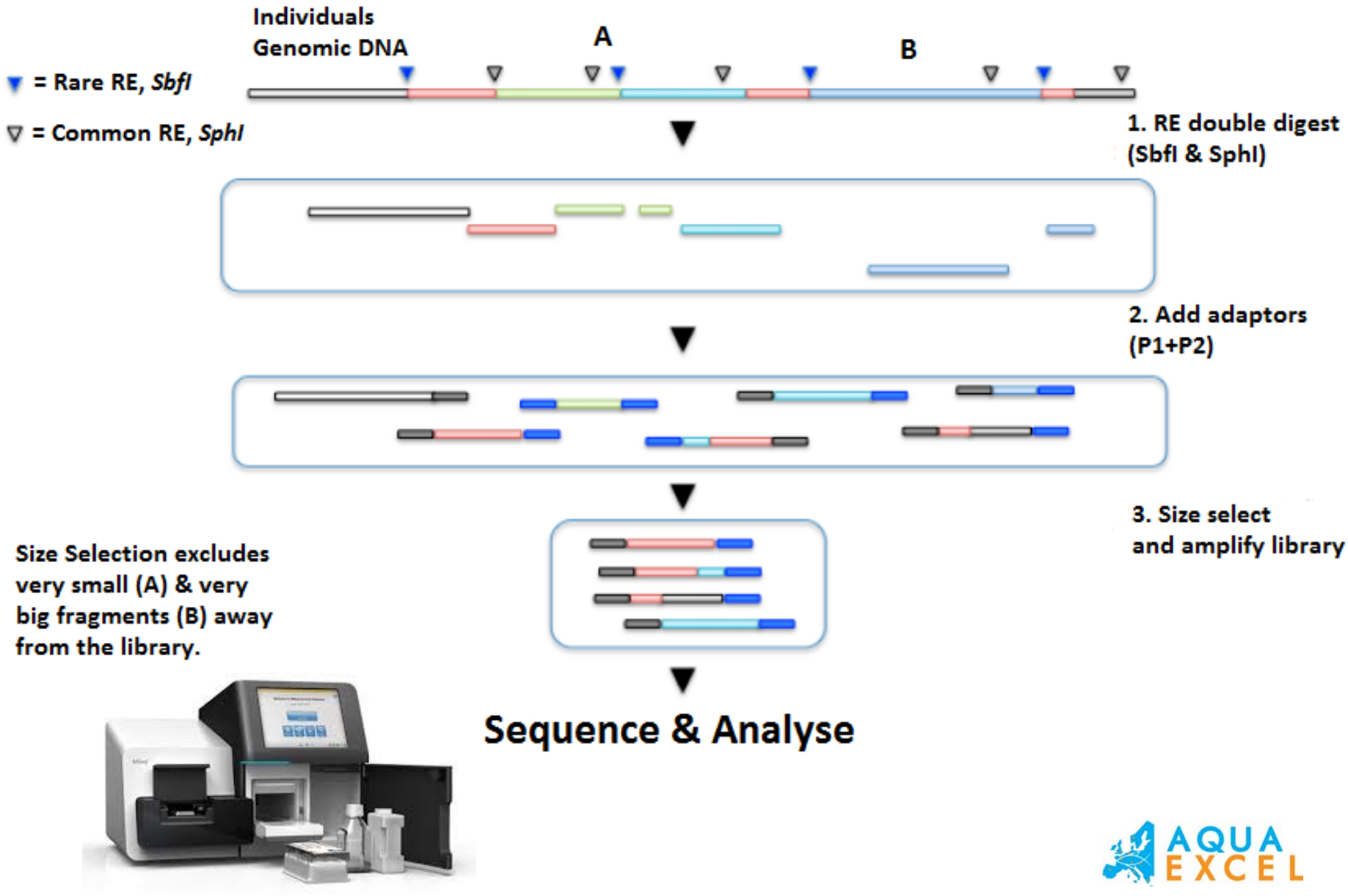
II.Material Methods: Double Digest RAD seq (ddRAD)



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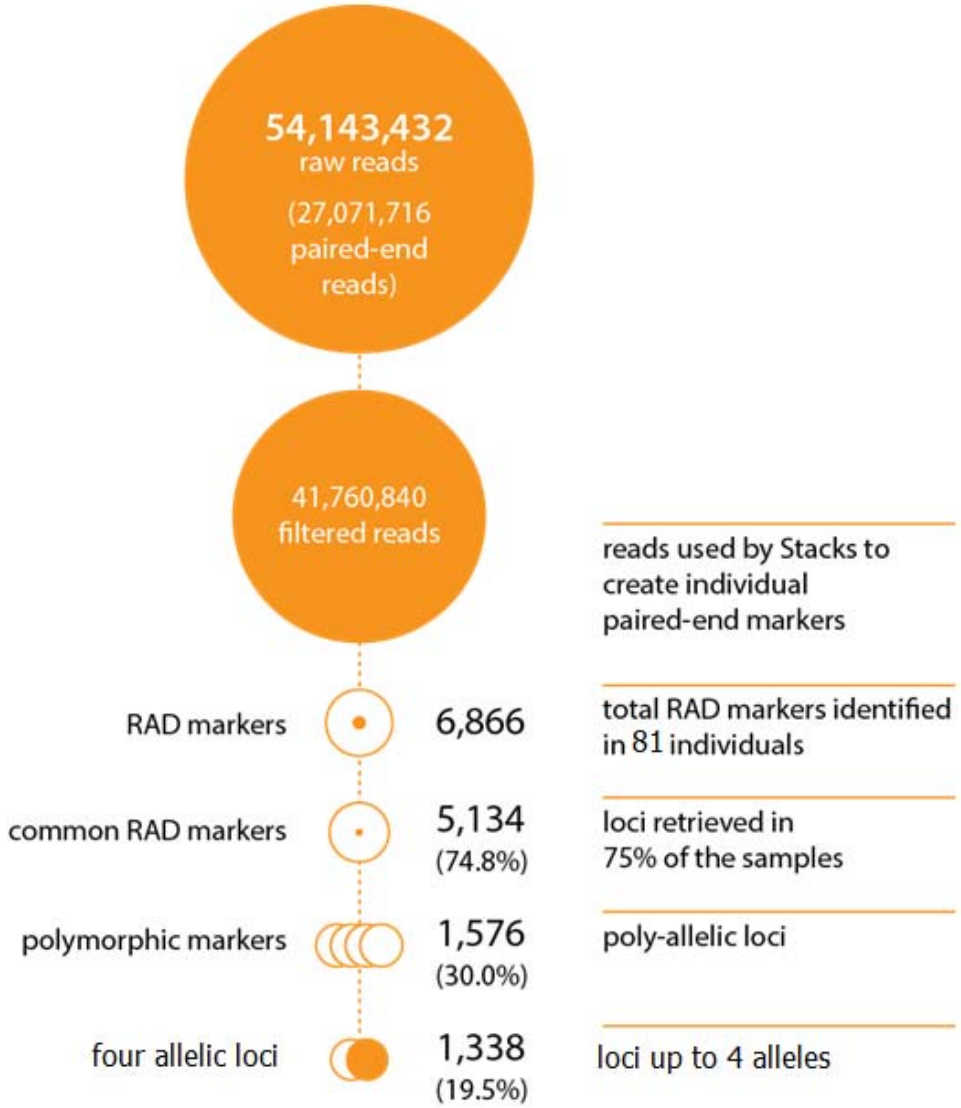
III.Results: Sequencing & RAD tag summary



From purified gDNA to the genotypes takes 1 week.

EBI Sequence Read Archive (SRA) with the accession number of: ERP006697.

***Raw Reads**: All reads have been produced by sequencer.*
***Filtered reads**: Reads with right barcodes & adapters combination.*
Stacks package (Catchen et al., 2011).

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II.Material Method: Genetic Linkage Map

- 340 male specific markers (no paternal inheritance detected)
- **R/OneMap** v2.0-4 (Margarido *et al*.,2007) & **TMAP** v1.1 (Cartwright *et al*., 2007).
  - *804 female heterogametic Input file: Outcross (Kosambi function*
  - *rf.2pts*
  - *LOD=4, max.rf=0.5* ***(draft seabass genome assembly)***
  - *order.seq (ser,rcd,rec,ug)*
  - *safe & force*
  - *rf.graph.table*
- *Final genetic map:* Genetic-Mapper v0.7 (Bekaert, 2012).

Table 2: Notation used to identify markers and genotypes

			Parent			Offspring	
		crosstype	Cross	Observed bands		Observed bands	Segregation
A		1	$ab \times cd$	$ab \times cd$		ac, ad, bc, bd	1:1:1:1
		2	$ab \times ac$	$ab \times ac$		a, ac, ba, bc	1:1:1:1
		3	$ab \times co$	$ab \times c$		ac, a, bc, b	1:1:1:1
		4	$ao \times bo$	$a \times b$		ab, a, b, o	1:1:1:1
B	B_1	5	$ab \times ao$	$ab \times a$		$ab, 2a, b$	1:2:1
	B_2	6	$ao \times ab$	$a \times ab$		$ab, 2a, b$	1:2:1
	B_3	7	$ab \times ab$	$ab \times ab$		$a, 2ab, b$	1:2:1
C		8	$ao \times ao$	$a \times a$		$3a, o$	3:1
D	D_1	9	$ab \times cc$	$ab \times c$		ac, bc	1:1
		10	$ab \times aa$	$ab \times a$		a, ab	1:1
		11	$ab \times oo$	$ab \times o$		a, b	1:1
		12	$bo \times aa$	$b \times a$		ab, a	1:1
		13	$ao \times oo$	$a \times o$		a, o	1:1
	D_2	14	$cc \times ab$	$c \times ab$		ac, bc	1:1
		15	$aa \times ab$	$a \times ab$		a, ab	1:1
		16	$oo \times ab$	$o \times ab$		a, b	1:1
		17	$aa \times bo$	$a \times b$		ab, a	1:1
		18	$oo \times ao$	$o \times a$		a, o	1:1

Refer to: Wu *et al*. (2002) Simultaneous maximum likelihood estimation of linkage and linkage phases in outcrossing species.

Physical Map

- 804 female heterogametic SNP markers as well as 11 microsatellites have been assigned in to European sea bass draft genome contigs across 24 LGs.
- Has been used to define recombination points.

MB_microsatellites position on physical map
Marker_ID,Phsical_Map_(Genome_Location),Phsical_Map_Position_(bp)

Dla0003,LG19,8127283
Dla0006,LG6,25269003
Dla0104,LG2,24689183
Dla0105,LG8,10127559
Dla0106,LG2,25784997
Dla0112,LG5,12681454
Dla0119,LG14,8349079
Labrax17,LGX,16504896
Labrax29,LG18-21,9732294
Labrax3,LG13,6001586
Labrax8 ,LG16,2733963

(Chistiakov *et al*., 2005)
(García De León *et al*., 1995)

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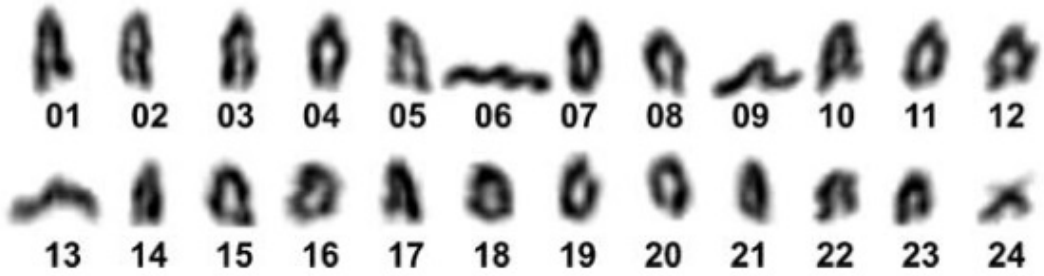
III.Results: Meiogynogenetic Linkage Map (R/OneMap)



Basic karyotype, 2n=48

LGs	Number of Markers	Length (cM)
LG1A	45	78.04
LG1B	29	51.30
LG2	30	61.83
LG3	24	22.79
LG4	34	44.10
LG5	38	68.19
LG6	26	55.59
LG7	26	72.31
LG8	31	47.92
LG9	23	46.00
LG10	30	34.68
LG11	42	54.26
LG12	29	47.25
LG13	27	54.03
LG14	31	66.67
LG15	37	61.31
LG16	37	49.89
LG17	45	55.03
LG18-21	15	30.23
LG19	30	56.96
LG20	46	45.82
LG22-25	39	62.70
LG24	19	39.07
LGX	31	45.05
Total	**764**	**1252.02**

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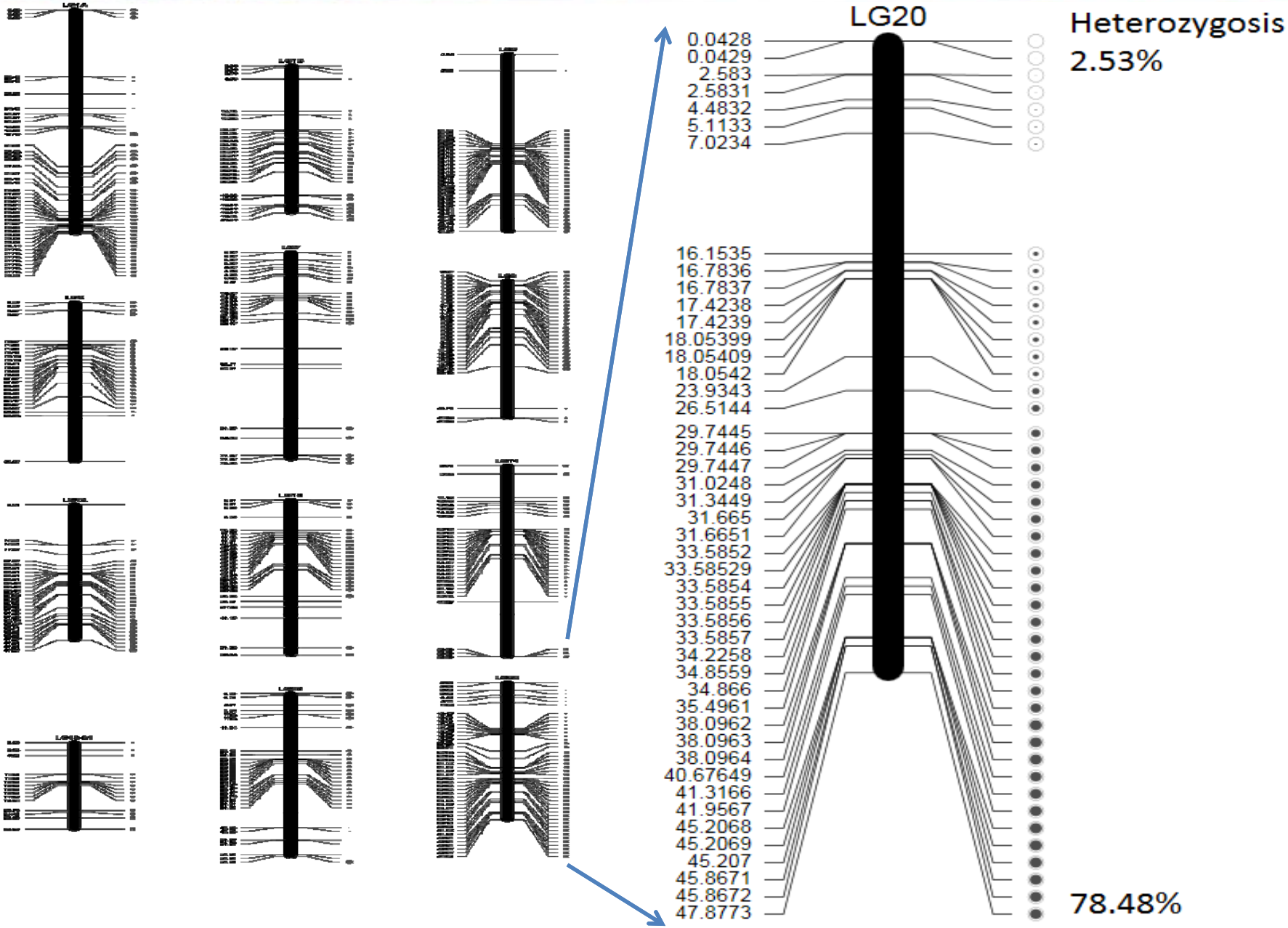
III.Results: Final *D.labrax* Meiotic Genetic Map

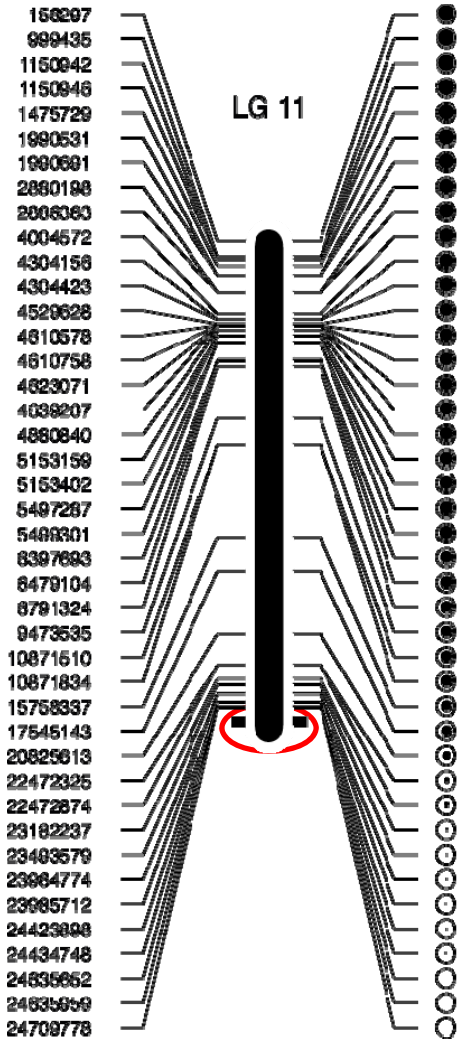
Empty circles: Homozygote
Black dots: Heterozygote



LG11

Empty circles: Homozygote
Black dots: Heterozygote

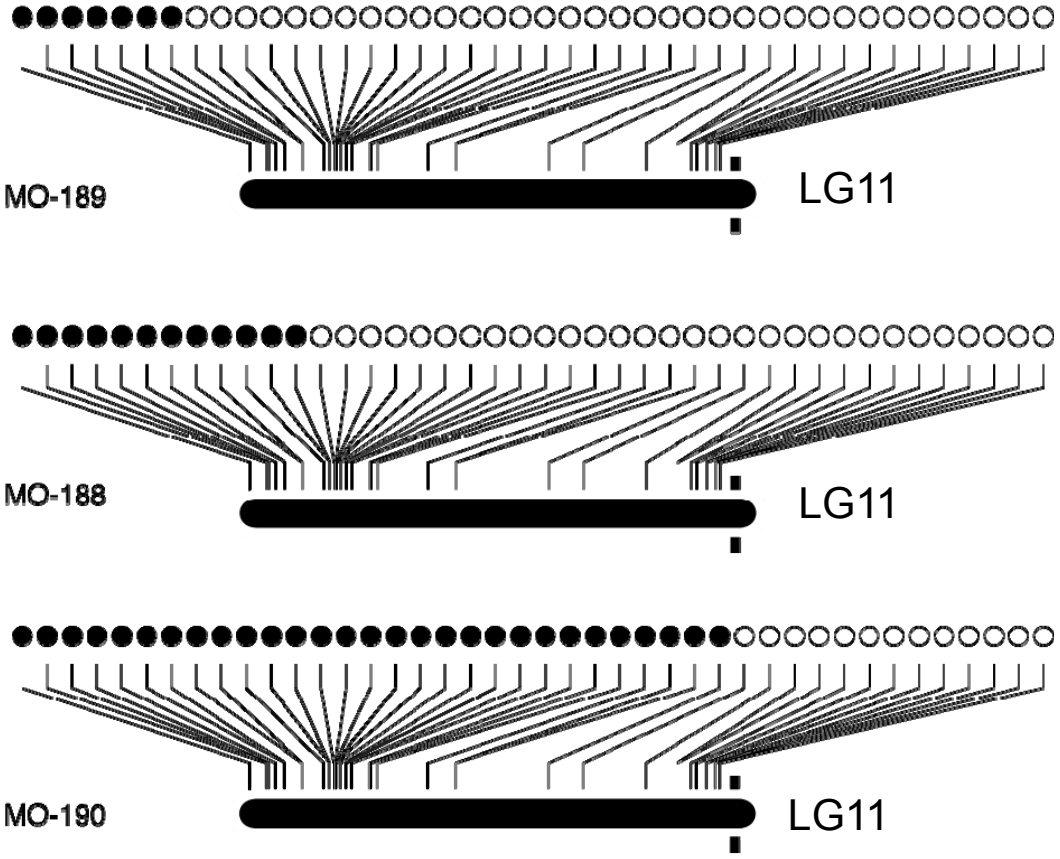
Overall



Heterozygosity
Max:87.17%

Min:3.84%

Individual progeny



Average 0.90±0.08 crossover per chromosome arm.

IV. Conclusion

- 764 SNP markers have been identified and mapped from the single meiogynogenetic *D. labrax* family with 79 offspring+parents.
- Crossover points per chromosome arm have been identified per linkage group with an average of 0.90±0.08 crossover per chromosome arm.
- Particularly those markers closer to telomere are of interest to differentiate between meiotic and mitotic gynogenesis for the reliable production of isogenic clonal lines (meiotic gynogenetics need to be detected and eliminated).
- Cost effective and quick (<£10 per individual, up to 100 individuals and >500 SNPs in one sequencing lane)
- Genotyping by (RAD) sequencing rather than isolating and assaying these individually or in multiplexes.

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V. Future work

- Application to development of Isogenic clonal lines in:
  - Seabass (*Dicentrarchus labrax*),
  - Common carp (*Cyprinus carpio*),
  - the Atlantic salmon (*Salmo salar*)
  - (Nile Tilapia (*Oreochromis niloticus*))

- Gametic inactivation in each species using UV irradiation and perhaps X-rays (androgenesis – chromosome fragments?).
- Distinguishing between meiotic and mitotic gynogenetics
- Genetic Linkage map.
- Verification of isogenic lines through RADseq (starting from outbred founders (parents of G1/A1 fish) to G1/A1 clone founders (with biparental control) and to isogenic lines.

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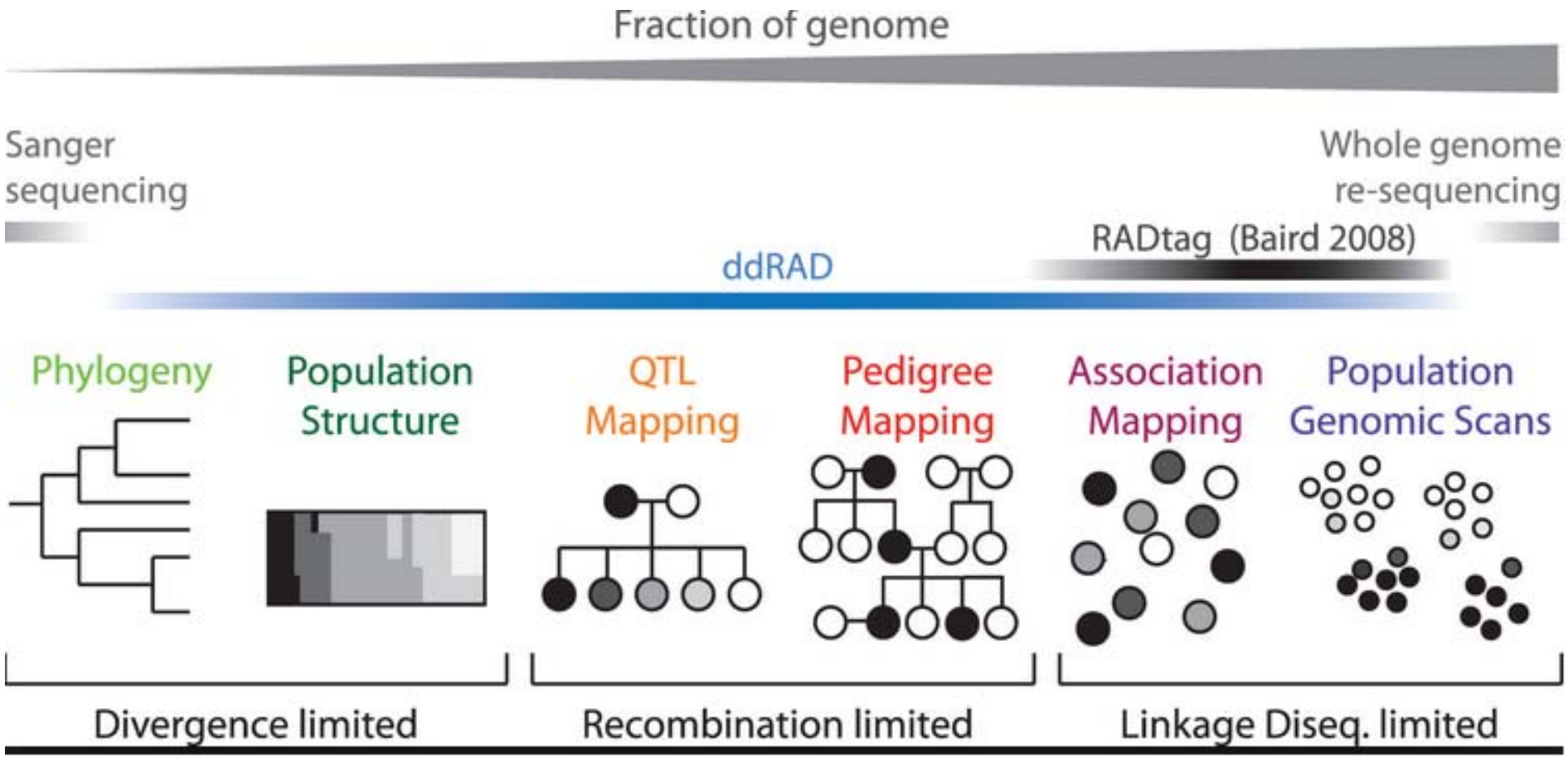
Experimental Approaches by using Genotyping by Synthesis



Baird *et al*., 2008 (RAD seq) & Peterson *et al*., 2012 (ddRAD seq)

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Would you like to find out more?

AQUAEXCEL INDUSTRY WORKSHOP:

Research Infrastructures: adding value to European aquaculture industry

Friday, 17th October
Kicking off at 10.30am
Room 11 (Exhibition Area)

See you there!

Contact us

Thank you for your attention

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DISCLAIMER

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