ASSEMBLE

ASSOCIATION OF EUROPEAN MARINE BIOLOGICAL LABORATORIES EXPANDED

JRA3 – Functional genomics

2nd General Assembly, Galway, October 10th-12nd 2018

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 730984. This output reflects the views only of the author(s), and the European Union cannot be held responsible for any use which may be made of the information contained therein.

CONTEXT

- The combination of systemic and small-scale approaches to establish links between genomic information and phenotypes is a central issue in modern biology

- While systemic approaches (i.e. genomics, transcriptomics, epigenomics) have been (or are being) developed for several model marine organisms, small-scale functional approaches are significantly lagging.

- In this JRA3 we propose to fill this gap by implementing (and where necessary adapting) functional genomics approaches for a panel of emerging marine model organisms.

- Several techniques for generation of genetic resources are well established in the laboratories of partners participating in this JRA.

- Examples:
  - *in situ* protocols are established in all metazoan models
  - transgenesis is working efficiently for ascidians, the cnidarian *Clytia hemisphaerica*, sea urchins and the cephalochordate amphioxus.
  - RNAi and/or targeted KO approaches have already been developed for the brown macroalga *Ectocarpus* and some microalgae
  - transient transformation with conjugation already exists for some bacterial species..

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Description of work:

Task 1: Functional genomics in marine metazoans (the cnidarian *Clytia hemisphaerica*, the acoel *Symsatigifera roscoffensis*, the sea urchin *Paracentrotus lividus* and *Strongylocentrotus purpuratus*, the cephalochordate *Branchiostoma lanceolatum*, and the ascidians (*Ciona intestinalis, Phallusia mammillata*).

Task 2: Functional genomics in macroalgae (*Ectocarpus* and kelps, especially *Saccharina latissima*)

Task 3: Functional genomics in microorganisms (the diatoms *Seminavis, Cylindrotheca, Phaeodactylum tricornutum and Pseudo-nitzschia multistriata*, the picoeukaryotes *Ostreococcus sp, Bathycoccus* and *Micromonas*, the cyanobacteria *Synechococcus* and the bacteria *Marinomonas*)

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Participants:

Task 1: Functional genomics in marine metazoans
Roscoff (SBR), Naples (SZN), Banyuls (OOB), St Andrews (SOI), Villefranche (OOV)

Task 2: Functional genomics in macroalgae
Oban (SAMS), Roscoff (SBR), Plymouth (MBA)

Task 3: Functional genomics in microorganisms
Banyuls (OOB), Plymouth (MBA), Paris (UPMC), Ghent, Naples (SZN)

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Participants/activities:

Work package	Partner	PI	Activity
Task1: metazoans	Roscoff (SBR)	X. Bailly	CRISPR *S. roscoffensis.*
	Naples (SZN)	F. Ristoratore	CRISPR *C. intestinalis*/sea urchin.
	Banyuls (OOB)	H. Escriva/S. Darras	CRISPR *P. mammillata*/amphioxus. *In situ* profiling ascidians/amphioxus.
	St Andrews (SOI)	D. Ferrier	In situ profiling amphioxus.
	Villefranche (OOV)	H. Yasuo	CRISPR *Clytia.*
	Common activity		Integration into database (Bioself Communication).
Task2: macroalgae	Oban (SAMS)	C. Gachon	CRISPR *Ectocarpus*. RNAi brown algae.
	Roscoff (SBR)	M. Cock	CRISPR *Ectocarpus*. RNAi brown algae.
	Plymouth (MBA)	C. Brownlee	Kelp viral elements for strain improvement, targeted delivery and improved product yield.
Task3: microbes	Banyuls (OOB)	F.Y. Bouget	Gene inactivation in microalgae.
	Banyuls (OOB)	R. Lamy	Gene inactivation in *Marinomonas.*
	Roscoff (SBR)	L. Garczarek	Gene inactivation in *Synechococcus*
	Naples (SZN)	M. Ferrante	CRISPR diatoms.
	Ghent	W. Vyverman	CRISPR diatoms.

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Deliverables:

- Protocols for genetic transformation of novel emerging metazoan, macroalgal and microalgal model organisms available via ASSEMBLE-plus web portal (M24)

-Protocols for the deployment of CRISPR/Cas9 system for novel emerging metazoan, macroalgal and microalgal model organisms available via ASSEMBLE-plus web portal (M36?)

- Gene expression pattern and phenotypic data available on-line (M48)

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Task 1: Functional genomics in marine metazoans

Roscoff (SBR)	X. Bailly
Naples (SZN)	F. Ristoratore
Banyuls (OOB)	H. Escriva/S. Darras
St Andrews (SOI)	D. Ferrier
Villefranche (OOV)	H. Yasuo

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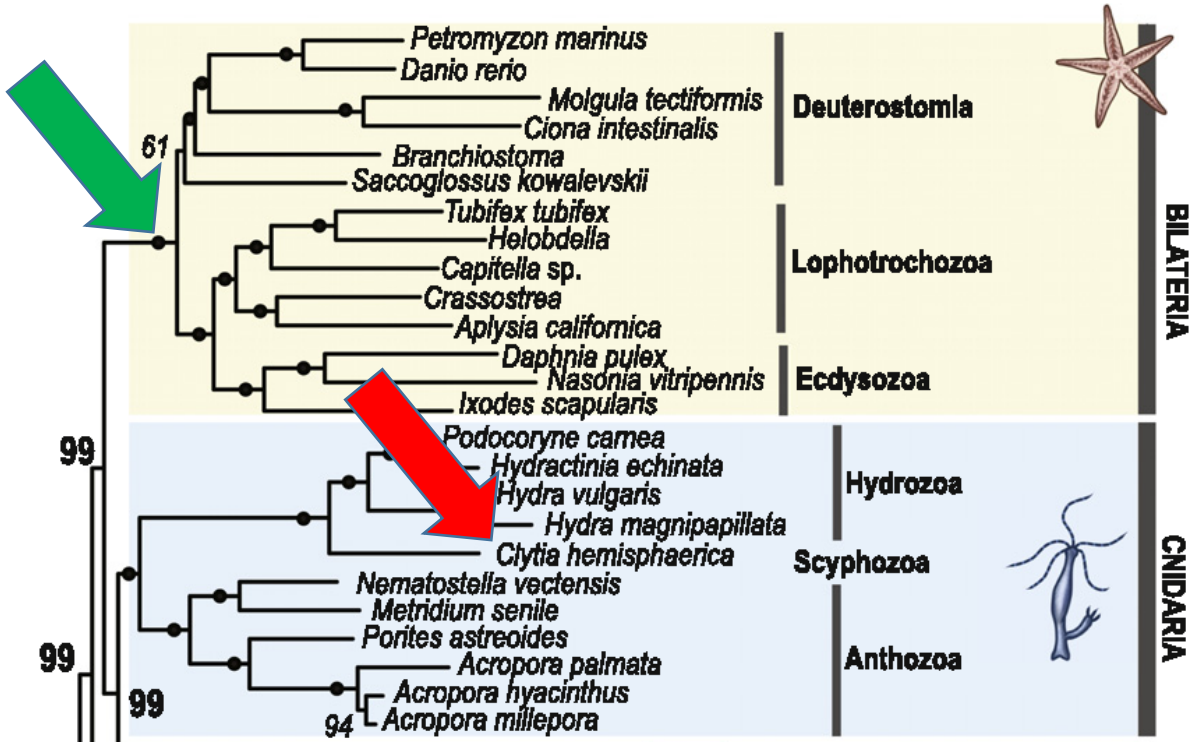

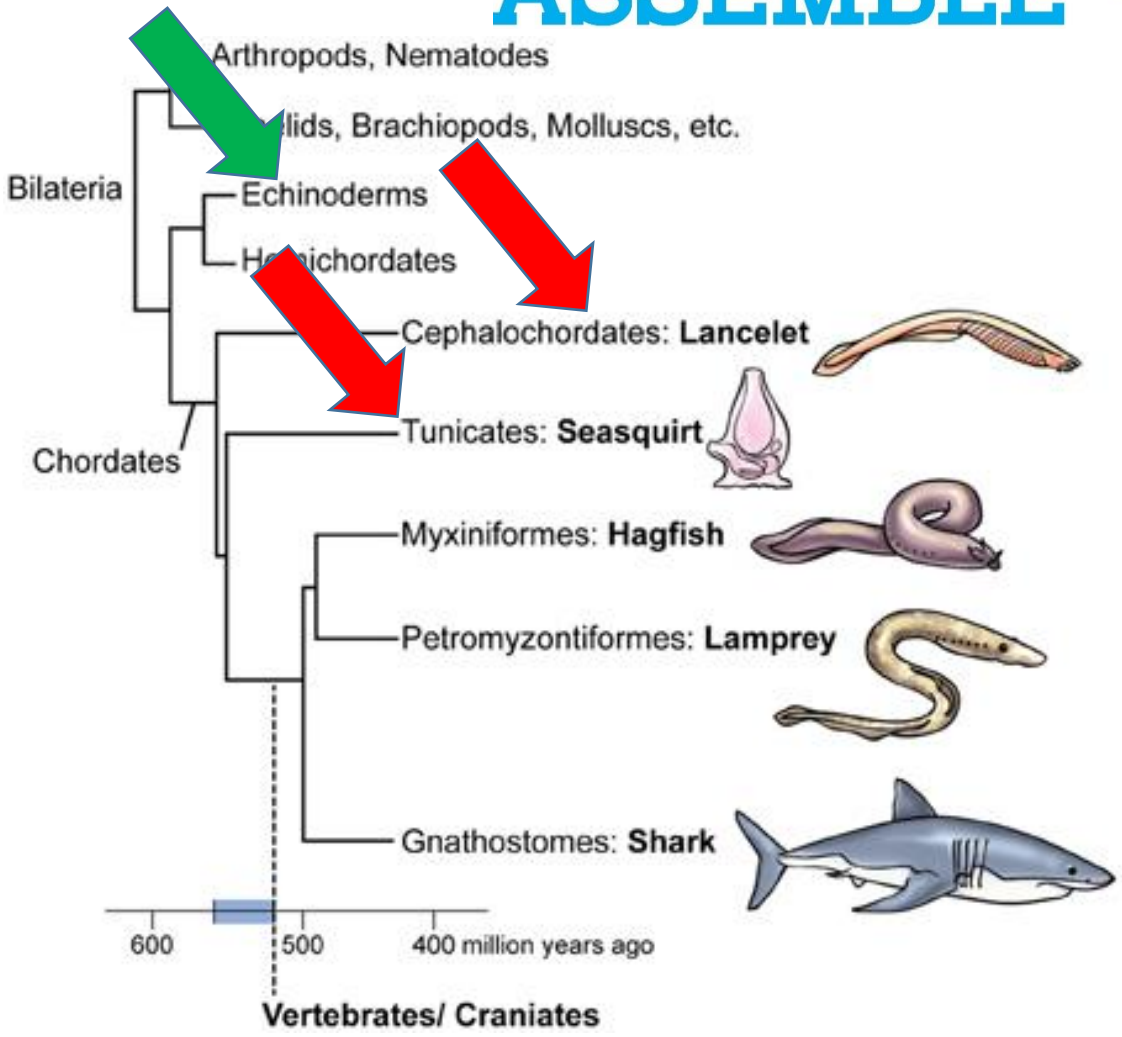

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Clytia hemisphaerica: State of the art

- Genome sequence will be available soon (CORBEL Marimba database)
- Gene knockout (KO) protocol by CRISPR/Cas9 has been fully established.
- KO-efficiency reaches virtually 100% (3 days after injection)
- Nearly "non-mosaic" F0 (depending on the condition)
- *Opsin9* gene KO (Quiroga-Artigas et al., 2018) and genotype convergence (submitted 2018)
- Gene knock-in (KI) does NOT work
- Transgenesis by Tol2 transposon vector is functional

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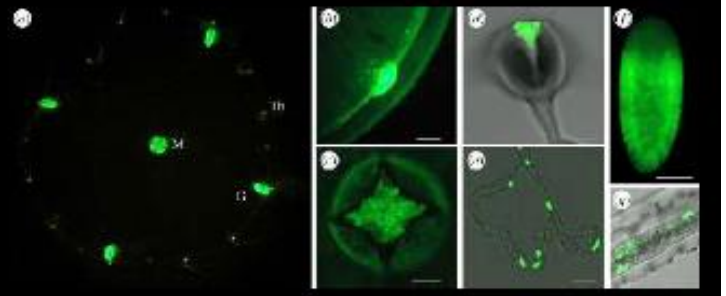
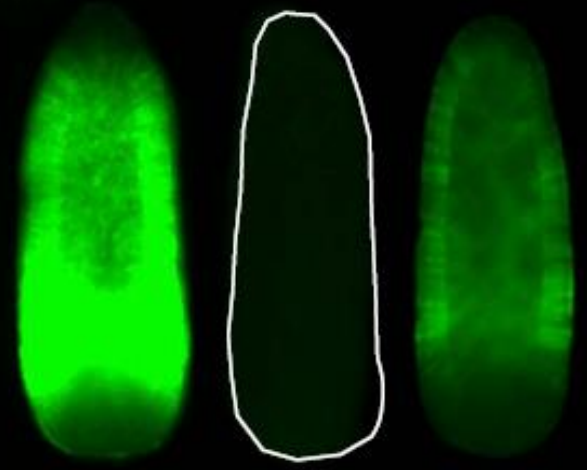
gfp1 KO demonstrates 100% biallelic mutation in *Clytia*



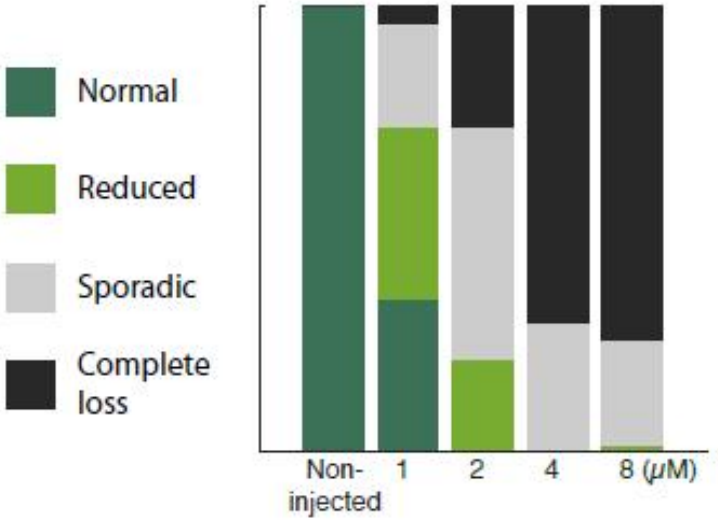
wildtype

Cas9/sgRNA injected



complete loss (4 µM)

reduced (1 µM)



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How about "insertional" gene editing?

- No insertion worked with CRISPR/Cas9-mediated KI
- long (500 bp~) and short (10~100 bp) homology arms
- double strand DNA and ssDNA
- Tol2-mediated random insertion works (efficiency: 10~30%) and can replace most of the CRSPR-KI.
- Collaboration Brady Weissbourd and David Anderson (Caltech), Jason Junge (USC, Fraser Lab)

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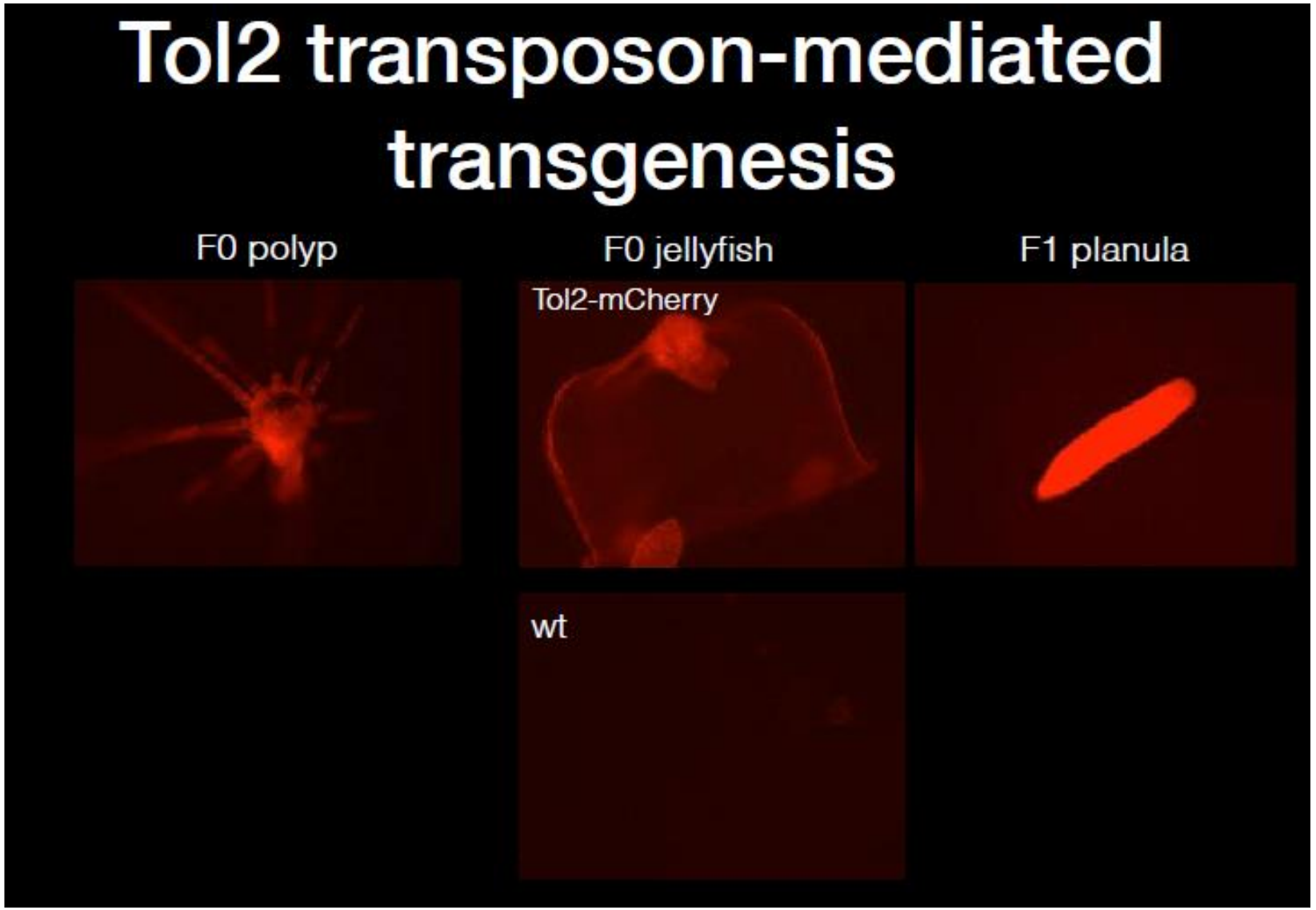

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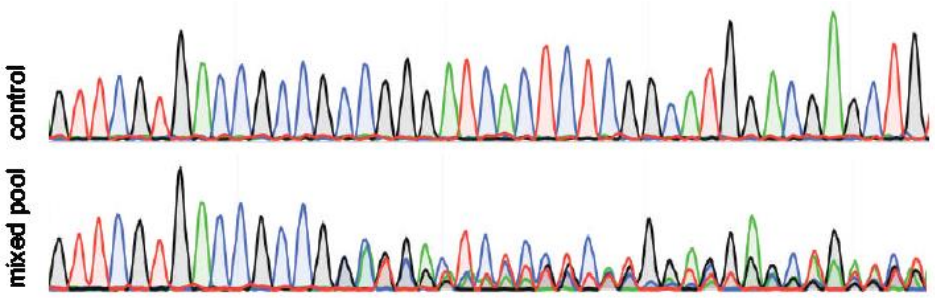
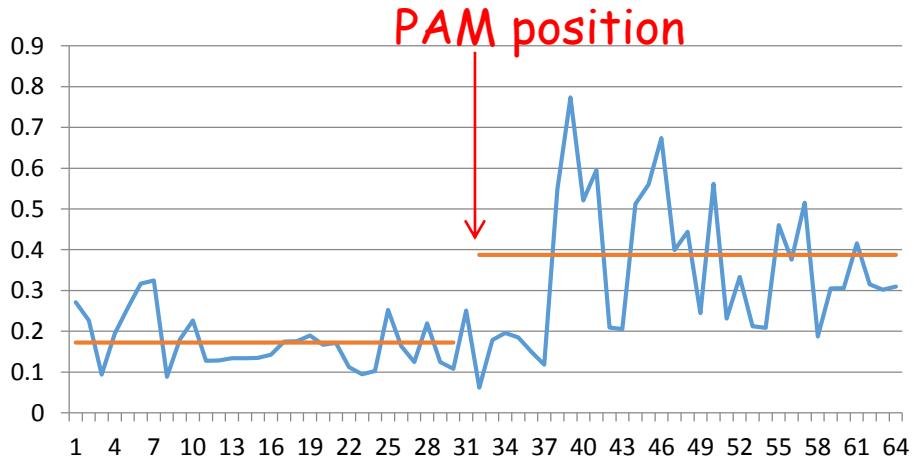
- **Some preliminary results in amphioxus:**

Decomposition of sequence data after PCR fragment direct sequencing starting from one embryo using TIDE (Tracking of Indels by Decomposition)

TIDE is a web tool which rapidly assesses genome editing of a target locus by CRISPR-Cas9. Based on the quantitative sequence trace data from two standard capillary (Sanger) sequencing reactions, the TIDE software quantifies editing efficacy and identifies the predominant types of insertions and deletions (indels) in the DNA of a targeted cell pool.



Brinkman et al. 2014 Nucl. Acids Res.



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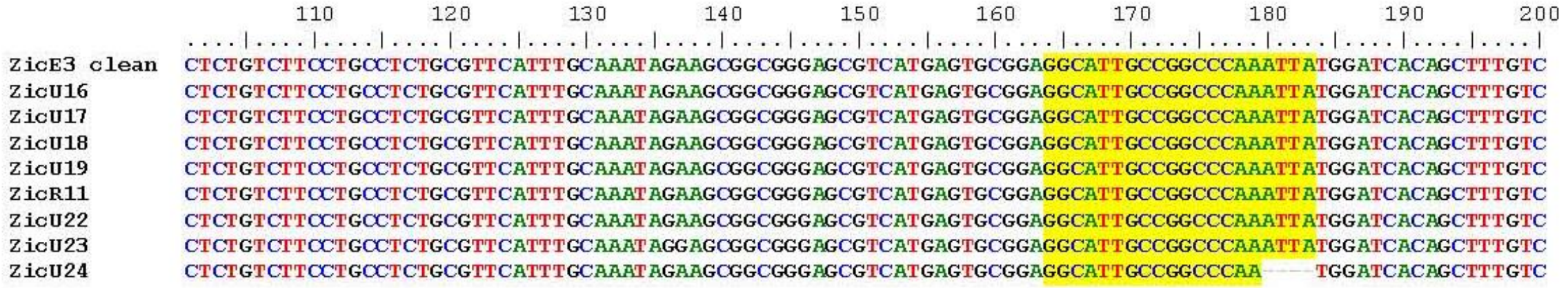
Sequencing of individual clones from one embryo



In yellow: guide sequence

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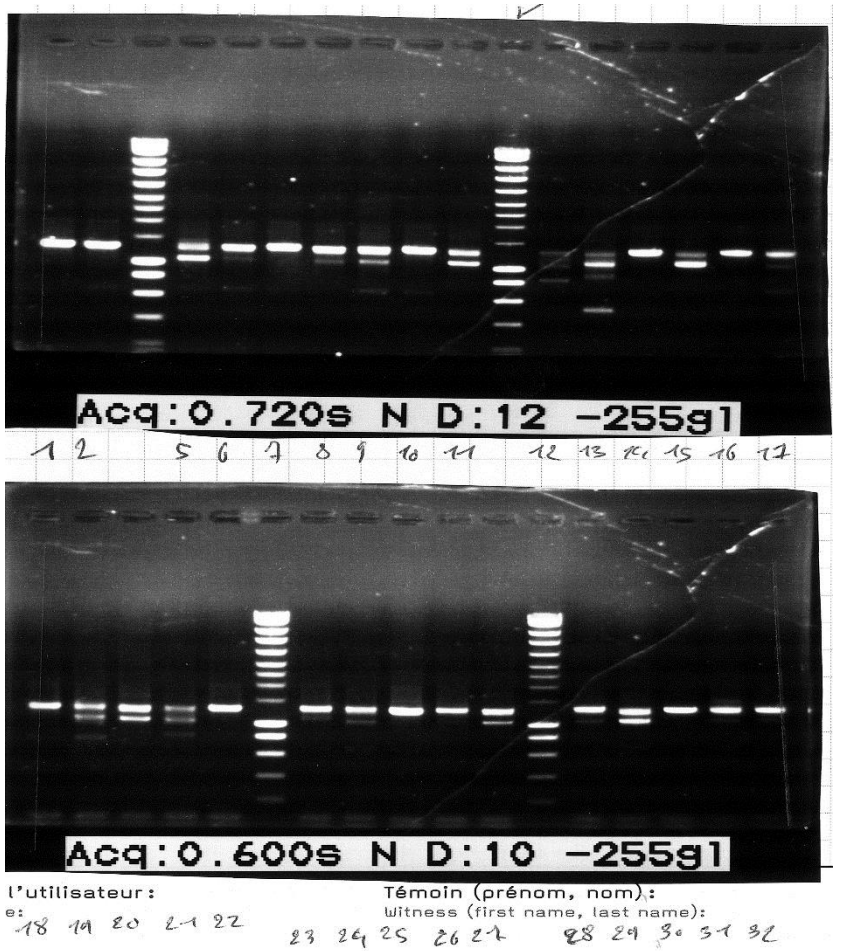
CRISPR/Cas9: *Tlx*

Bl-Tlx-Ex1-sg02	336forw	ACTTCCCGTGGATGGACCAACGG	55	65	70	85
	396forw	CACGTCTTAAAAGTGTCACCCGG	NotEnoughFla	NotEnoughFla	NotEnoughF	80
	337forw	CTTCCCGTGGATGGACCAACGGG	50	56	67	77
Bl-Tlx-Ex1-sg01	95forw	ACTGGGAGCACCACGAGTCCCGG	50	47	62	77
	320rev	TGGCCCGTTGGTCCATCCACGGG	56	86	64	68
	169rev	ACATATGGACGGATGTGACAGGG	66	30	45	68
	349rev	CTGACCTGCTAGACCTTCCTTGG	42	38	75	67
	101forw	AGCACCACGAGTCCCGGCCCGGG	53	50	61	66
	104rev	TACTGAAGCTAATGTTCTCCTGG	49	-1	56	62
	78forw	GCATCATGGAATCGGAGACTGGG	54	46	58	58
	100forw	GAGCACCACGAGTCCCGGCCCGG	38	65	57	58
	288forw	TGATCAGAGTACCGGCCCACCGG	59	61	66	57
	168rev	CATATGGACGGATGTGACAGGGG	73	61	42	57
	184rev	ACTGGGCTGGGACATACATATGG	46	43	65	55
	170rev	TACATATGGACGGATGTGACAGG	53	31	46	55
	248forw	TGGCCCACGACGCCGTCCTGTGG	57	71	82	54
	228forw	TCTACTCCCCTCACCTCTCCTGG	45	10	67	53
	70forw	CATGGTTGGCATCATGGAATCGG	45	60	57	53
	365forw	CTCGCCAAGGAAGGTCTAGCAGG	NotEnoughFla	NotEnoughFla	NotEnoughF	50
	201rev	AGGTGAGGGGAGTAGAAACTGGG	56	77	67	49
	180rev	GGCTGGGACATACATATGGACGG	63	44	67	48
	77forw	GGCATCATGGAATCGGAGACTGG	47	33	59	48
	240rev	TTCGCCACGCTGCCACAGGACGG	56	57	72	47
	202rev	GAGGTGAGGGGAGTAGAAACTGG	35	49	67	47
	99rev	AAGCTAATGTTCTCCTGGCCCGG	48	55	58	47
	342forw	CGTGGATGGACCAACGGGCCAGG	48	62	64	46
	131rev	AGCTTTGACTGTTCAAAATTTGG	28	31	58	45
	197rev	GAGGGGAGTAGAAACTGGGCTGG	50	32	69	44
	64forw	TGTGTACATGGTTGGCATCATGG	56	33	58	43
	280forw	GAACGGCGTGATCAGAGTACCGG	61	60	75	42
	232rev	GCTGCCACAGGACGGCGTCGTGG	57	35	61	42
	324forw	CCCTCAACGCGCACTTCCCGTGG	54	43	66	41
	403forw	TAAAAGTGTCACCCGGCTTGAGG	NotEnoughFla	NotEnoughFla	NotEnoughF	40
	196rev	AGGGGAGTAGAAACTGGGCTGGG	62	51	69	40
	321rev	CTGGCCCGTTGGTCCATCCACGG	50	55	66	40
	352forw	CCAACGGGCCAGGCTCGCCAAGG	54	45	75	38
	328forw	CAACGCGCACTTCCCGTGGATGG	52	42	67	35
	305rev	TCCACGGGAAGTGCGCGTTGAGG	60	59	65	35
	231rev	CTGCCACAGGACGGCGTCGTGGG	54	72	65	33
	340rev	TAGACCTTCCTTGGCGAGCCTGG	38	36	65	33
	98rev	AGCTAATGTTCTCCTGGCCCGGG	49	37	61	33
	244rev	GCCGTTCGCCACGCTGCCACAGG	49	60	81	32
	215rev	TCGTGGGCCAGGAGAGGTGAGGG	51	72	62	31
	303rev	CACGGGAAGTGCGCGTTGAGGGG	59	58	69	30
	26rev	ACACACACGCGCGCGCTGCACGG	NotEnoughFla	NotEnoughFla	NotEnoughF	29
	356forw	CGGGCCAGGCTCGCCAAGGAAGG	50	54	79	25
	304rev	CCACGGGAAGTGCGCGTTGAGGG	49	54	65	25
	297rev	AAGTGCGCGTTGAGGGGAGGTGG	48	72	62	25
	332rev	CCTTGGCGAGCCTGGCCCGTTGG	44	51	73	22
	85rev	CTGGCCCGGGCCGGGACTCGTGG	46	61	56	20
	292rev	CGCGTTGAGGGGAGGTGGCGCGG	42	51	67	18
	291rev	GCGTTGAGGGGAGGTGGCGCGGG	41	55	65	18
	214rev	CGTGGGCCAGGAGAGGTGAGGGG	60	78	62	17
	221rev	ACGGCGTCGTGGGCCAGGAGAGG	57	74	69	16
	106forw	CACGAGTCCCGGCCCGGGCCAGG	38	59	54	16
	216rev	GTCGTGGGCCAGGAGAGGTGAGG	52	69	61	15
	94rev	AATGTTCTCCTGGCCCGGGCCGG	42	54	65	13
	263forw	TCCTGTGGCAGCGTGGCGAACGG	47	83	63	12
	52forw	AGCGCGCGCGTGTGTGTACATGG	46	64	64	10
	226rev	ACAGGACGGCGTCGTGGGCCAGG	41	58	66	9
	300rev	GGGAAGTGCGCGTTGAGGGGAGG	52	74	66	8
	56forw	CGCGCGTGTGTGTACATGGTTGG	50	56	60	7
	256forw	GACGCCGTCCTGTGGCAGCGTGG	67	39	66	5
	284rev	GGGGAGGTGGCGCGGGCCGGTGG	18	92	66	3
	279rev	GGTGGCGCGGGCCGGTGGGCCGG	34	63	70	2
	93rev	ATGTTCTCCTGGCCCGGGCCGGG	39	65	64	2
	283rev	GGGAGGTGGCGCGGGCCGGTGGG	40	80	65	1
	287rev	TGAGGGGAGGTGGCGCGGGCCGG	43	74	71	0



19/28 with a deletion

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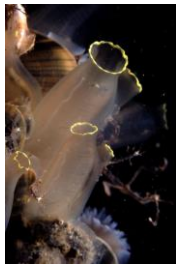

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CRISPR/Cas9 technology for gene knock-out and knock-in in the two ascidian models

• CRISPR/Cas9 system in place of Morpholino antisense oligos for functional gene studies

• Knock-in for protein dynamics (FP-tagging) and transcription dynamics (MS2 system)

>> Microinjection of Cas9/gRNA RNPs into eggs

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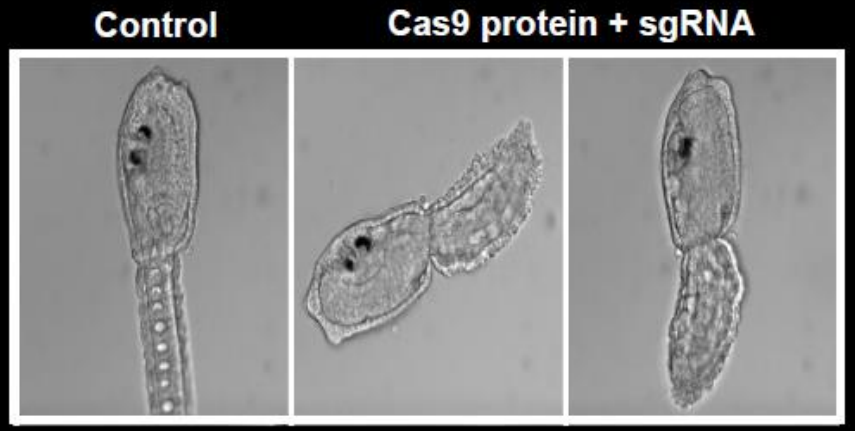
CRISPR/Cas9-mediated *Bra* knockout in *Ciona intestinalis*

- *"no-tail"* phenotype : 12/14 (86%)
- efficiency: 1 out of 4 guide RNAs

CRISPR/Cas9-mediated *Bra* knockout in *Phallusia mammillata*



- phenotype : 4/6
- efficiency: 1 out of 3 guide RNAs

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Task 2: Functional genomics in macroalgae

What about CRISPR project in the brown algae Ectocarpus ?

Oban (SAMS)	C. Gachon
Roscoff (SBR)	M. Cock
Plymouth (MBA)	C. Brownlee

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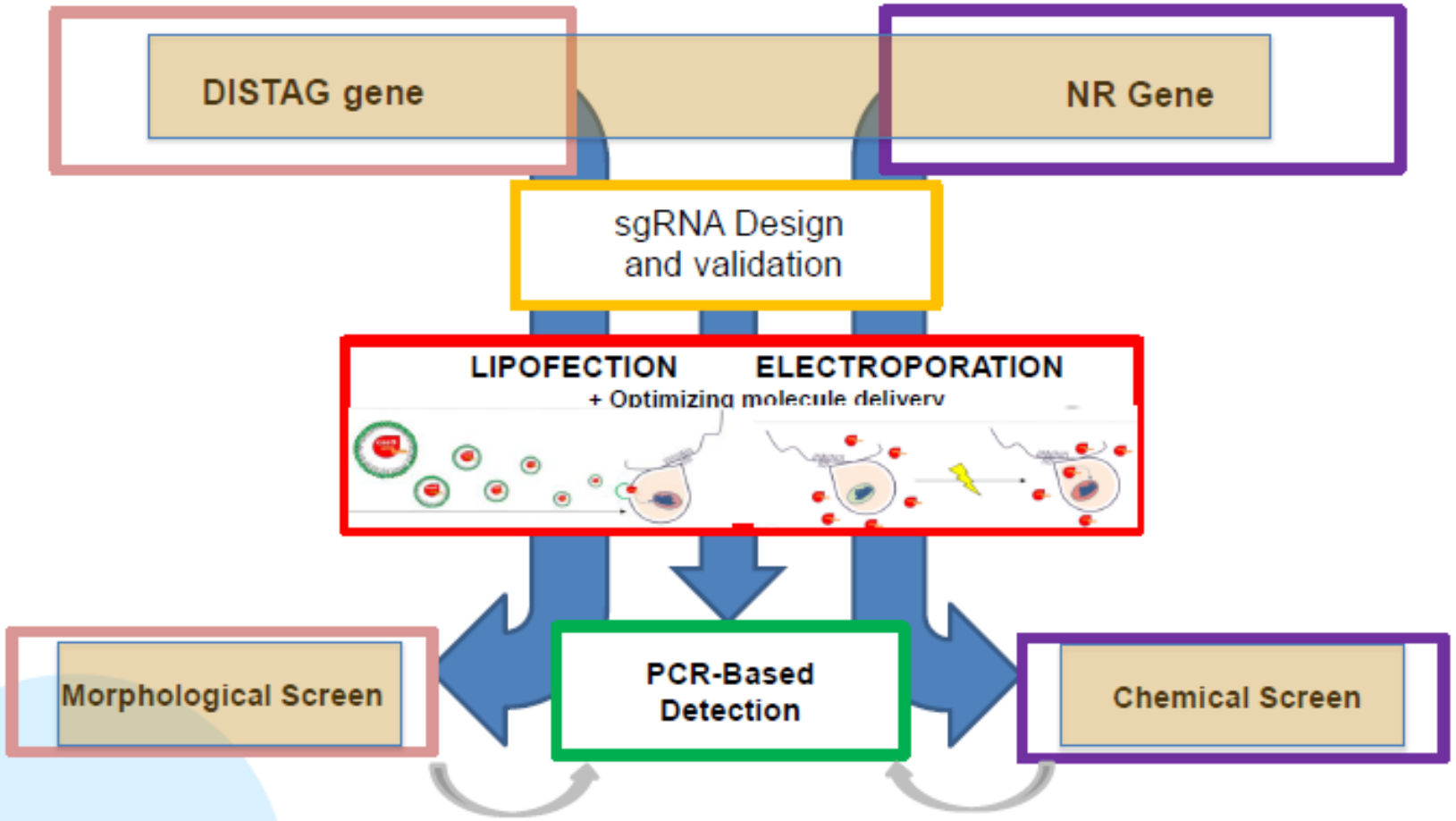

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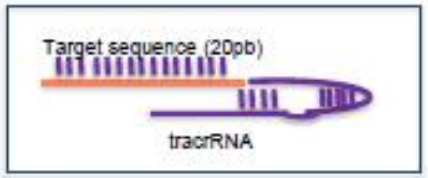
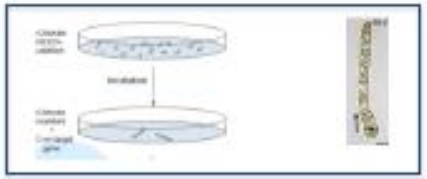
All material ready to assay Crispr-CAS9 on Ectocarpus

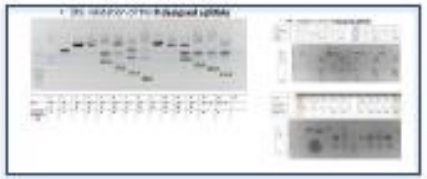
2 target gene sequences



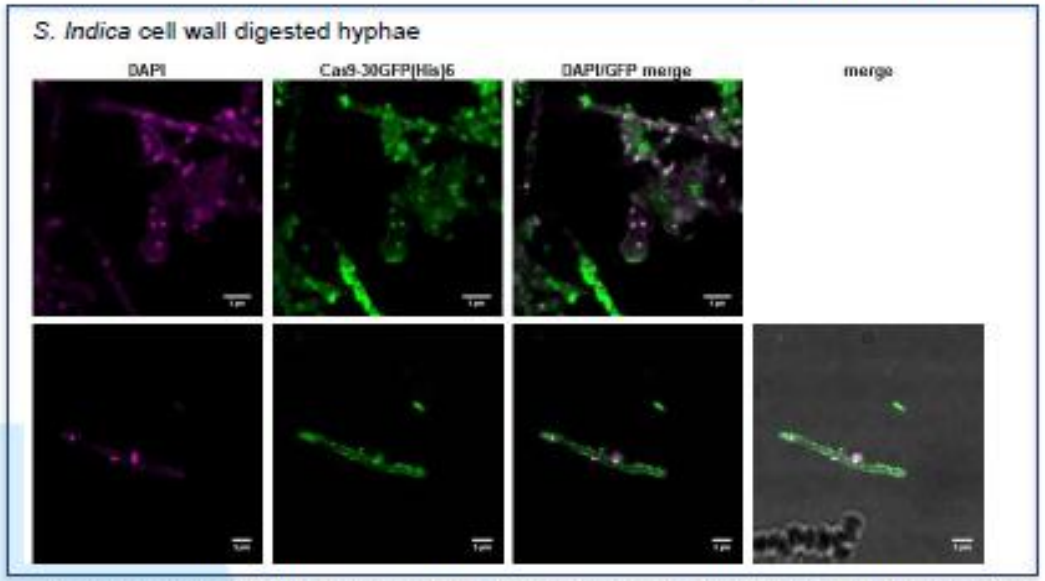
Validated sgRNAs



2 screens for detection of mutants (chemical&phenotypic)



Primers for Detection of Genomic Deletions



Collaboration with Stephan Wawra for production of CAS9 proteins with specific NLS and/or fused with GFP

Transfection systems

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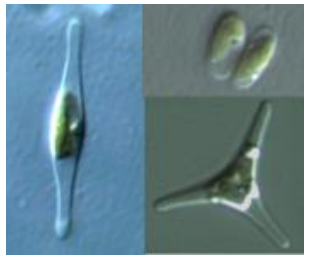

Task 3: Functional genomics in microorganisms

Banyuls (OOB)	F.Y. Bouget
Banyuls (OOB)	R. Lamy
Roscoff (SBR)	L. Garczarek
Naples (SZN)	M. Ferrante
Ghent	W. Vyverman

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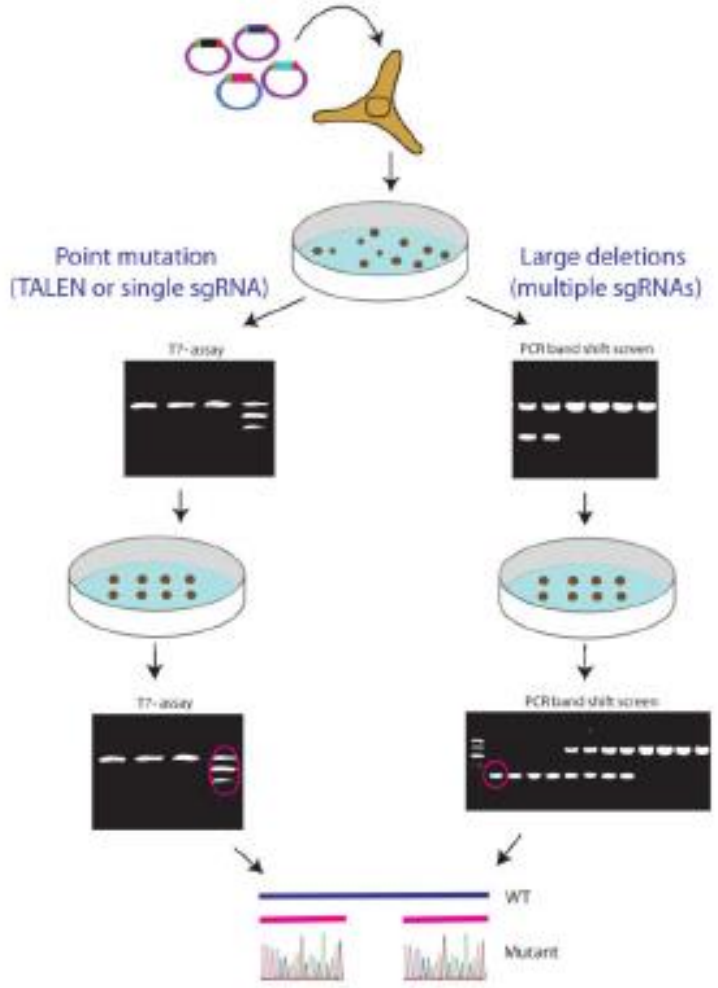
Current Methodology in diatoms (Phaeodactylum)



Stable co-transformation by biolistics

Transformant selection

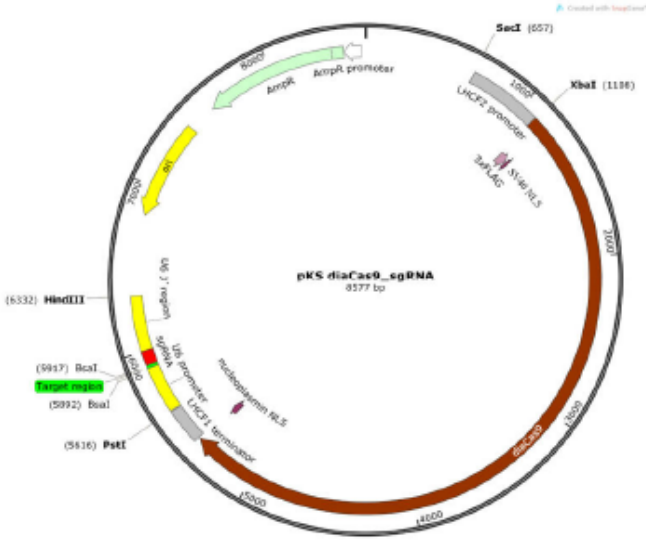
Screen for colonies conaining mutants

Single cell colony isolation

PCR screen for biallelic mutants

Sequencing of identified mutants

Delivery as episome



Cas9 gene codon optimized for *Phaeodactylum tricornutum*.

sgRNA driven by the *P. tricornutum* U6 promoter.

Target sites inserted by adapter ligation.

(Nymark et al., 2016)

Vector available through Addgene
(Plasmid #74923)
pKS diaCas9_sgRNA

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- The objective is to set up the CRISPR/Cas9 technology in the diatom Pseudo-nitzschia multistriata, optimizing the bacterial conjugation method to deliver the Cas9 enzyme as an episome

- This technology already works in the diatom Phaeodactylum tricornutum and obtained P. tricornutum KO strains

-Modyfing the conjugation plasmid in order for it to be functional in P. multistriata, by replacing the P. tricornutum promoter with the P. multistriata H4. The team also tested microalga/bacteria incubation conditions (they are species-specific).

- CRISPR/CAS genome editing in diatoms: C. closterium and S. robusta. Transformation protocols are ongoing

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Other microorganisms

- Implementation of transgene introduction in Micromonas cells.
  Conditions for electroporation have been optimized in a trehalose osmoticum.

- Conjugation and/or electroporation have already allowed to obtain a first set of random transposition mutants in *Synechococcus* sp. (strain WH7803).
  Generation of inactivation mutants in A15-62 is ongoing.

- Bacterial mutants for quorum sensing. The strain MOLA401 has been selected, and various receptors of quorum sensing compounds will be targeted through functional experiments

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Thank You

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