JRA3 – Functional genomics

ASSEMBLE Plus Kick-off Meeting

October 19th-20th, 2017

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..

Objectives

-to implement/adapt specific protocols for generation of genetic resources for a panel of emerging/prospective marine model organisms

-to generate a reference set of carefully phenotyped or genotyped genetic resources of different marine organisms ranging from bacteria to metazoans

- to produce and provide access to the phenotypic or genotypic data necessary for the functional description of the genetic resources

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*, *Phaullusia 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*)

Participants:

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)

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.*
	All	All	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.

Deliverables:

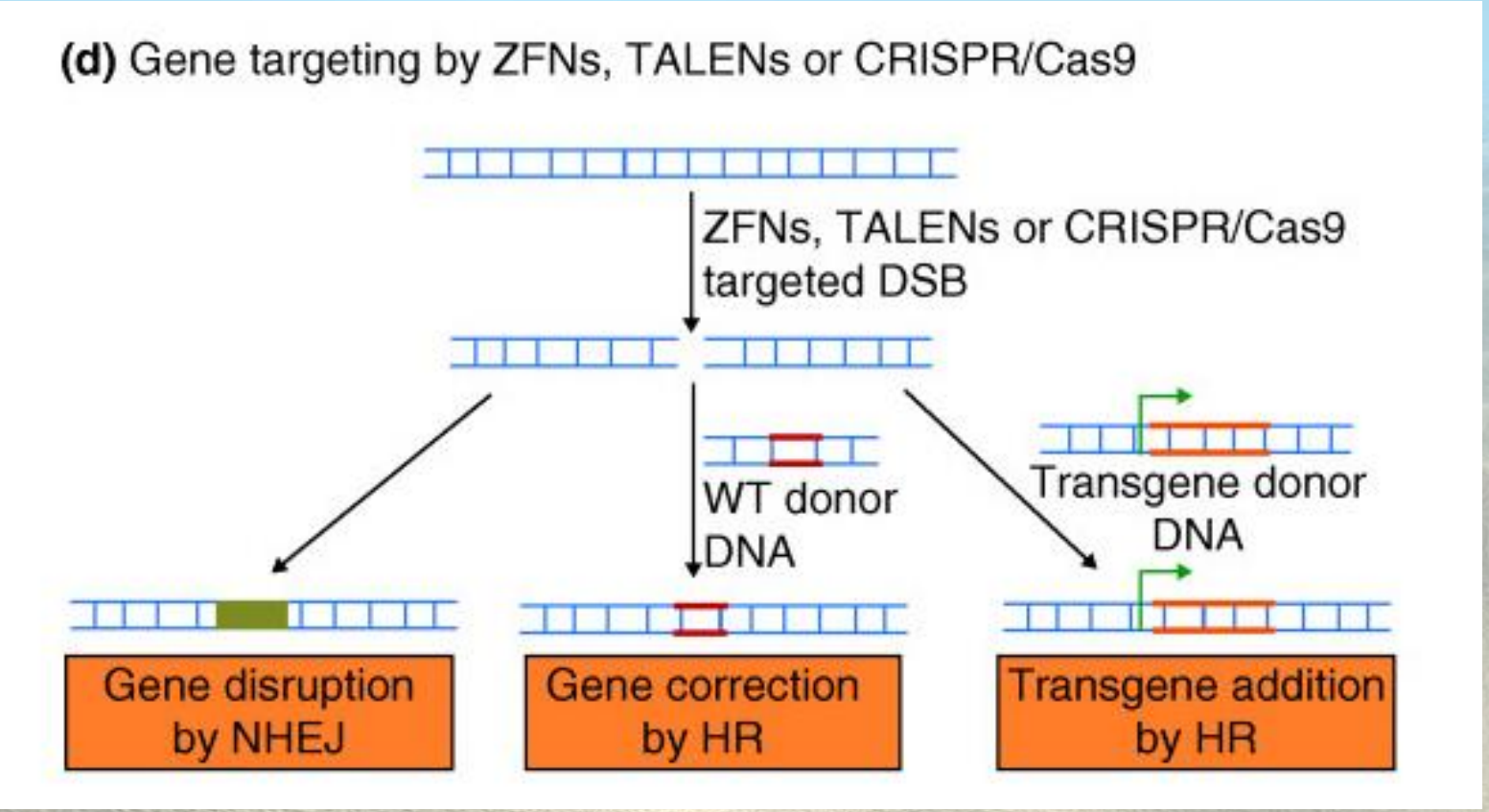
- Protocols for genetic transformation of novel emerging metazoan, macroalgal and microalgal model organisms available via ASSEMBLE-plus web portal (M24)

-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)

- A collection of mutant and transgenic/enhancer trap lines for metazoan, macroalgal and microalgal model organisms (M48).

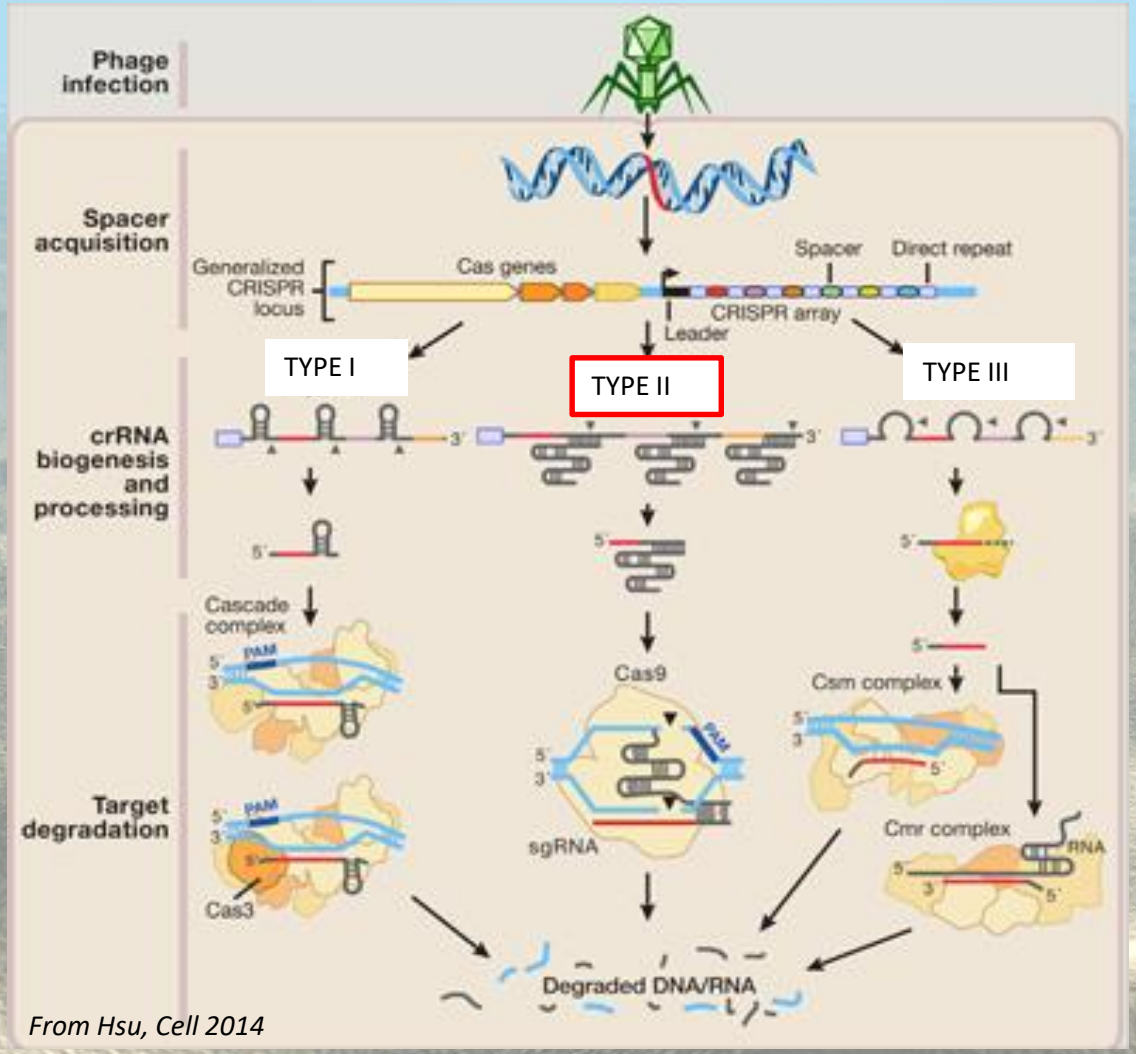
CRISPR/Cas9 overview:



From Ramalingam, Genome Biology, 2013

CRISPR/Cas9 overview:

CRISPR : **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeat
CAS : **C**RISPR **AS**sociated genes



From Hsu, Cell 2014

The CRISPR/Cas system is a prokaryotic immune system that confers resistance to foreign genetic elements such as plasmids and phages and provides a form of acquired immunity. CRISPR spacers recognize and cut these exogenous genetic elements in a manner analogous to RNAi in eukaryotic organisms.

Type I and Type III use multiprotein interference complex
while
Type II needs only Cas9

(Streptococcus pyogenes)

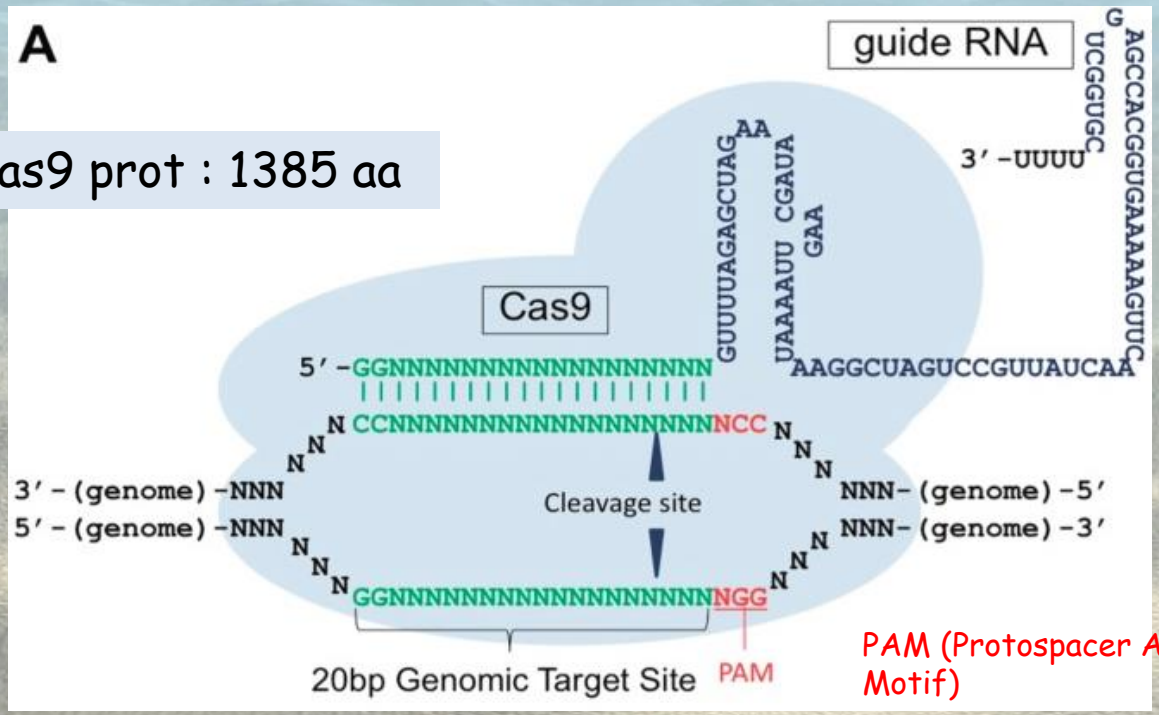
CRISPR/Cas9 overview:

VARIABLE SEQUENCE
specific target :
"cr RNA" 20nt

CONSTANT SEQUENCE
"tracr RNA" : 80 nt

1 **gRNA** = crRNA + tracr RNA
≈ 100nt

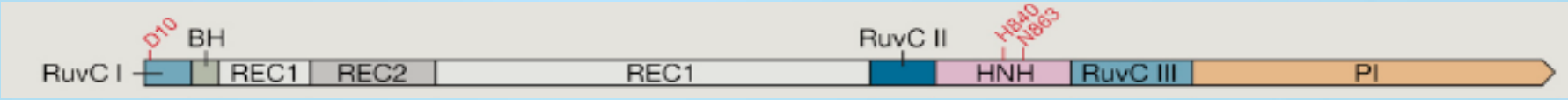
Cas9 prot : 1385 aa



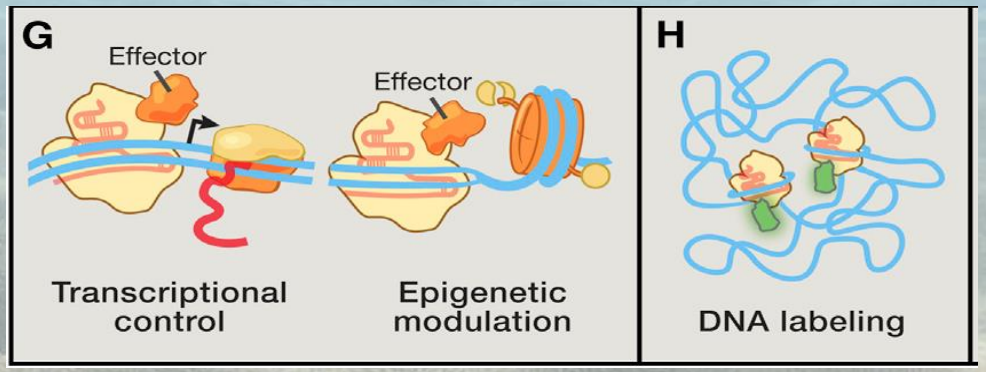
PAM (Protospacer Adjacent Motif)

From Nakanishi PlosOne, 2014

CRISPR/Cas9 overview:



D10A + H840A => dead-Cas9 (dCas9) => **Binding** but **NO** DNA strand Break



dCas9 Fused with transcriptional repressors/activators
=> modify gene expression level

dCas9 Fused with histone modifiers enzymes (acethylase/deseacythylase/methylase)
=> modify epigenetic context
=> act on transcription

dCas9 Fused with reporter gene
=> visualise site of fixation

From Hsu, Cell 2014

CRISPR/Cas9 overview:

Table 1 Published examples of cell types and organisms modified by Cas9

Cell type or organism	Cas9 form	Cell type	Reference numbers
Human cells	Cas9 nuclease	HEK293FT, HEK293T, HEK293, K562, iPSC, HUES9, HUES1, BJ-RiPS, HeLa, Jurkat, U2OS	9,13–16,47, 49–51,54,59, 84,85
	Cas9 nickase	HEK293FT, HEK293T	13,14,47,49
	dCas9 (gene regulation)	HEK293FT, HEK293T	70–72,74,82
	dCas9 (imaging)	HEK293T, UMUC3, HeLa	81
Mouse or mouse cells	Cas9 nuclease	Embryos	14,24–26
	Cas9 nickase	Embryos	47
	dCas9 (gene regulation)	NIH3T3	74
Rat	Cas9 nuclease	Embryos	26,36
Rabbit	Cas9 nuclease	Embryos	27
Frog	Cas9 nuclease	Embryos	28
Zebrafish	Cas9 nuclease	Embryos	17,33,37,60,85
Fruit fly	Cas9 nuclease	Embryos	29,30,61
Silkworm	Cas9 nuclease	Embryos	31
Roundworm	Cas9 nuclease	Adult gonads	32,62–67
Rice	Cas9 nuclease	Protoplasts, callus cells	21,23
Wheat	Cas9 nuclease	Protoplasts	21
Sorghum	Cas9 nuclease	Embryos	23
Tobacco	Cas9 nuclease	Protoplasts, leaf tissue	19,20,23
Thale cress	Cas9 nuclease	Protoplasts, seedlings	19,23
Yeast	Cas9 nuclease	*Saccharomyces cerevisiae*	18
Bacteria	Cas9 nuclease	*Streptococcus pneumoniae*, *E. coli*	8
	dCas9 (gene regulation)	*E. coli*	69,70

HEK, human embryonic kidney; iPSCs, induced pluripotent stem cells; UMUC3, human bladder cancer.

From Sander & Joung, Nat.Biotechnology 2014

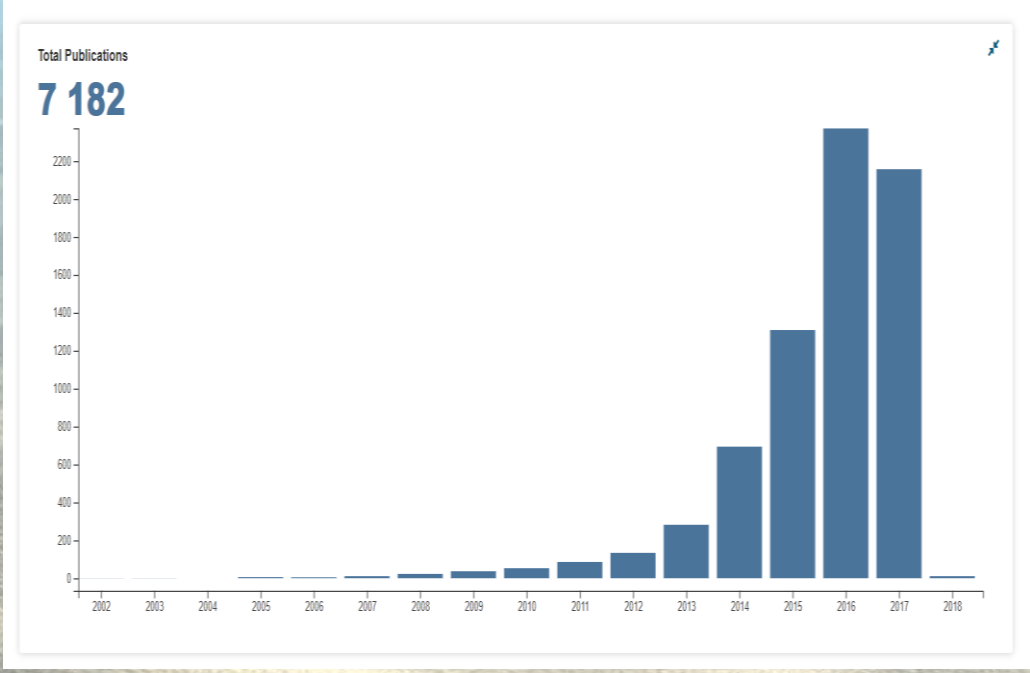
CRISPR/Cas9 overview:

Advantages of CRISPR/Cas9

- Allow GENOME EDITING : **modify *in vivo* genomic context at precise loci**
- works in different models, animal/plants, in culture cell or embryo
- Simple system : 1 gRNA + 1 protein
- low cost : plasmid available (addgene)
  short gRNA : -> "order custom oligos" (100nt)
  -> replace only crRNA (variable seq- 17 to 20nt) in a vector already containing tracrRNA seq.(invariant seq)
  -> "Do it yourself" by PCR

CRISPR/Cas9 overview:

Nb of publication/year



5614 articles since 5 years

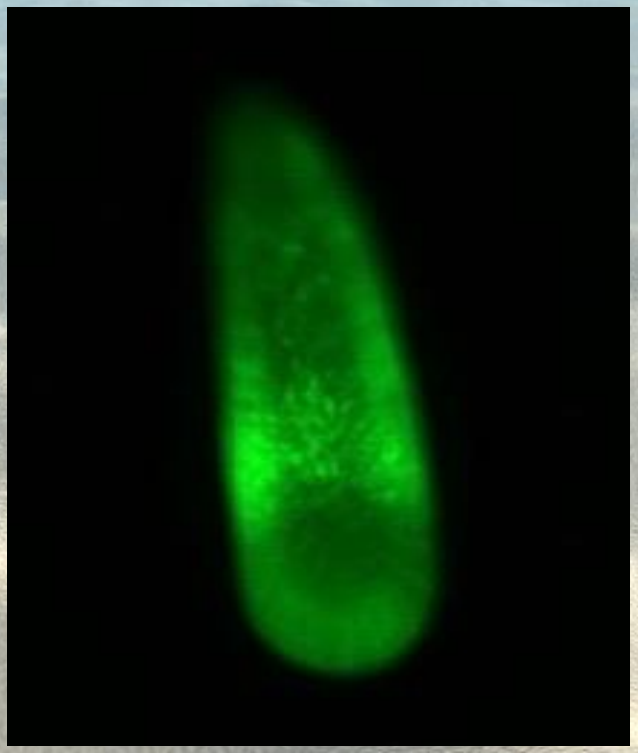
4529 in the last 2 years

CRISPR slides, thanks Agnes Roure

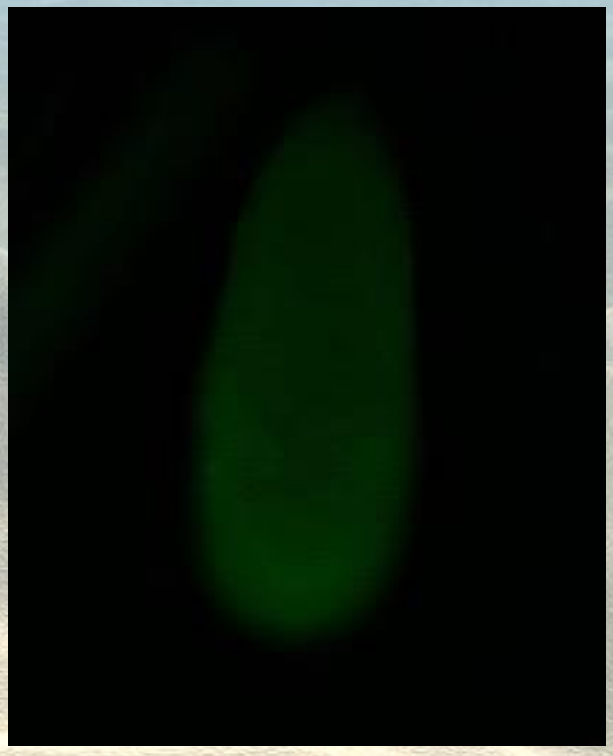
Some preliminary results:

***gfp1* gene knockout**
GFP observed 3 days after injection

Non-injected



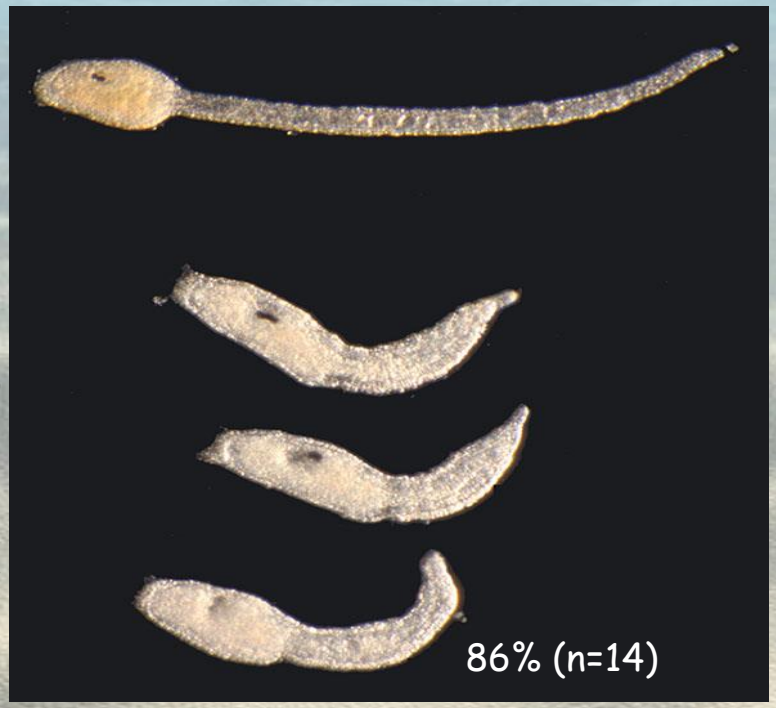
Cas9 + sgRNA (GFP1-n4) injected



Thanks: Tsuyoshi Momose

Some preliminary results:

Bra* gene knockout in *Ciona intestinalis
"no tail" phenotype at the larval stage

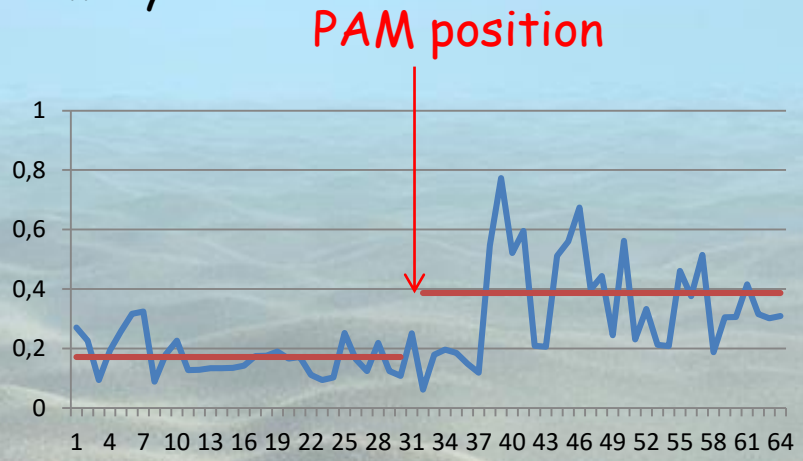


Thanks: Hitoyoshi Yasuo

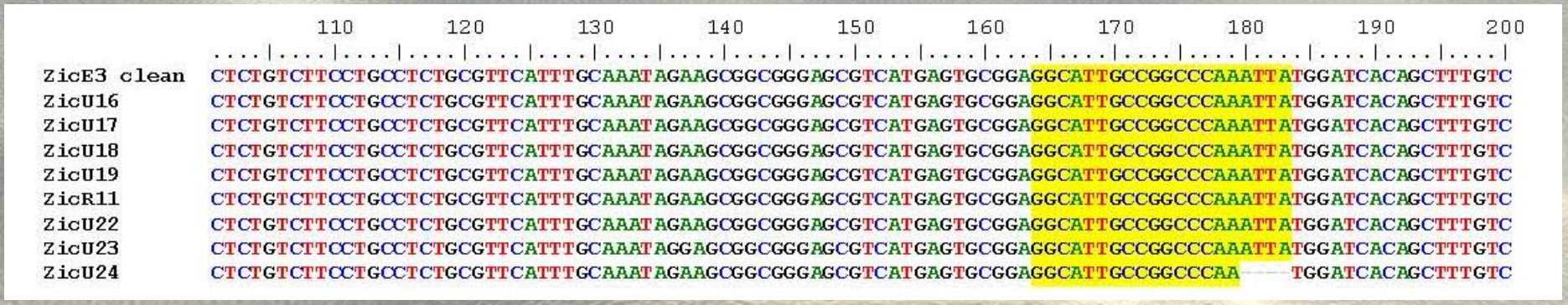
Some preliminary results:

Amphioxus Zic

Decomposition of sequence data after PCR fragment direct sequencing starting from one embryo



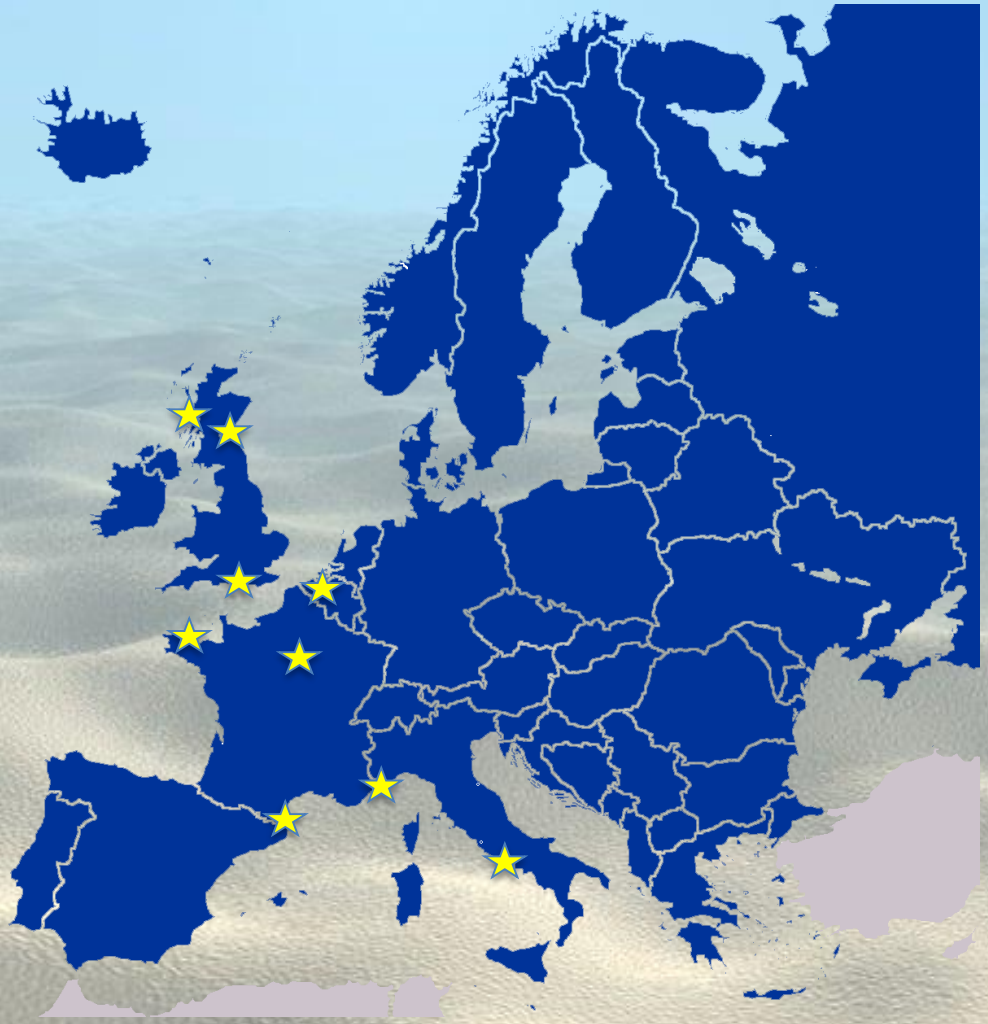
Sequencing of individual clones from one embryo



Thanks: Stephanie Bertrand

In yellow: guide sequence

Thanks:



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Tsuyoshi Momose
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Stephanie Bertrand