ASSEMBLE

ASSOCIATION OF EUROPEAN MARINE BIOLOGICAL LABORATORIES EXPANDED

Acronym: ASSEMBLE Plus

Title: Association of European Marine Biological Laboratories Expanded

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Deliverable D9.3

« Gene expression pattern and phenotypic data available online (WP9)".

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Lead parties for Deliverable: [UPMC**]**
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GENERAL DATA

Acronym: **ASSEMBLE Plus**

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Dissemination Type

Report	☒
Websites, patent filling, etc.	☐
Ethics	☐
Open Research Data Pilot (ORDP)	☐
Demonstrator	☐
Other	☐

Confidential, only for members of the consortium (including the Commission Services)	☐

Dissemination Level

Public	☒

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Document properties

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Abstract

The general objectives of this WP:

- **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.**

During the time we worked on the project the different participants in this WP have developed the various activities and experimental approaches that were detailed in the project. Depending on the model, the final results are at more or less advanced stages of development, but overall, we can estimate that this WP has generated numerous results for each model studied. Particularly, concerning this deliverable, we have developed or adapted protocols for the deployment of CRISPR/Cas9 system in the different biological models used in this JRA3. This process of genomic modifications is essential if we want generate GMOs for the different biological models. In this last deliverable we intended to produce also gene expression data for different metazoan models in order to facilitate the study of the phenotype of mutants. Most if not all of these gene expression data are now published in different reviews.

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1. Introduction

In this deliverable we present a collection of gene expression patterns for the different metazoan models, worked by the different partners of JRA3:

1) Cnidarians: Gene expression in *Clytia hemisphaerica*
2) Ascidians: Gene expression in *Phallusia mammillata*
3) Cephalochordates: Gene expression in amphioxus, *Branchiostoma lanceolatum*

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2. Objective

The objectives of this deliverable of JRA3 are:

1- To collect gene expression data from different metazoans
2- To render available the gene expression data through scientific publications
3- To use gene expression as marquers of morphological parts of the different metazoans to easily study phenotypic changes induced by CRISPR-Cas9 KO.

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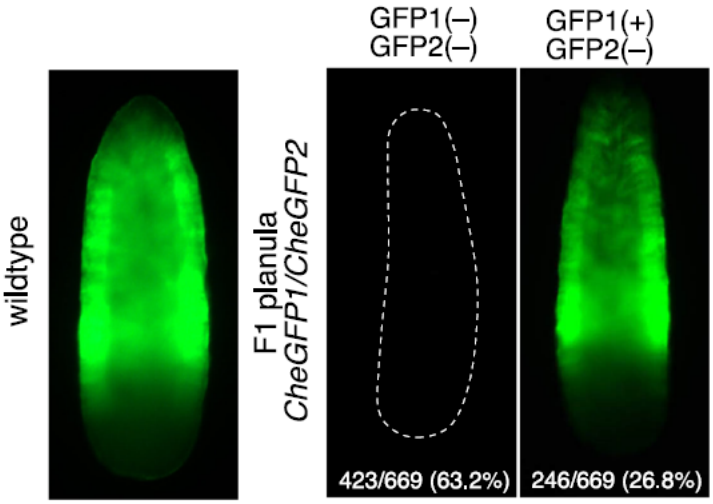
3. Gene expression in *Clytia hemisphaerica*

3.1 Gene expression in *Clytia hemisphaerica*

In Clytia hemisphaerica, the endogenous expression of GFP was used as test for CRISPR-Cas9 knock-out. First GFP expression was obtained by the natural fluorescence



GFP fluorescence in a wildtype planula (left) and in F1 planulae from male and female CheGFP1/CheGFP2 double KO founder jellyfish, which were either completely GFP free (CheGFP1−/− centre) or almost identical to wildtype (CheGFP1+/− or CheGFP1+/+, right). In both cases, functional maternal CheGFP2 protein was absent according to (C).

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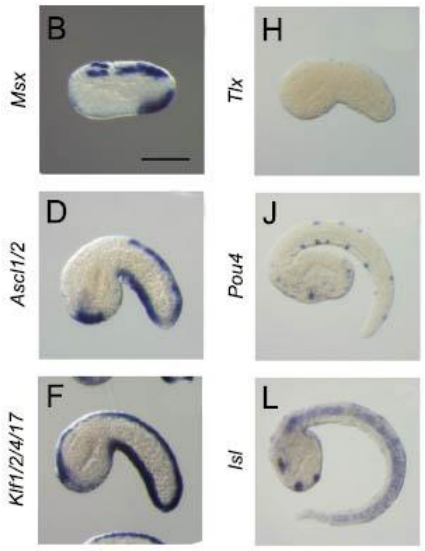
3.2 Gene expression in Phallusia mammillata

In *Phallusia mammillata*, gene expression was studied for different gene marquers by ISH.



In situ hybridization for Msx (B) at initial tailbud stage for *P. mammillata*, for Ascl1/2 (D), for Klf1/2/4/17 (F), for Tlx (H) at mid tailbud *P. mammillata* embryos, for Pou4 (J) and Isl (L) at late tailbud *P. mammillata* embryos. Embryos are shown in lateral view with anterior to the left and dorsal to the top.

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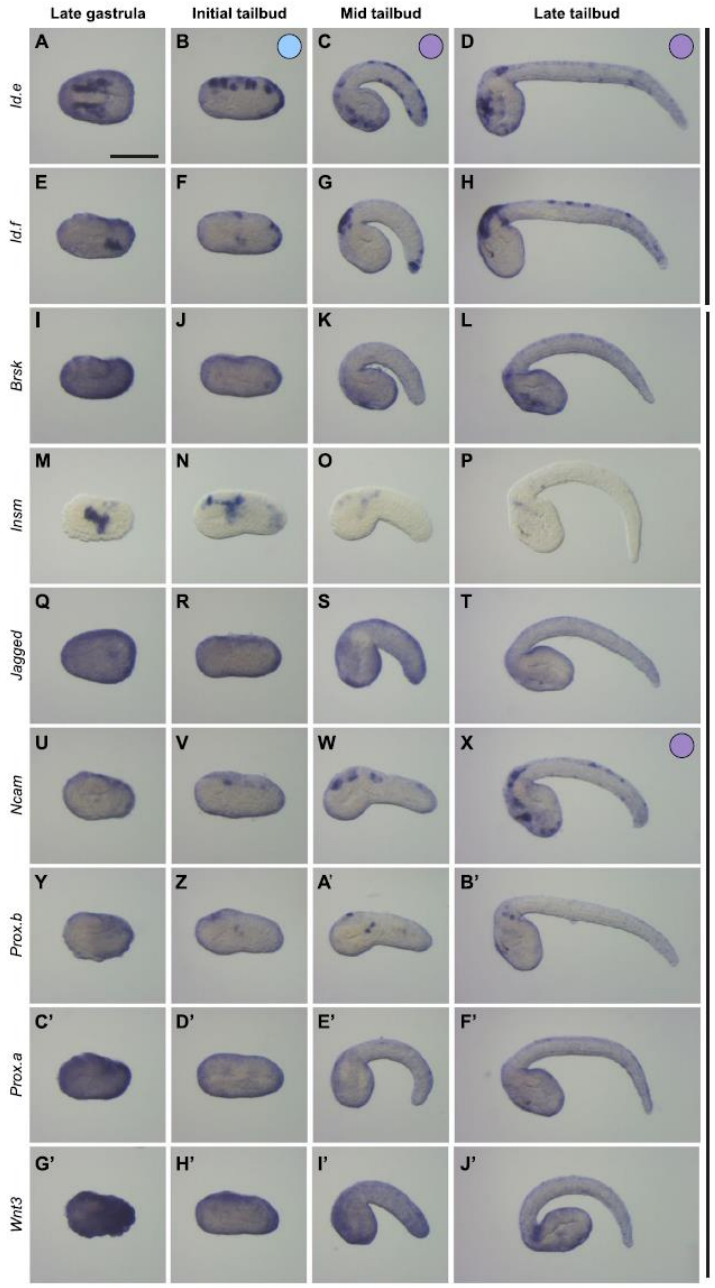

In situ hybridization at several developmental stages for *P. mammillata* genes: Id.e (A-D), Id.f (E–H)), Brsk (I–L), Insm (M–P), Jagged (Q–T), Ncam (U–X), Prox.b (Y–B'), Prox.a (C'–F') and Wnt3 (G'–J'). Genes expressed in the VML are represented by a blue circle and genes expressed in ventral ESNs by a purple circle. Embryos are shown in lateral view with dorsal to the top and anterior to the left except for A and Q which are in dorsal view with anterior to the left and right side to the top. Scale bar: 50 µm

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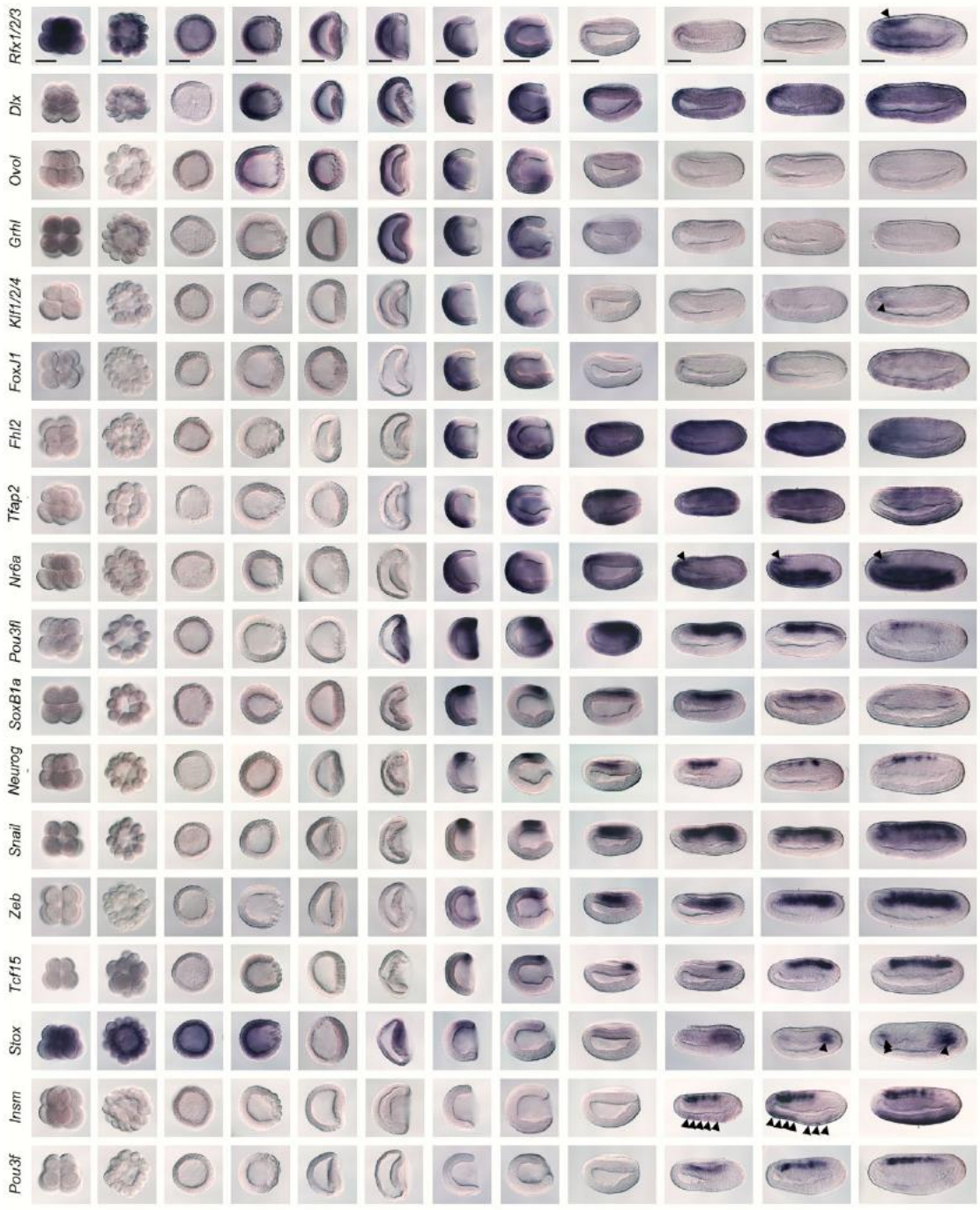
3.3 Gene expression in Amphioxus

In Branchiostoma lanceolatum, gene expression was studied for different gene marquers by ISH.



Expression patterns of different genes by in situ hybridization. The expression of epidermal genes (Rfx1/2/3, Dlx, Ovol, Grhl, Klf1/2/4, FoxJ1, Fhl2, Tfap2) and neural genes (Pou3fl, SoxB1a, Neurogenin, Snail, Zeb, Tcf15, Stox, Insm, Pou3f) were analyzed at eight-cell, morula, blastula, G0, G1, G2, G4, G5,

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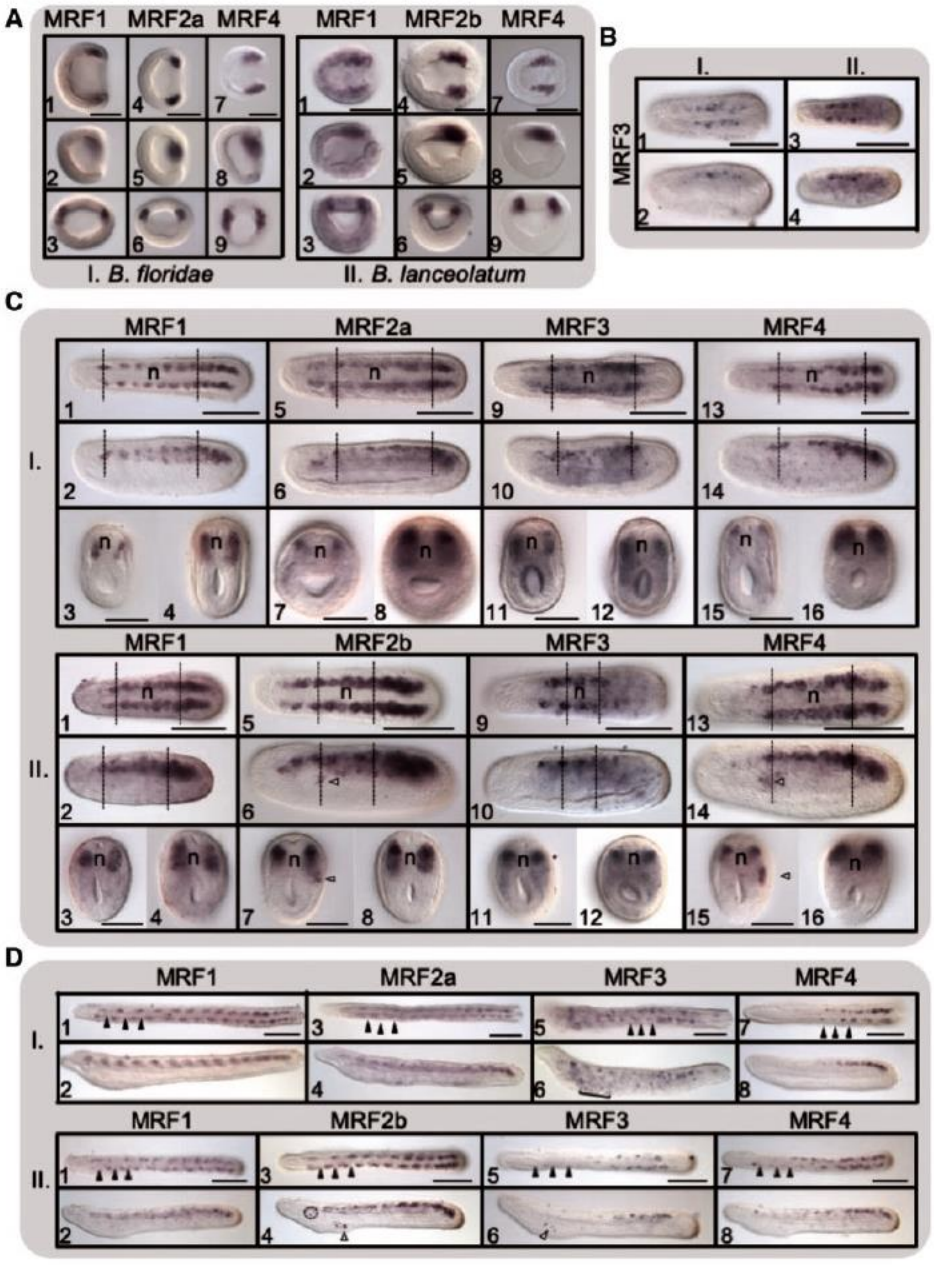
N0, N2, N3, and N4 stages. Side views are presented with anterior to the left and dorsal to the top for gastrula and neurula stages. At N4 stage, Rfx1/2/3 is expressed in the cerebral vesicle (arrowhead) and Klf1/2/4 in the anterior endoderm (arrowhead). Nr6a is expressed during neurulation in the cerebral vesicle region (arrowheads). Stox is expressed at N3 and N4 stages in the posterior endoderm (arrowheads) and in the anterior endoderm at N4 stage (double arrowhead). Insm is expressed in the neural plate/tube as well as in the epidermal sensory neurons at neurula stages (arrowheads). Scale bar=50 µm.



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Expression time-course of amphioxus MRFs. WMISH of (A) late gastrula, (B) early neurula (up to 8 somites), (C) mid-late neurula (10–12 somites), and (D) early larva (12–15 somites) stages of (I) Branchiostoma floridae and (II) B. lanceolatum embryos. For both species at all stages, the top rows show dorsal views and the second rows show lateral views (anterior is to the left, scale bars represent 100 mm). For (A.I.) and (A.II.), the bottom row is the posterior view from the blastopore (dorsal to top). For (C.I.) and (C.II.), the bottom rows are cross-sections through the dotted lines in the anterior (left) and posterior (right) somites (scale bars represent 50 mm). Full arrowheads in (D.I.) and (D.II.) represent somite boundaries. Nonmyotomal expression is denoted in the neurulae with the open arrows (C.II.6 and II.7: expression of MRF2b in the somatic [parietal] layer left of the third somite and C.II.14 and II.15: expression of MRF4 in the somatic [parietal] layer of mesoderm of the third anterior left somite) and in the larvae with the square bracket (D.I.6: pharyngeal expression of MRF3), open arrowheads (D.II.4: expression of MRF2b in pharyngeal gill slits and D.II.6: expression of MRF3 in the mouth rudiment) and the circle (D.II.4: expression of MRF2b in the wall of the preoral pit).

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4. Conclusion

Here we present a few gene expression patterns for three different metazoans used as models in the present JRA. This expression patterns have been published in:

Momose, T., De Cian, A., Shiba, K. et al. High doses of CRISPR/Cas9 ribonucleoprotein efficiently induce gene knockout with low mosaicism in the hydrozoan Clytia hemisphaerica through microhomology-mediated deletion. Sci Rep 8, 11734 (2018).

Chowdhury R, et al (2022). Highly distinct genetic programs for peripheral nervous system formation in chordates. BMC Biology 20, 152.

Meister L, et al. Functions of the FGF signalling pathway in cephalochordates provide insight into the evolution of the prechordal plate. Development. 2022;149(10):dev200252.

Aase-Remedios, et al. (2020). More than one-to-four via 2R: evidence of an independent amphioxus expansion and two-gene ancestral vertebrate state for MyoD-related Myogenic Regulatory Factors (MRFs). Mol. Biol. Evol. 37(10): 2966-2982.

The need of known gene expression patterns is obvious for understanding phenotypes produced by CRISPR-Cas9 KO. The different laboratories implicated in this JRA, will certainly continue developing this activity in the future.

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