

Stage-resolved analysis of posterior regeneration in *Ophryotrocha labronica* reveals early pluripotency activation and conserved regenerative programs

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Supplementary Information includes:

Supplementary Information 1. Include Supplementary Figures 1 and 2 and Supplementary Tables 1 and 2.

Supplementary Information 2. edgeR results for all pairwise comparisons, including normalised expression values and BLAST annotations.

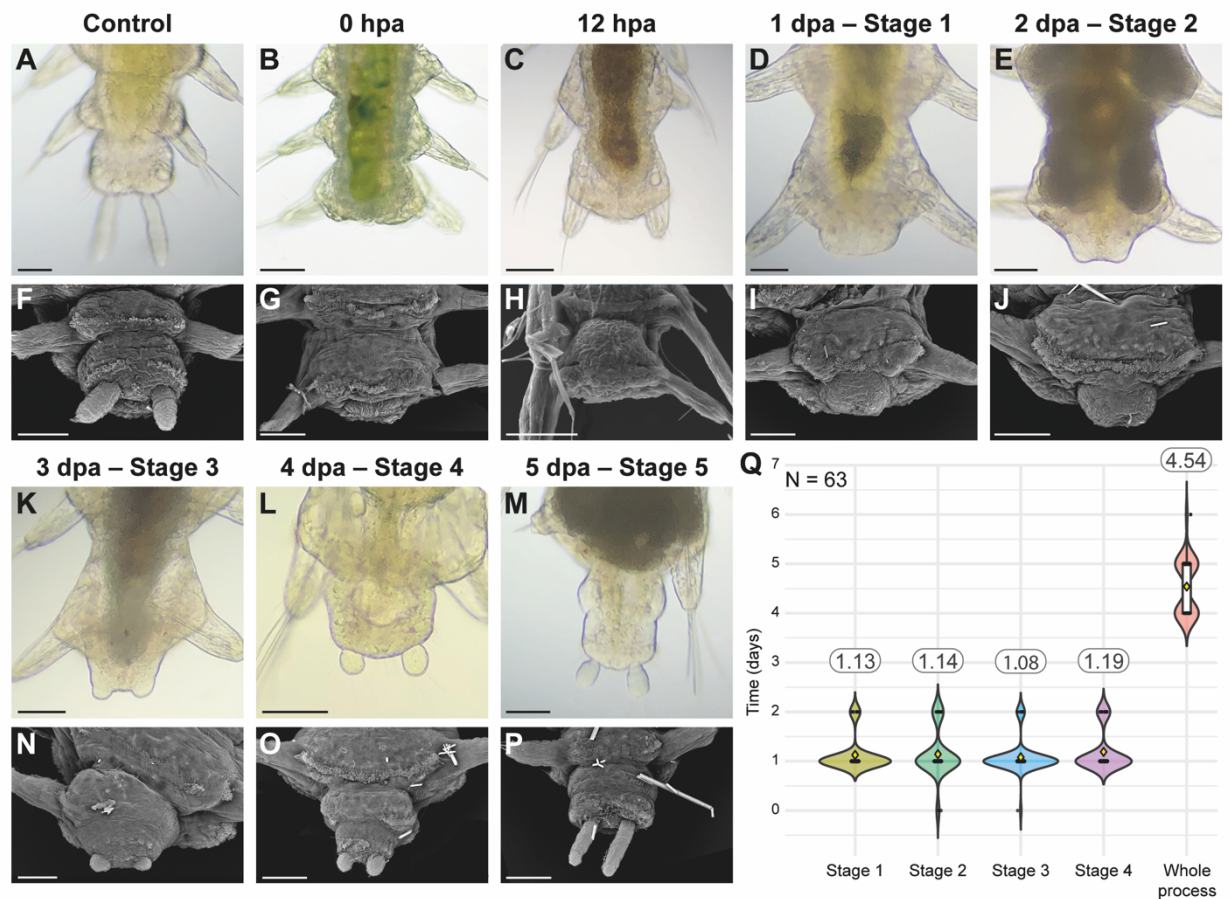
Supplementary Information 3. MFuzz cluster expression plots across posterior regeneration stages (m= 1.4, 30 clusters).

Supplementary Information 4. MFuzz cluster core gene lists for α -core ≥ 0.5 .

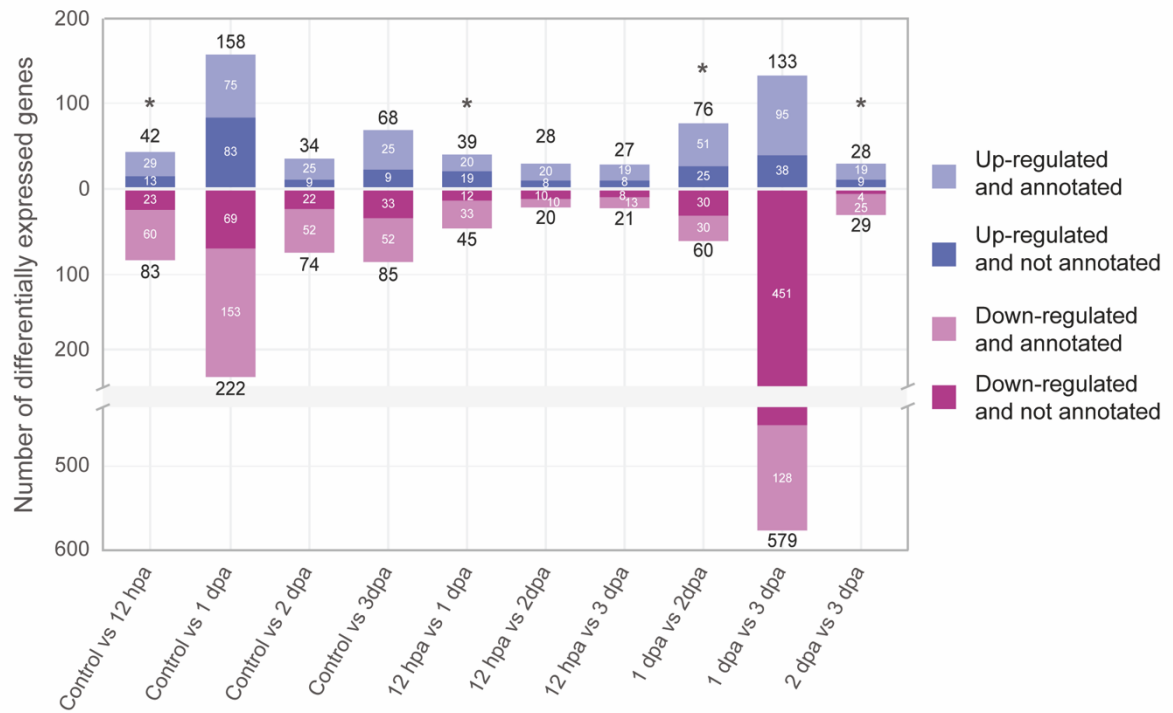
Supplementary Information 5. Gene ID annotation of MFuzz clusters peaking at control, 12 hpa, and 1–3 dpa, corresponding to clusters 4, 16, 23, 18, and 26, respectively. Genes unique to that cluster are highlighted in yellow.

Supplementary Information 6. Enriched GO terms associated with the unique genes identified in each of the MFuzz clusters peaking at at control and 12 hpa, and 1–3 dpa.

Supplementary Information 7. List of previously reported regeneration genes in other annelid species, showing their expression across regeneration timepoints in the annelid *O. labronica*.



Supplementary Figure 1. Morphological characterisation of posterior regeneration in *O. labronica* from 0 to 5 dpa. All panels show posterior ends of amputated individuals at each stage, indicated above each image. (A-E, K-M) bright-field images, dorsal view and (F-J, N-P) SEM micrographs, ventral view. (Q) Violin plot showing the time spent at each stage per individual, as well as the total regeneration time up to the addition of the first segment (i.e., stage 5). Scale bars: A-E, K-M: 100 μm ; F-J, N-P: 50 μm .



Supplementary Figure 2. Differential gene expression across regeneration stages in *O. labronica*. Bar plots show the total number of upregulated and downregulated genes identified across all pairwise comparisons, separated into annotated and non-annotated genes. Asterisks indicate sequential comparisons.

Supplementary Table 1. Summary of the libraries generated in this study, including sample details and corresponding SRA accession numbers (PR, posterior regeneration).

Experimental condition	ID	SRA Accession number	Tapestation concentration (ng/μl)	Raw reads	Trimmed reads	% retained
Control	OIC_1		12,5	20541458	19883758	96.80
	OIC_2		10,8	19658969	19028693	96.79
PR 12 hpa	OIPR12_1		5,69	22287461	21634664	97.07
	OIPR12_2		15,4	24892071	23915603	96.08
	OIPR12_3		8,75	17315680	17013445	98.25
PR 24 hpa	OIPR24_1		8,4	23551096	22855046	97.04
	OIPR24_2		3,46	17124490	16250973	94.90
	OIPR24_3		9,67	15785837	15177341	96.15
PR 48 hpa	OIPR48_1		3,4	18133797	17160281	94.93
	OIPR48_2		24,6	22995353	22326434	97.00
	OIPR48_3		5,57	20816171	19804188	95.14
PR 72 hpa	OIPR72_1		5,87	23001565	22321817	97.04
	OIPR72_2		4,77	22336381	21687597	97.10
	OIPR72_3		14,2	19085644	17582884	92.13
TOTAL:				287525973	276642724	96.21

Supplementary Table 2. De novo Trinity assembly and BUSCO completeness statistics.

Length Statistics and Composition:	
Total assembled reads	276219078
Total assembled bases	468455302
Trinity transcripts	535801
Longest sequence (bp)	33544
Shortest sequence (bp)	169
Average sequence length (bp)	874.31
Median sequence length (bp)	423
Contig N10 (bp)	5426
Contig N20 (bp)	3906
Contig N30 (bp)	2962
Contig N40 (bp)	2256
Contig N50 (bp)	1673
L50 sequence count	73038
Ex90N50 (bp)	1919
Number of sequences > 1K (bp)	123854 (23.1% of total number)
Number of sequences > 10K (bp)	369 (0.1% of total number)
Number of sequences > 100K (bp)	0 (0.0% of total number)
Number of sequences > 1M (bp)	0 (0.0% of total number)
Number of sequences > 10M (bp)	0 (0.0% of total number)
Base composition (%)	A: 29.93
	T: 30.00
	G: 19.89
	C: 20.17
	N: 0.00
	Other: 0.00
GC-content (%)	40.06
Number of non-ACGTN (bp)	0
Completeness Assessment Results:	
Total number of core genes queried	954
Number of core genes detected	
Complete	948 (99.37%)
Complete + Partial	950 (99.58%)
Number of missing core genes	4 (0.42%)
Average number of orthologs per core genes	3.06
% of detected core genes that have more than 1 ortholog	77.32
Scores in BUSCO format	C:99.3%[S:22.5%, D:76.8%], F:0.2%, M:0.5%