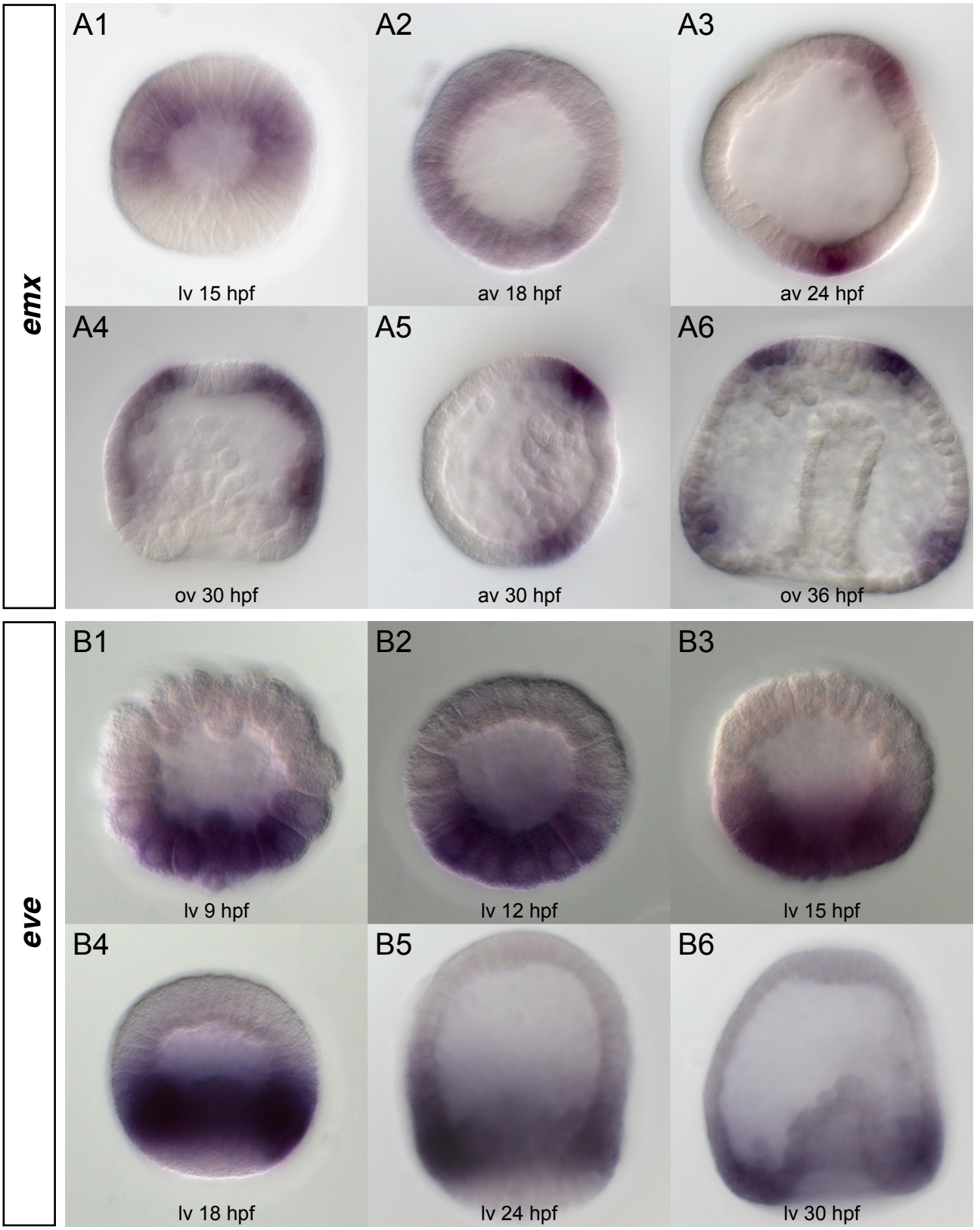
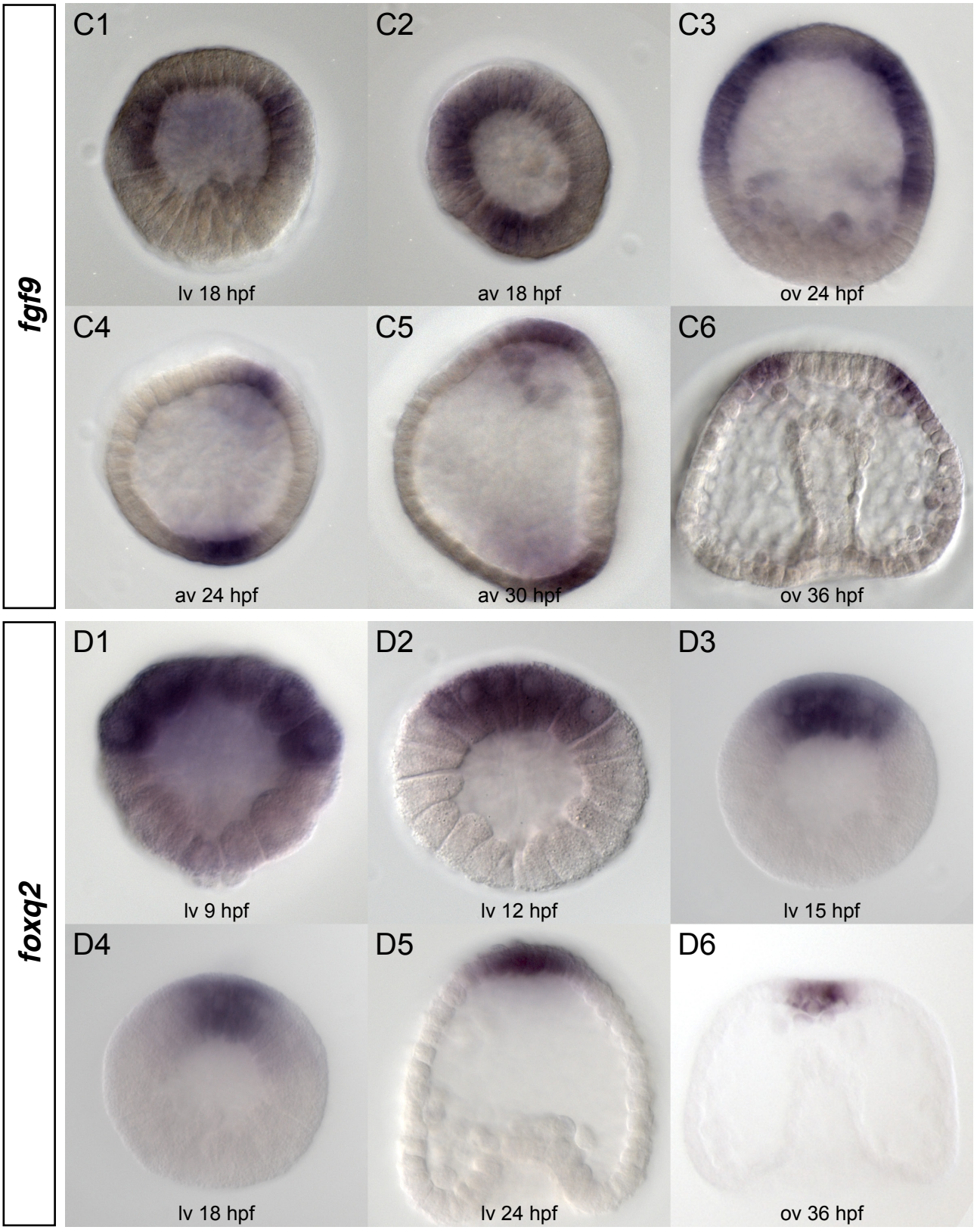
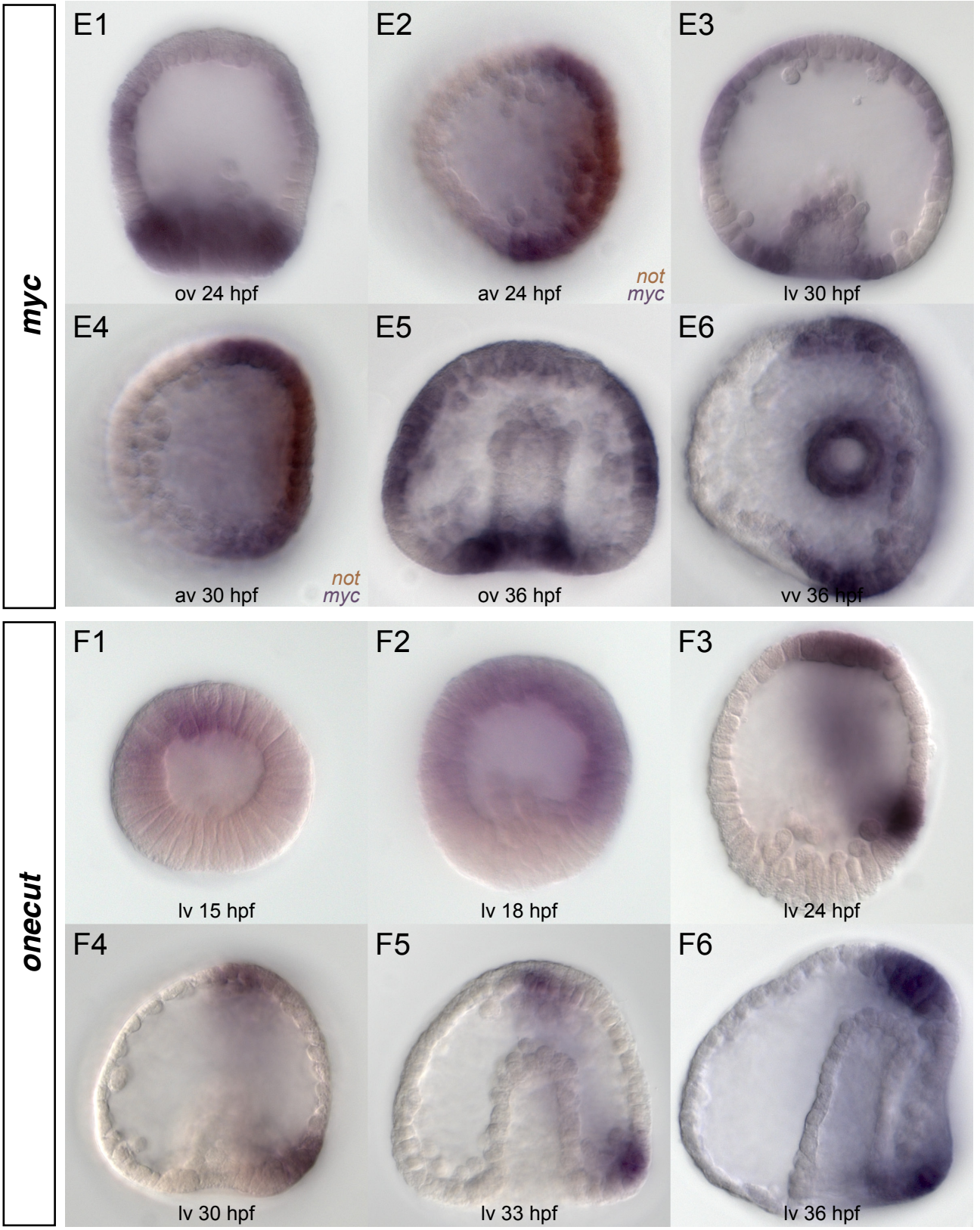
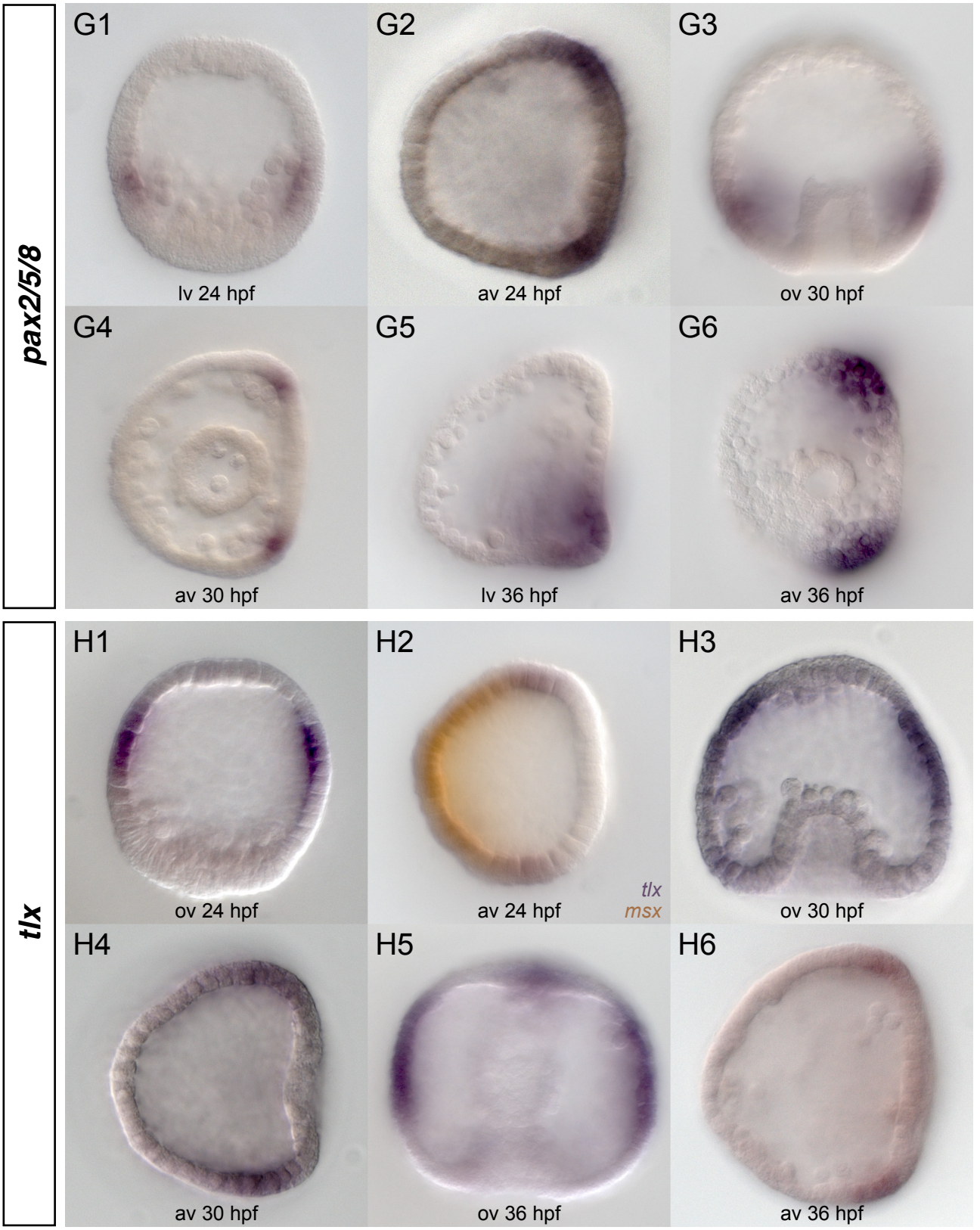
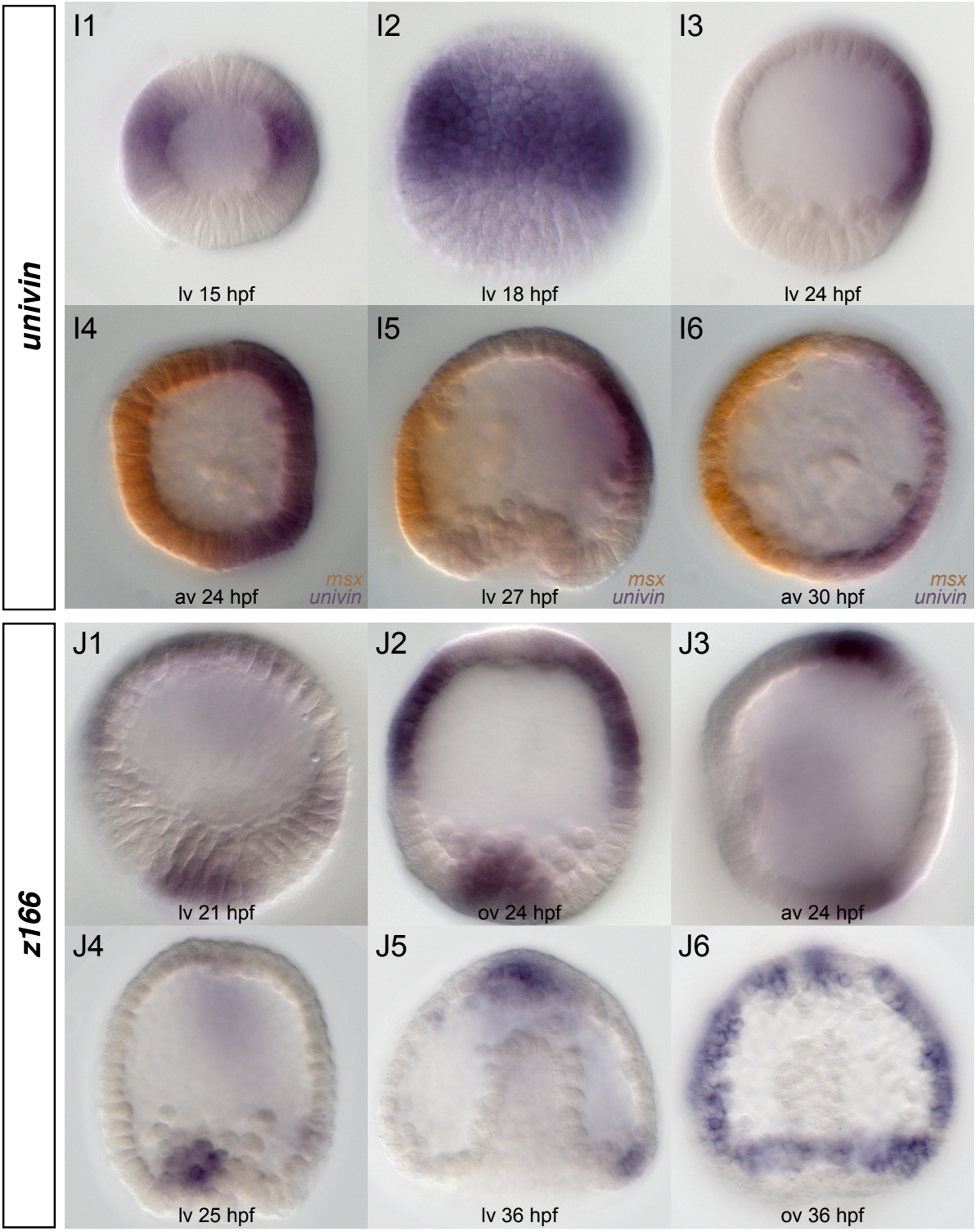

emx
A1
lv 15 hpf
A2
av 18 hpf
A3
av 24 hpf
A4
ov 30 hpf
A5
av 30 hpf
A6
ov 36 hpf
eve
B1
lv 9 hpf
B2
lv 12 hpf
B3
lv 15 hpf
B4
lv 18 hpf
B5
lv 24 hpf
B6
lv 30 hpf

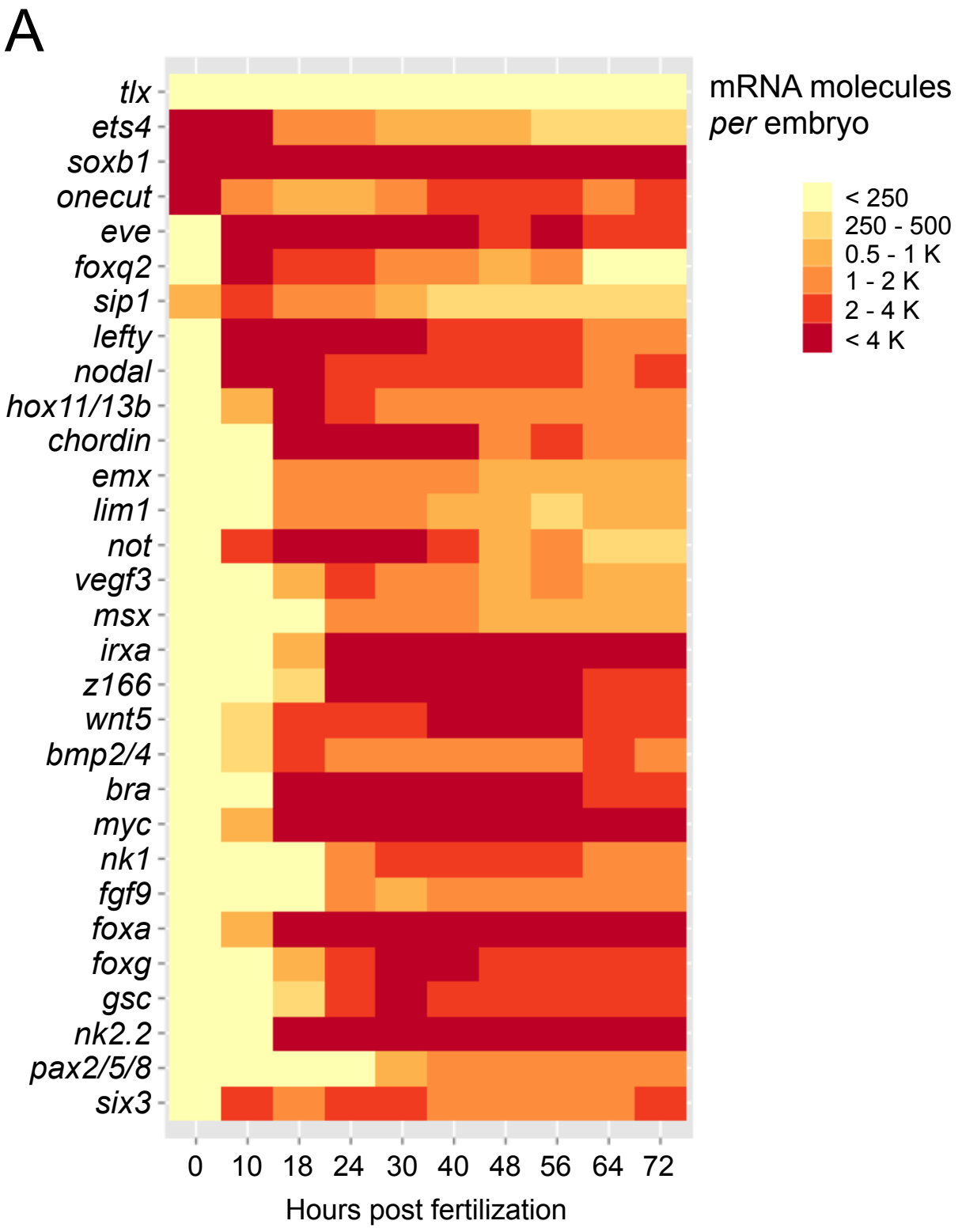

fgf9
C1
lv 18 hpf
C2
av 18 hpf
C3
ov 24 hpf
C4
av 24 hpf
C5
av 30 hpf
C6
ov 36 hpf
foxq2
D1
lv 9 hpf
D2
lv 12 hpf
D3
lv 15 hpf
D4
lv 18 hpf
D5
lv 24 hpf
D6
ov 36 hpf

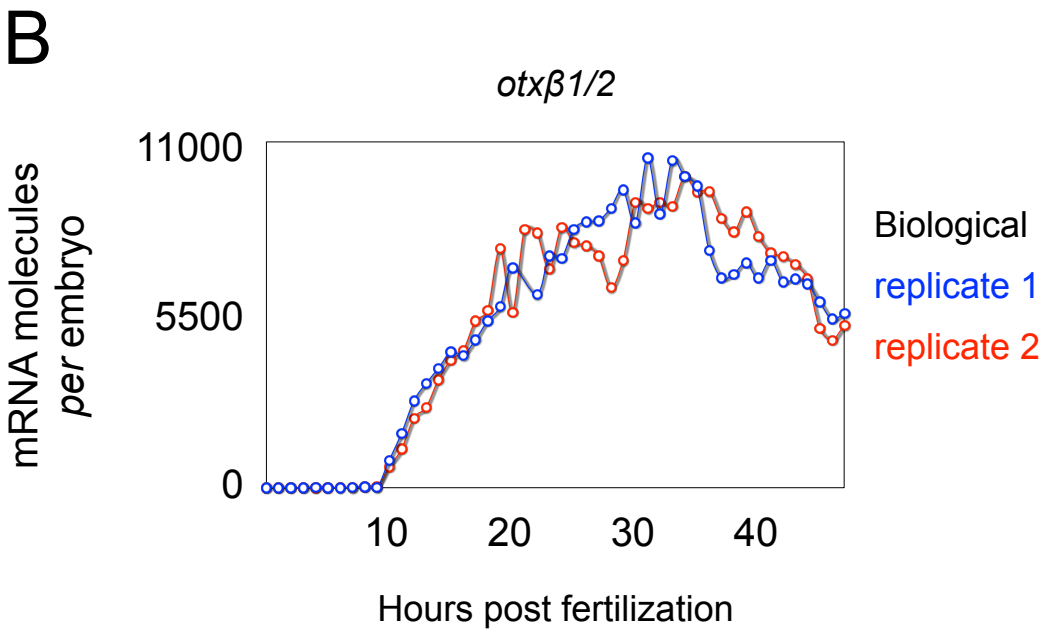

myc
E1
ov 24 hpf
E2
av 24 hpf
not myc
E3
lv 30 hpf
E4
av 30 hpf
not myc
E5
ov 36 hpf
E6
vv 36 hpf
onecut
F1
lv 15 hpf
F2
lv 18 hpf
F3
lv 24 hpf
F4
lv 30 hpf
F5
lv 33 hpf
F6
lv 36 hpf


pax2/5/8
G1
lv 24 hpf
G2
av 24 hpf
G3
ov 30 hpf
G4
av 30 hpf
G5
lv 36 hpf
G6
av 36 hpf
tlx
H1
ov 24 hpf
H2
av 24 hpf
tlx
msx
H3
ov 30 hpf
H4
av 30 hpf
H5
ov 36 hpf
H6
av 36 hpf



Supplementary Fig. S1. Gene expression patterns. (A-J) RNA *in situ* hybridization series for relevant genes (A) *emx*, (B) *eve*, (C) *fgf9*, (D) *foxq2*, (E) *myc*, (F) *onecut*, (G) *pax2/5/8*, (H) *tlx*, (I) *univin*, (J) *z166*. Abbreviations: av, apical pole view; lv, lateral view; ov, oral view; vv, ventral view; hpf, hours post fertilization. Oral ectoderm faces towards the right, except in panels marked ov, where the oral ectoderm is viewed head-on.


A
tlx
ets4
soxb1
onecut
eve
foxq2
sip1
lefty
nodal
hox11/13b
chordin
emx
lim1
not
vegf3
msx
irxa
z166
wnt5
bmp2/4
bra
myc
nk1
fgf9
foxa
foxg
gsc
nk2.2
pax2/5/8
six3
0
10
18
24
30
40
48
56
64
72
Hours post fertilization
mRNA molecules
per embryo
< 250
250 - 500
0.5 - 1 K
1 - 2 K
2 - 4 K
< 4 K



Supplementary Fig. S2. Gene expression kinetics. (A) Level of mRNA abundance throughout development for all genes modeled in Fig. 7. Heat-map reflecting the expression profile for each gene listed. Absolute values of mRNA levels are inferred from the color code key, at right. Measurements are from our previous study (Tu et al., 2014), and are plotted using the online tool described therein. (B) Expression time course for the *otxβ1/2* splice variant (this transcript is not included in A). Data are from previously published measurements (Materna et al., 2010).

Table S1. Nanostring codeset information (mRNA targets 1-197)

Click here to Download Table S1

Supplementary Methods

Normalization

mRNA raw code counts (RCCs) were normalized as described (Barsi et al., 2014).

Differential gene expression

Given the vast experience our laboratory has amassed over the years concerning this technology and the fact that we contributed toward it's pioneer study (Geiss et al., 2008), hundreds of technical replicates have been compared using the nCounter analysis system. In no instance has the code count between technical replicates, for any given mRNA species in our codeset, deviated beyond twofold (with the exception of insignificantly low-level transcripts). From this, we conclude that differential gene expression is biologically relevant if a delta larger than twofold is observed for any given transcript. We acknowledge that our assumption is conservative, given that transcripts with a smaller observed delta could potentially be statistically significant. Nevertheless, for the purpose of this study, there is no question that a twofold difference (or higher) in gene expression accurately reflects the biological repercussion of a MASO perturbation experiment. The envelope delineated by a red line in Fig. 4 reflects the empirically derived confidence threshold described above. Data points found beyond this envelope are of biological import to this study.

References

Barsi, J. C., Tu, Q. and Davidson, E. H. (2014). General approach for in vivo recovery of cell type-specific effector gene sets. *Genome Res*. **24**, 860-868.

Geiss, G. K., Bumgarner, R. E., Birditt, B., Dahl, T., Dowidar, N., Dunaway, D. L., Fell, H. P., Ferree, S., George, R. D., Grogan, T. et al. (2008). Direct multiplexed measurement of gene expression with color-coded probe pairs. *Nat. Biotechnol.* **26**, 317-325.

Materna, S. C., Nam, J. and Davidson, E. H. (2010). High accuracy, high-resolution prevalence measurement for the majority of locally expressed regulatory genes in early sea urchin development. *Gene Expr. Patterns* **10**, 177-184.