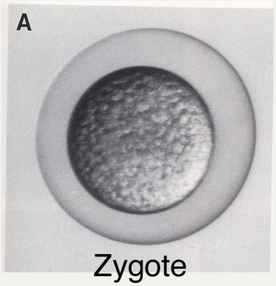
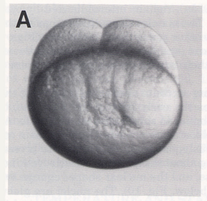
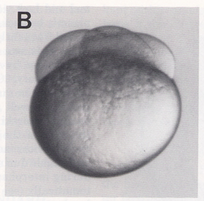


Zygote

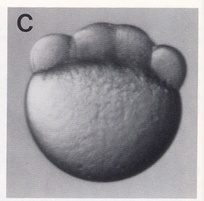
Fertilized



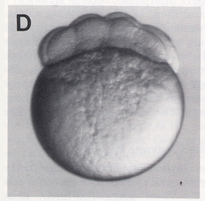
Two cells



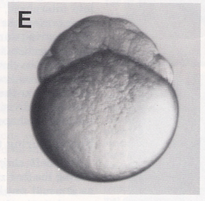
Four cells



Eight cells

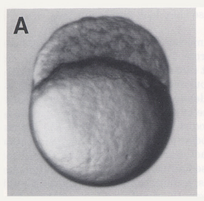


Sixteen cells

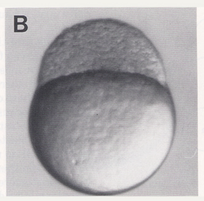


Thirty two

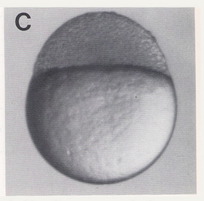
Sixty four



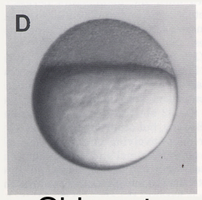
256



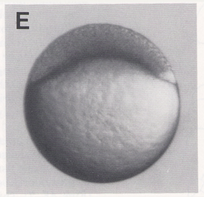
High stage



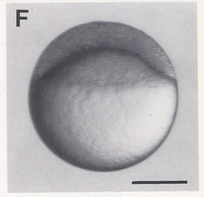
High to oblong transition



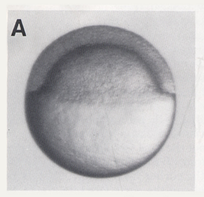
Oblong to sphere



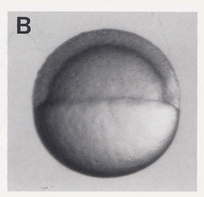
Dome



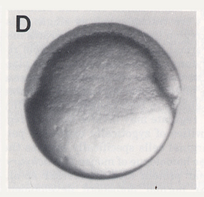
30% epiboly



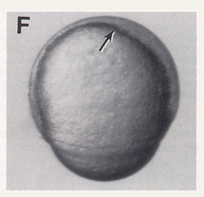
50% epiboly



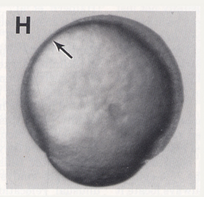
Germ ring



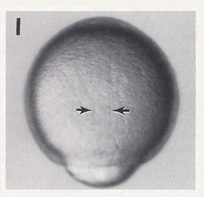
Shield



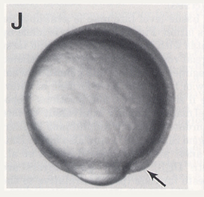
70% epiboly



75% epiboly

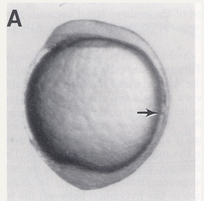


80% epiboly

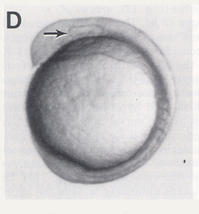


90% epiboly

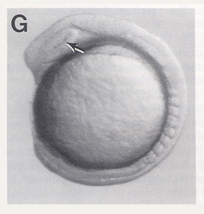
Bud stage



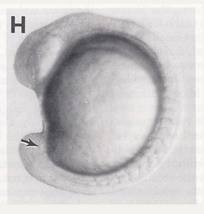
2-somite



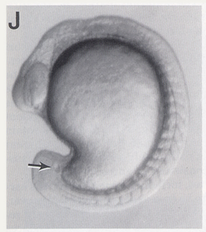
4-somites



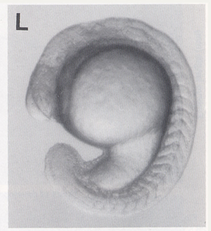
8-somites



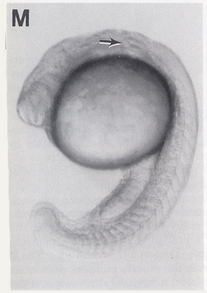
13-somites



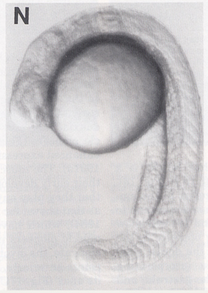
15-somites



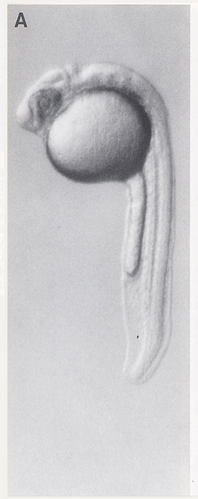
17-somites



20-somites

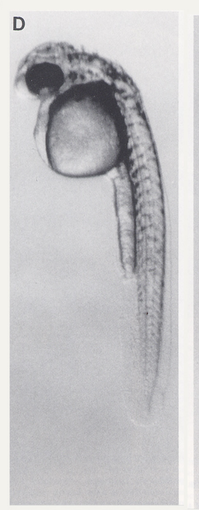


25-somites

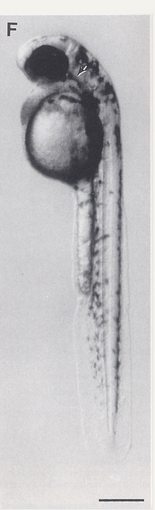


24 hr

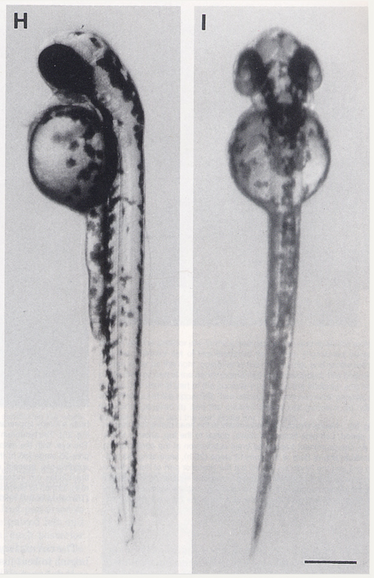
28 hr



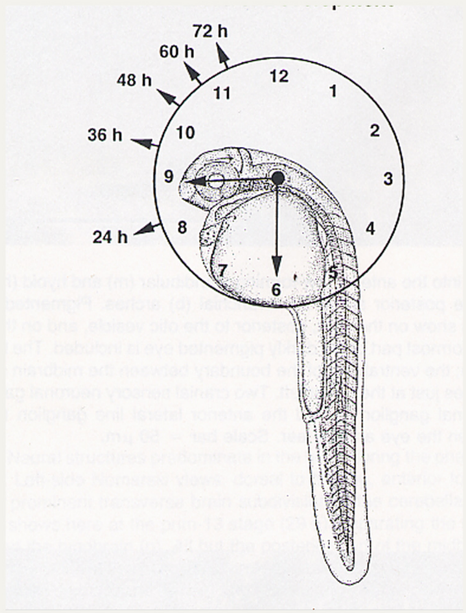
33 hr



36 hr



48 hr



From: Kimmel et al. Stages of embryonic development of the zebrafish
Dev. Dyn. 203:253-310, 1995

Whole mount in situ hybridization

Solutions

10 x PBS

NaCl	80 g
KCl	2 g
Na2PO4	14.4 g
KH2PO4	2.4 g
	/ 1 liter

Adjust to pH 7.4

Sterile by autoclaving

PBST : PBS containing 0.1% Tween 20

4% PFA/PBS

20% PFA	10 mL
10 x PBS	5 mL
DW	up to 50 mL

Store at -20˚C.

Hybridization buffer		final
Formamide	25mL	50%
Tween 20	50 ul	0.1%
50 ug/ul yeast tRNA	50 ul	50 ug/ml
50 ug/ul heparin	50 ul	50 ug/ml
20 x SSC	12.5 mL	5x
DEPC treated DW	up to 50 mL	

RNase buffer

5 M NaCl	5 mL
1 M Tris-HCl (pH 8.0)	500 ul
Tween 20	50 ul
DW	up to 50 ML

RNase solution

20 ug/ul RNase A	10 ul
5 U/ul RNase T1	20 ul
RNase buffer	10 mL

Blocking solution

10% Blocking reagent	5 mL
10 x PBS	5 mL
Tween 20	100 ul
DW	up to 50 mL

10% Blocking reagent

Blocking reagent	10 g	Roche cat# 1 096 176
Buffer 1	100 mL	

mix well, then autoclave it.

after autoclave, mix well again until the solution is hot.

(This is very important to dissolve the powder.)

Store at 4 ˚C

Buffer 1

Maleic acid	23.2 g
NaCl	17.53 g
DW	1.5 L

adjust pH 7.5 with NaOH, then add DW up to 2L
to adjust pH, you can add 15 g of solid NaOH first.
The solution should be autoclaved.

Antibody solution
Dig-antibody : Blocking solution = 1: 4000

AP buffer

1 M Tris-HCl(pH 9.5)	5 mL
1 M MgCl2	2.5 mL
5 M NaCl	1 mL
Tween 20	50 ul
DW	up to 50 mL

20 x SSC

NaCl	175.3g
Tri-sodium citrate	88.2g
	/1 liter

5 x SSCT/50% formamide

Formamide	25 mL
20 x SSC	12.5 mL
Tween 20	50 ul
DW	up to 50 mL

2 x SSCT/50% formamide

Formamide	25 mL
20 x SSC	5 mL
Tween 20	50 ul
DW	up to 50 mL

2 x SSCT

20 x SSC	5 mL
Tween 20	50 ul
DW	up to 50 mL

0.2 x SSCT

20 x SSC	0.5 mL
Tween 20	50 ul
DW	up to 50 mL

Procedures

1st day

Transfer embryos in 100% MetOH into Eppendorf tubes.	
Wash with 75% MetOH	5 min
Wash with 50% MetOH	5 min
Wash with 25% MetOH	5 min
Wash in PBST	5 min x 4

Embryos older than 18 somites should be treated with ProtK (10 μg/ml in PBST):

5 min for 18som-24hr, 10 min for up to 1.5 day, 20-30 minutes for up to 4 day larvae

Refix embryos with 4% PFA/PBS	20 min
Wash in PBST	5 min
Add 400 μl (pre)hybridization buffer	1-3 hours at 65°C
Add 1 ul of probe to 400 μl hyb. buffer	5 min at 65°C
Exchange prehyb buffer to probe solution	ON at 65°C

2nd day

Rinse embryos with 5 x SSCT/50% formamide	
Wash with 2 x SSCT/50% formamide	1h at 65°C
Wash with 2 x SSCT	10 min x 2 at RT (room temperature)
Wash with RNase buffer	10 min
Rnase treatment	10 min
Wash with 2 x SSCT	10 min
Wash with 2 x SSCT/50% formamide	1h at 65°C
Wash with 2 x SSCT	15 min at 65°C
Wash with 0.2 x SSCT	15 min at 65°C
Was with PBST	5 min at RT
Rinse with Blocking solution	
Incubate in Blocking solution	1h (RT)
Incubate embryos in antibody solution	ON at 4 °C

3rd day

Wash with PBST	15 min x 6 (at RT)
Was with AP buffer	5 min x 3

Incubate embryos in BM-purple

(Note: before using BM-purple centrifuge it at 13,000 rpm for 1 min and use only the supernatant)

Check embryos every 30 min

Stop the staining reaction with several washes in PBST

Fix embryos in 4% PFA/PBS 4°C ON or 20 min at RT

Wash with PBST several times

Move into 90% glycerol, store in dark