

Figure S1

NF stage	Animal		Vegetal/Ventral		Dorsal		Lateral	
	NF	Zahn	NF	Zahn	NF	Zahn	NF	Zahn
1 (egg)	✓	✓	✓	✓	✓	✓	□	□
2-	✓	□	□	✓	□	✓	□	□
2 (2-cell)	✓	✓	✓	✓	✓	✓	□	□
3 (4-cell)	✓	✓	□	□	□	□	✓	✓
4 (8-cell)	✓	✓	✓	✓	✓	✓	□	□
5 (16-cell)	✓	✓	□	□	✓	✓	□	□
6 (32-cell)	✓	✓	✓	✓	✓	✓	□	□
6.5	✓	✓	✓	✓	✓	✓	□	□
7	✓	✓	✓	✓	✓	✓	□	□
8	✓	✓	✓	✓	✓	✓	✓	✓
9	□	✓	✓	✓	✓	✓	□	□
Vegetal-Posterior								
10	✓	✓	✓	✓	✓	✓	✓	✓
10.25	□	□	□	□	□	□	□	□
10.5	✓	✓	✓	✓	✓	✓	✓	✓
11	✓	✓	✓	✓	✓	✓	✓	✓
11.5	✓	✓	✓	✓	✓	✓	✓	✓
12	✓	✓	✓	✓	✓	✓	✓	✓
12.5	✓	✓	✓	✓	✓	✓	✓	✓
Anterior								
Anterior		Ventral		Dorsal		Lateral		
NF	Zahn	NF	Zahn	NF	Zahn	NF	Zahn	
13	□	✓	□	□	✓	✓	□	□
14	□	□	□	□	✓	✓	✓	✓
15	✓	✓	✓	✓	✓	✓	✓	✓
16	✓	✓	✓	✓	✓	✓	✓	✓
17	✓	✓	✓	✓	✓	✓	✓	✓
18	✓	✓	✓	✓	✓	✓	✓	✓
19	✓	✓	✓	✓	✓	✓	✓	✓
20	✓	✓	✓	✓	✓	✓	✓	✓
21	✓	✓	✓	✓	✓	✓	✓	✓
22	□	✓	✓	✓	✓	✓	✓	✓
23	✓	✓	✓	✓	✓	✓	✓	✓
24	✓	✓	✓	✓	✓	✓	✓	✓
25	✓	✓	✓	✓	✓	✓	✓	✓
26	✓	✓	✓	✓	✓	✓	✓	✓
27	✓	✓	✓	✓	✓	✓	✓	✓
28	✓	✓	✓	✓	✓	✓	✓	✓
29-30	✓	✓	✓	✓	✓	✓	✓	✓
31	✓	✓	✓	✓	✓	✓	✓	✓
32	✓	✓	✓	✓	✓	✓	✓	✓
33-34	✓	✓	✓	✓	✓	✓	✓	✓
35-36	✓	✓	✓	✓	✓	✓	✓	✓
37-38	✓	✓	✓	✓	✓	✓	✓	✓
39	✓	✓	✓	✓	✓	✓	✓	✓
40	✓	✓	✓	✓	✓	✓	✓	✓
41	✓	✓	✓	✓	✓	✓	✓	✓
42	✓	✓	✓	✓	✓	✓	✓	✓
43	✓	✓	✓	✓	✓	✓	✓	✓
44	✓	✓	✓	✓	✓	✓	✓	✓
45	✓	✓	✓	✓	✓	✓	✓	✓
46	✓	✓	✓	✓	✓	✓	✓	✓
47	✓	✓	✓	✓	✓	✓	✓	✓
Anterior								
Anterior		Ventral		Dorsal		Lateral		
NF	Zahn	NF	Zahn	NF	Zahn	NF	Zahn	
48	□	✓	□	□	✓	✓	✓	n/a n/a
49	□	□	✓	✓	□	□	✓	n/a n/a
50	✓	✓	✓	✓	✓	✓	✓	✓
51	✓	✓	✓	✓	✓	✓	✓	✓
52	✓	✓	✓	✓	✓	✓	✓	✓
53	✓	✓	✓	✓	✓	✓	✓	✓
54	✓	✓	✓	✓	✓	✓	✓	✓
55	✓	✓	✓	✓	✓	✓	✓	✓
56	✓	✓	✓	✓	✓	✓	✓	✓
57	✓	✓	✓	✓	✓	✓	✓	✓
58	✓	✓	✓	✓	✓	✓	✓	✓
59	✓	✓	✓	✓	✓	✓	✓	✓
60	✓	✓	✓	✓	✓	✓	✓	✓
61	✓	✓	✓	✓	✓	✓	✓	✓
62	✓	✓	✓	✓	✓	✓	✓	✓
63	✓	✓	✓	✓	✓	✓	✓	✓
64	✓	✓	✓	✓	✓	✓	✓	✓
65	✓	✓	✓	✓	✓	✓	✓	✓
66	✓	✓	✓	✓	✓	✓	✓	✓
Hindlimbs								
Hindlimbs		Forelimbs						
NF	Zahn	NF	Zahn	NF	Zahn	NF	Zahn	

**Fig. S1. A comparison of available illustrations of *Xenopus laevis* developmental stages from Nieuwkoop and Faber's Normal Table (1994 edition) and those by Natalya Zahn.** Check boxes show drawings which illustrate each stage in the view indicated in column headers (i.e., animal vegetal/ventral, vegetal-posterior, anterior, ventral dorsal, lateral). Note that Nieuwkoop and Faber's NF stage 3 and NF stage 4 'dorso-lateral' views are recorded in the 'lateral' view column, and NF stage 13, 14 15 and 16 'post-dorsal' views are recorded here in the 'dorsal' view column. Light grey shaded cells indicate that line drawings (n= 56) are available for specified stage/view. Darkly shaded cells indicated drawings are the those commissioned from XenHead project (Zahn et al., 2017). Open box: drawing not available. n/a: not applicable. Total Nieuwkoop and Faber illustrations = 125, and total Zahn illustrations = 142 (plus an additional 56 simple line illustrations), when counting each limb bud as an individual drawing in both series. Many of the Zahn drawings (but not all) are available as both complex, fully shaded illustrations and as simple unshaded line drawings. All drawings are available on Xenbase, but note that only the Zahn drawings are open access, and can be reused using this citation: Illustration © 2021 Natalya Zahn, CC BY-NC 4.0.

## Figure S2

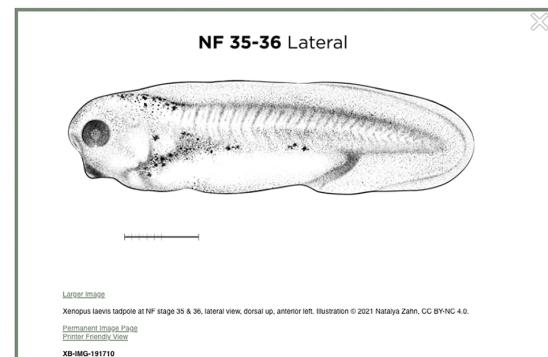
A

This screenshot shows the Xenbase XAO page for NF stage 28. The top navigation bar includes links for Summary, Stage Literature (517), Attributions, and Wiki. The main title is XB-STAGE-46. Below the title, the stage is identified as Name: NF stage 28 and XAO Id: 1000044. A detailed definition is provided: "Nieuwkoop and Faber stage 28, corresponding to an embryo of age 1 day, 8 hr 30 min at 22-24 Celsius and having the following external morphological criteria: Fin extending up to anus. Fin broadened and distinctly divided into outer transparent and inner translucent band. Length: 3.8 - 4.0 mm." Below the definition, it says Preceded By: NF stage 27 and Succeeded By: NF stage 29 and 30. To the right of the text is a small Zahn drawing of an embryo, and below it is a photograph of a tadpole.

B

This screenshot shows the Xenbase XAO page for NF stage 35 and 36. The top navigation bar includes links for Summary, Stage Literature (2), Attributions, and Wiki. The main title is XB-STAGE-51. Below the title, the stage is identified as Name: NF stage 35 and 36 and XAO Id: 1000049. A detailed definition is provided: "Nieuwkoop and Faber stage 35/36, corresponding to an embryo of age 2 days, 2 hr at 22-24 Celsius and having the following external morphological criteria: Stomodeal invagination roundish. Eye entirely black, choroid fissure nearly closed. Formation of two gill rudiments, anterior one nipple-shaped. Melanophores appearing on back. Posterior outline of proctodeum still curved. Length of tail bud about three times its breadth. Beginning of hatching. Length: 5.3 - 6.0 mm." Below the definition, it says Preceded By: NF stage 33 and 34 and Succeeded By: NF stage 37 and 38. To the right of the text is a large photograph of a tadpole.

C



**Fig. S2. Xenbase XAO pages for *Xenopus* embryo stages.** Each XAO page for embryonic stages on Xenbase has a definition, based on the Nieuwkoop and Faber Normal Table, and is populated with Zahn drawings and other images (e.g., photographs from stereomicroscopy) when available. A. XAO page for NF stage 28. B. XAO page for NF stage 35 & 36, illustrated with a *Xenopus* community submitted image taken by Matthew Kofron (used here with their kind permission). C. Pop-up window from NF stage 35 & 36, activated by double click on the image, shows a larger view and has a left-right scroll arrow to view all images posted to this page, including Zahn drawings. Links from the pop-up will enlarge an image further or give researchers a printer friendly view.

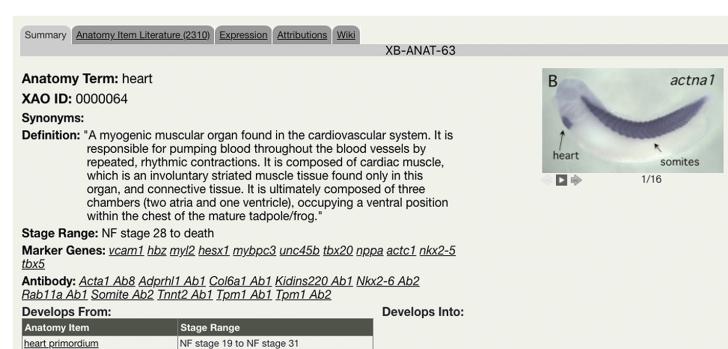
Figure S3

**A**

Summary Anatomy Item Literature (2310) Expression Attributions Wiki XB-ANAT-63

**Anatomy Term:** heart  
**XAO ID:** 0000064  
**Synonyms:**  
 Definition: "A myogenic muscular organ found in the cardiovascular system. It is responsible for pumping blood throughout the blood vessels by repeated, rhythmic contractions. It is composed of cardiac muscle, which is an involuntary striated muscle tissue found only in this organ, and connective tissue. It is ultimately composed of three chambers (two atria and one ventricle), occupying a ventral position within the chest of the mature tadpole/frog."

**Stage Range:** NF stage 28 to death  
**Marker Genes:** *vcam1 hbx myl2 hessx1 mybpc3 unc45b tbx20 npga actc1 nkx2-5 tbx5*  
**Antibody:** *Acta1 Ab8 Adprhl1 Ab1 Col6a1 Ab1 Kidins220 Ab1 Nkx2-6 Ab2 Rab17a Ab1 Somite Ab2 Tnni2 Ab1 Tpm1 Ab1 Tpm1 Ab2*  
**Develops From:** heart primordium  
**Develops Into:** heart


**B**

Summary Anatomy Item Literature (2310) Expression Attributions Wiki XB-ANAT-63

**Genes expressed in heart**  
 100 displayed out of 3237 [View all](#)

	Images	Clones	Papers	Total
<i>nkx2-5</i>	78	0	198	276
<i>tnn13</i>	68	0	72	140
<i>myh6</i>	42	0	68	110
<i>actc1</i>	37	0	78	115
<i>hand1</i>	30	0	22	52
<i>tpm1</i>	28	0	24	52
<i>bmp4</i>	21	0	80	101
<i>tbx5</i>	20	0	53	73
<i>tbx20</i>	20	0	26	46
<i>tnt2</i>	18	0	15	33
<i>myl2</i>	17	0	29	46
<i>aplnr</i>	16	0	34	50
<i>pitx2</i>	14	0	70	84
<i>alcam</i>	12	0	6	18
<i>myh1</i>	10	0	11	21
<i>adprhl1</i>	9	0	2	11
<i>h3-3a</i>	9	0	11	20
<i>jag1</i>	8	0	4	12
<i>wnt11</i>	7	0	25	32
<i>cdh2</i>	7	0	9	16
<i>casz1</i>	7	0	5	12
<i>myl7</i>	7	0	13	20
<i>rps12</i>	7	0	0	7
<i>rack1</i>	7	0	1	8
<i>rps9</i>	7	0	0	7
<i>crip3</i>	7	0	1	8
<i>hand2</i>	6	0	19	25

**Fig. S3. Xenbase XAO anatomy pages.** Anatomical entities (i.e., primary germ layers, cell types, tissues, organs, structures) are individual terms in the *Xenopus* anatomy ontology, the XAO. XAO terms used in the Landmarks Table link to the specific XAO page where information about that term is collated. A. XAO page (Summary tab) for heart (XAO:0000064), where the definition is based on Nieuwkoop and Faber's (1956) Normal Table criteria, and a curated list of molecular markers from Xenbase gene expression data are given. B. Expression tab of XAO page for heart collates all curated gene expression data. Columns are sortable based on number of primary sources, predominantly images (curated gene expression data from published research articles) and papers (where gene and anatomy are co-referenced). Click 'Images' heading to sort genes with the most curated gene expression figures from primary sources (and therefore the best candidates for molecular markers) at the top of the list.

**Table S1.** *Xenopus laevis* staging landmarks. For a live online Xenbase version see:

<https://www.xenbase.org/entry/landmarks-table.do>

Developmental Process	NF stage number	NF stage name	LANDMARKS		MOLECULAR MARKERS [gene: anatomy]
			EXTERNAL	INTERNAL	
FERTILIZATION	n/a	mature egg	1 cell; ovulated unfertilized egg; <b>animal hemisphere</b> dark, <b>vegetal hemisphere</b> light; animal-vegetal axis in random orientation; soft texture	large nucleus, ' <b>germinal vesicle</b> ' in <b>animal hemisphere</b>	<i>nanos1</i> : mitochondrial cloud; <i>vegt</i> : vegetal cortex
	1	1-cell, fertilized egg	1 cell; <b>vitelline membrane</b> swells; eggs rotate with dark <b>animal hemisphere</b> up, light <b>vegetal hemisphere</b> down; firm texture; sperm entry point indicated by pigment condensation; <b>germinal vesicle</b> breakdown leaving pale spot in animal hemisphere	cleavage has not begun; <b>germinal vesicle</b> breaks down and pronuclei fuse; visible cortical layer thickest on animal and dorsal sides.	
CLEAVAGE	2-		first cleavage begins as a top to down <b>furrow</b> in <b>animal hemisphere</b>	<b>cleavage furrow</b> has not yet reached the <b>vegetal pole</b>	
	2	2-cell	2 cells; 1st cleavage plane is meridional along the dorso-ventral plane dividing right and left halves		<i>sox3</i> , <i>stp4a</i> : animal hemisphere; <i>vegt</i> : vegetal hemisphere
	3	4-cell	4 cells; 2nd cleavage is meridional, perpendicular to the 1st cleavage; in many embryos, the 2 dorsal blastomeres ( <b>left</b> and <b>right</b> ) are smaller and lighter (on animal surface) than 2 larger darker ventral blastomeres (also <b>left</b> and <b>right</b> ) <sup>[1]</sup>	cleavage cavity present	center
	4	8-cell	8 cells; 3rd cleavage plane is equatorial giving 4 smaller animal blastomeres ( <b>micromeres</b> ) and 4 larger vegetal blastomeres ( <b>macromeres</b> ); animal dorsal cells are lighter and ventral cells are darker in some embryos		<i>nanos1</i> : germ plasm; <i>hwa</i> : dorsal Wnt signalling
	5	16-cell	16 cells; 4th cleavage is again meridional; <b>animal blastomeres</b> smaller than <b>vegetal blastomeres</b> ; dorsal blastomeres lighter than ventral.		
	6	32-cell	32 cells; 5th cleavage equatorial, giving 4 rows each with 8 blastomeres; <b>animal pole</b> with smallest rosette of <b>micromeres</b> , 2 middle rows of irregular shaped cells, larger vegetal rosette of <b>macromeres</b>		
	6.5	morula	64 cells; 6th cleavage; cleavages becoming asynchronous; animal/dorsal blastomeres divide before vegetal blastomeres		<i>gdf1</i> : vegetal hemisphere; <i>shroom1</i> , <i>tfap2a</i> : animal hemisphere
	7	early blastula	128-512 cells; 7th-9th cleavages, no longer possible to count cells reliably; size of animal cells used to distinguish embryo stage	clear delineation of the <b>3 primary germ layer tissues</b> ; <b>notochord</b> begins to form from mediolateral convergence of <b>dorsal presumptive axial mesoderm</b> under the neural ectoderm	<i>nodal5</i> ( <i>nr5</i> ): earliest zygotic transcription
BLASTULA	8	middle blastula	~1000-4000 cells; 10-12+ cleavages; cell number no longer a stage guide; <b>animal hemisphere</b> with small dark pigmented cells referred to as ' <b>animal cap</b> '; animal surface looks 'pebbly'	at NF stage 8.5, after 12th cell divisions the <b>mid-blastula transition (MBT)</b> occurs; asynchronous internal cell division; zygotic genome activation	<i>gs17</i> , <i>nr1</i> : major initiation of zygotic transcription
	9	late blastula	<b>animal hemisphere</b> still darkly pigmented; animal surface more like 'grains of sand' than 'pebbles' of NF stage 8	<b>blastocoel</b> now maximum size; 3 germ layers becoming distinct; <b>ectoderm</b> in the animal 1/3 of the embryo, a ring of <b>mesoderm</b> in the middle and yolkly <b>endoderm</b> on the vegetal 1/3	<i>sox17a</i> : endoderm; <i>tbxt</i> : mesoderm
	10	initial gastrula	darker pigment from <b>bottle cells</b> on the dorsal vegetal surface indicate <b>dorsal blastopore lip</b> formation, between '11 & 1 o'clock'; blastopore groove where cell ingress, is not yet visible	initial formation of Spemann Organizer at the <b>dorsal marginal zone/upper blaspose lip</b> .	<i>gsc</i> : upper blastopore lip; <i>tbxt</i> : marginal zone
	10.25	early gastrula	<b>blastopore lip</b> extends laterally ~1/4 around the circumference, from ~'10 to 2 o'clock'; indentation of <b>blastopore</b> visible as a groove	<b>marginal zone involutes</b> on dorsal side; internally, dorsal <b>endomesoderm</b> begins to migrate anteriorly along blastocoel surface; vegetal rotation of endoderm mass	
	10.5	crescent-shaped blastopore	arch of pigmented <b>blastopore lip</b> extends laterally from dorsal to lateral sides ~1/4 to 1/2 around the circumference; yolk plug almost round	medial-lateral intercalation of dorsal mesoderm; ' <b>Brachet's cleft</b> ' forms between internal leading edge <b>endomesoderm</b> and the dorsal mesoderm; neural induction begins, dorsal-ventral patterning	<i>chrd1</i> , <i>lhx1</i> : involuted dorsal mesoderm; <i>ventx2.1</i> , <i>wnt8a</i> : ventro-lateral marginal zone; <i>hhex</i> : anterior endomesoderm
	11	horse-shoe shaped blastopore	pigmented <b>blastopore lip</b> ~ half the circumference, pigmented <b>bottle cells</b> extend to the ventral side; yolk plug ~1/2 diameter of embryo, is slightly elongated in the dorsal-ventral direction	<b>Brachet's cleft</b> begins to open forming the archenteron; <b>ectoderm</b> , <b>mesoderm</b> and <b>endoderm</b> germ layers specified; <b>blastocoel</b> becoming smaller, <b>mesodermal mantle</b> undergoes convergent extension and endoderm is internalized	<i>sox17a</i> : endoderm; <i>tbxt</i> : mesoderm; <i>sox2</i> : neurectoderm; <i>krt12.4</i> : non-neural ectoderm
	11.5	large yolk plug	<b>blastopore lip</b> extends all the way around yolk plug; <b>lower/ventral blastopore lip</b> more darkly pigmented; yolk plug not quite round, ~1/3 of embryo diameter elongated in the dorsal-ventral direction	<b>blastocoel</b> displaced to the ventral side; involuting ' <b>endomesoderm</b> ' mantle continues to extent anteriorly	
	12	medium yolk plug	areas of light and dark pigment radiating from yolk plug (flower petal like); yolk plug small and circular, a little less than 1/4 of egg diameter, diameter decreasing; <b>neurectoderm</b> transforming into the discernible <b>neural plate</b>	<b>blastocoel</b> beginning to close as the <b>archenteron</b> expands	<i>sox2</i> , <i>sox3</i> : neural plate
GASTRULATION	12.5	small yolk plug	darker pigment lines on dorsal surface indicate future <b>neural groove</b> and <b>neural plate</b> ; blastopore slit is slightly open, diameter decreasing and oval shaped	clear delineation of the <b>3 primary germ layer tissues</b> ; notochord forms from mediolateral convergence of dorsal axial mesoderm under the neural ectoderm	<i>chrd1</i> , <i>nog</i> , <i>shh</i> : axial mesoderm, notochord
	13	slit blastopore	<b>blastopore</b> completely closed to a 'slit'; <b>neural plate</b> on dorsal side clearly outlined	<b>neural crest</b> form at the <b>neural plate border</b> , i.e., the boundary between the neural and non-neural ectoderm	<i>sox2</i> , <i>sox3</i> : neural plate; <i>ednra</i> , <i>msx1</i> : neural plate border; <i>sna1</i> : neural crest; <i>lhx2</i> : cement gland primordium; <i>myf5</i> : paraxial mesoderm; <i>rax</i> : optic field
	13.5	initial neural plate	sharp demarcation of <b>anterior neural plate</b> ; anterior aspect of neural plate bent down; <b>yolk plug internalized</b>		<i>tubb2b</i> : differentiating neural progenitors; <i>msx1</i> : anterior neural plate border
	14	neural plate	<b>neural plate</b> obvious; dorsal midline thin with <b>neural folds</b> thickening anteriorly and laterally; <b>neural folds</b> begin to elevate; convergence extension begins to narrow neural plate posteriorly	<b>blastocoel</b> continues to close in the <b>ventral foregut</b> region as <b>archenteron</b> expands on the internal dorsal side	<i>pax6</i> : optic field, neural plate, lens placode; <i>pax2</i> : between anterior and posterior neural plate
	15	early neural fold	<b>neural folds</b> distinct; <b>anterior neural fold</b> round; demarcation of neural plate clear caudally, narrowing in middle and caudal regions; <b>neural groove</b> deepens; pigmented <b>cement gland primordium</b> faintly visible at the ventral-anterior border of neural plate, cells stand out from epithelial layer.	physical segregation of <b>cranial neural crest</b> from <b>anterior (pre-chordal) neural plate</b> ; blastocoel closes in the <b>ventral foregut</b>	<i>pax3</i> : neural fold/neural crest; <i>pax8</i> : intermediate mesoderm; <i>rax</i> , <i>otx2</i> , <i>six3</i> : optic field; <i>hhex</i> : foregut endoderm
	16	mid-neural fold	<b>eye primordia</b> [2] become discernable as indentations near the lateral edges of the <b>anterior neural plate</b> ; anterior neural plate 'rectangular' in shape; neural plate sharply constricted in the middle.	<b>right and left cardiac mesoderm</b> migrates to anterior-ventral midline; <b>foregut</b> diverticulum forms	<i>pax6</i> : optic field, anterior neural plate, and lens placode; <i>nkx2-5</i> : cardiac progenitor cells; <i>krt12.4</i> : ectoderm, non-neural ectoderm, epidermis
	17	late neural fold	<b>anterior neural plate</b> oblong, triangular, angles formed by <b>eye primordia</b> [2]; clear neurenteric canal along midline, posterior end of which continues over closed blastopore slit; neural folds closing in trunk region	delineation of <b>cranial neural crest</b> lateral to the <b>anterior neural plate</b> ; 1st indication of somite segregation from <b>presomitic mesoderm</b>	<i>sox2</i> , <i>sox3</i> : neural plate/neurenteric canal; <i>otx2</i> , <i>rax</i> , <i>pax6</i> , <i>six1</i> , <i>six2</i> : optic field; <i>lhx1</i> : intermediate mesoderm
	18	neural groove	<b>anterior part of neural plate</b> narrow, club shaped; parallel neural folds very close but not touching	<b>neural crest</b> segregation begins; 3-4 anterior <b>somites</b> segregate from <b>paraxial mesoderm</b>	<i>myoD</i> : early somites and presomitic mesoderm; <i>casz</i> : early somites; <i>sna1</i> , <i>egr2</i> , <i>twist1</i> : neural crest; <i>pax8</i> : optic placode
NEURULATION	19	initial neural tube	<b>neural folds</b> mostly closed forming a <b>neural tube</b> , except for an open slit in the anterior neural plate; <b>neureteric canal</b> deepening; dark pigmented <b>cement gland primordium</b> immediately ventral to anterior neural plate; lateral view of embryo	migration of 4 <b>neural crest streams</b> begins; 4-6 anterior <b>somites</b> segregated	<i>ag1</i> , <i>agr2</i> : cement gland primordium
	20	fused neural tube	<b>neural tube</b> fused anteriorly; no pharyngeal bulge visible; dark oval <b>cement gland primordium</b> below border of anterior neural plate; embryo starts to elongate; optic cup (eye primordium) forms; oral evagination (mouth) visible	<b>neural crest</b> extends to front to eye; paired <b>glomus primordia</b> present [2]; anterior 6-7 <b>somites</b> ; thickening of cardiac mesoderm	<i>rax</i> , <i>pax6</i> , <i>otx2</i> , <i>six3</i> : optic vesicle; <i>wt1</i> , <i>rgn</i> : glomus; <i>nkx2-5</i> : cardiac progenitor cells; <i>egr2</i> : rhombomeres R3, R5, and neural crest; <i>en2</i> : midbrain-hindbrain boundary; <i>sna1</i> : neural crest; <i>chrd1</i> , <i>nog</i> , <i>shh</i> : notochord
	21	neural tube	embryo has a dorsal curvature, with flat ventral surface; one <b>pharyngeal arch</b> bulge; <b>neural tube</b> completely closed; <b>optic vesicle</b> ('eyes') begin protuding, forming 2 oblique 'oval spots'; <b>multiciliated cells</b> form on the surface of the epidermis	8-9 <b>somites</b> ; first indication of <b>pronephric mesenchyme</b> ; <b>optic placodes</b> form posterior to <b>optic vesicle</b>	<i>pax8</i> , <i>lim1</i> : pronephric mesenchyme; <i>nrp1</i> : neural tube; <i>pax3</i> : hatching gland; <i>tuba4b</i> , <i>cflp206</i> , <i>foxi1</i> : multiciliated epidermal cell

			Developmental Stages			Molecular Markers
			External	Internal	Behavior & Physiology	
EARLY ORGANOGENESIS	22	early tailbud	embryo begins to elongate (convergent extension); ventral surface slightly concaved; two <b>pharyngeal arch</b> bulges; distinct <b>eye</b> protrusion; anal opening displaced to ventral side	9-10 somites; ventral blood island forms; segregation of <b>forebrain</b> , <b>midbrain</b> and <b>hindbrain</b>		<b>gsc1</b> , <b>sox3</b> : brain segments; <b>gata1</b> , <b>hba3</b> , <b>tail</b> : ventral blood island; <b>nodal1</b> : lateral plate mesoderm on the left side only; <b>tbx6</b> , <b>foxd4l1.1</b> : early tail bud
	23	early tailbud	ventral surface concave giving embryo a 'coffee bean' look; two <b>pharyngeal arch</b> bulges; <b>olfactory placodes</b> thicken between eyes; slight depression of <b>otic placode</b> ; jaw and gills separated by groove; "inverted Y shaped" <b>hatching gland</b> between eyes to cement gland	12 somites; forebrain regions <b>telencephalon</b> and <b>diencephalon</b> distinguishable		<b>cxcl14</b> , <b>astl3a.1</b> , <b>pax3</b> : hatching gland; <b>myod1</b> : somites and presomitic mesoderm; <b>six1</b> : olfactory placode; <b>pax2</b> , <b>pax6</b> , <b>vax2</b> : diencephalon; <b>foxt1</b> : telencephalon
	24	early tailbud	noticeable elongation of the embryo and <b>tail bud</b> outgrowth; in dorsal view, <b>eyes</b> protruding out laterally less than gills; gill primordium area smooth (ungrooved)	15 somites; primary germ cells detectable in cell trunk endoderm; primary and secondary heart fields indicated in <b>heart primordium</b>	initial motor reactions to external stimuli	<b>tbx1</b> , <b>lmo2</b> , <b>aplr</b> : tail bud; <b>tnn3</b> , <b>nkx2.5</b> : primary heart field; <b>bmp4</b> : secondary heart field; <b>grif2</b> , <b>pgf</b> : primordial germ cells; <b>pcdh8.2</b> : otic vesicle and <b>tail bud</b> ; <b>eya2</b> : otic vesicle
	25	early tailbud	embryo still convex dorsally and concave ventrally; <b>eyes</b> protruding out laterally equal to or more than gills, gills now grooved; otic vesicle pigmented	16 somites; head somite 1 diminished; <b>brain</b> flexure ~90°		<b>ag1</b> : cement gland; <b>tubb2b</b> : brain and spinal cord
	26	tailbud	If liberated from the <b>vitelline membrane</b> the embryo is straight, not convex dorsally; if the embryo remains in the vitelline membrane it is curved laterally; <b>tail bud</b> obvious; otic (ear) vesicle protruding	17 somites; head somite 1 disintegrated; <b>pronephros</b> distinct; myotomes distinct	spontaneous movements begin	<b>pax8</b> , <b>hnf1b</b> , <b>irx3</b> : pronephric mesenchyme
	27	tailbud	<b>tail bud</b> defined in lateral view; fin translucent; <b>lens</b> begins to form, eyes flatten laterally; <b>otic vesicle</b> closes	heart fields merge forming a triangular-shape at ventral midline, behind the <b>cement gland</b> primordium and anterior to <b>liver diverticulum</b> ; 19 somites		<b>pax6</b> , <b>sox3</b> , <b>prox1</b> , <b>foxe3</b> , <b>nrl</b> : lens; <b>neurod1</b> , <b>sox3</b> : epibranchial placodes
	28	tailbud	<b>tail bud</b> elongates distally and extends downward to <b>cloaca</b> ; <b>fin</b> divided into outer transparent ( <b>outer fin</b> ) and inner translucent bands ( <b>inner fin</b> ); black <b>cement gland</b> fully formed; <b>otic vesicle</b> separates from epidermis	heart primordium and pericardial cavity discernible; <b>pronephric nephrostomes</b> form; 20-22 somites; epibranchial placodes first segregate	embryos liberated from the <b>vitelline membrane</b> glide around due to multi-ciliated cell fluid flow	<b>hand2</b> , <b>actc1</b> : endocardial tube; <b>dlx2</b> , <b>sox9</b> , <b>sox10</b> : cranial neural crest; <b>pax2</b> , <b>lhx1</b> : nephrostomes; <b>neurog2</b> , <b>eya1</b> : epibranchial placodes; <b>fgf8</b> , <b>sox9</b> : otic vesicle
	29 & 30	late tailbud	<b>tail bud</b> distinct; <b>outer fin</b> edge transparent over entire length; gray disc of the <b>eye</b> cup now visible	23-25 somites segregated to <b>end of tail</b> ; lumen in pronephric kidney collecting duct; appearance of <b>glomus</b> and <b>thyroid</b> ; neural tube closure has formed the <b>spinal cord</b>		<b>runt1</b> : olfactory placode; <b>lxh9</b> : brain segments; <b>foxa2</b> , <b>tubb2b</b> : spinal cord; <b>nphs1</b> , <b>wt1</b> : glomus; <b>nkx2.1</b> : thyroid primordium; <b>not</b> : tail tip
	31	late tailbud	<b>tail bud</b> equal in length and height; <b>nasal/olfactory pits</b> first indicated	heart primordium extends ventrally and bends slightly to right; 22-23 post-otic somites; midbrain-hindbrain boundary distinct		<b>hey1</b> , <b>myod1</b> , <b>actc1</b> : somites; <b>en2</b> , <b>pax2</b> , <b>fgf8</b> : midbrain-hindbrain boundary
ORGANOGENESIS	32	late tailbud	<b>tail bud</b> ~1.5x longer than height; <b>eye</b> cup distinct, U-shaped (open); <b>mouth primordium</b> not visible	heart a linear tube with anterior <b>outflow tract</b> , left <b>ventricle</b> , <b>atrioventricular canal</b> and <b>atrium</b> ; 26 post-otic somites; <b>pronephric nephrostomes</b> form; <b>lung buds</b> visible		<b>pax2</b> , <b>vax1</b> : optic stalk; <b>clap161</b> : nephrostomes and multiciliated epidermal cells; <b>nkx2.1</b> : lung and thyroid progenitors; <b>aldh1a3</b> , <b>agr2</b> : otic vesicle
	33 & 34	late tailbud	<b>tail bud</b> ~2x longer than height; gut ~3x longer than tail; <b>eye cup</b> open C-shape with darker pigmentation dorsally; <b>mouth primordium</b> a shallow vertical groove; 32 post-otic somites; pigmented cells ( <b>melanophores</b> ) first appear on head (near hindbrain) and <b>anterior trunk</b> (near <b>pronephric kidney</b> )	heart looping begins; heart beat clearly observable; foregut begin to constrict at trachea-ophagus boundary; <b>cranial nerves</b> distinct; <b>thyroid primordium</b> discernible [2]; <b>pronephric kidney</b> and duct formed with surrounding vasculature; <b>thyroid</b> primordium detectable		<b>dab2</b> : pronephric sinus, posterior cardinal vein; <b>sox2</b> : dorsal foregut; <b>tubb2b</b> : cranial nerves; <b>myl2</b> , <b>bves</b> : heart; <b>actc1</b> , <b>mybpc3</b> : heart and somites; <b>pax2</b> , <b>lhx1</b> : pronephric kidney and pronephric nephrostomes [2]
	35 & 36	free swimming tadpole [3]	<b>tail bud</b> ~3x longer than height; gut ~2x longer than tail; outline of the <b>proctodeum</b> still curved; optic vesicle/retina completely black, <b>choroidal fissure</b> open; <b>cardiac mesoderm</b> starts to spontaneously contract; <b>mouth</b> invagination not quite round; 2 <b>gill lobes</b> ; 36 post-otic somites; <b>melanophores</b> extend over top of head and along dorsal trunk	heart S-shaped, with distinct <b>atrium</b> lying dorsal to <b>ventricle</b> ; <b>vasculature to head</b> and <b>tail</b> developing; <b>liver bud</b> visible posterior to heart; <b>pronephric duct</b> fused with <b>rectal diverticulum</b> , <b>pronephric nephrostomes</b> obvious	embryos naturally hatch from vitelline membrane to become free swimming	<b>apln</b> , <b>hbz</b> : blood vessels and heart; <b>oncet</b> , <b>nr1h5</b> , <b>hhex</b> : liver; <b>aldh1a1</b> : pronephric kidney/duct, choroidal fissure and olfactory bulb; <b>ta1</b> , <b>hba3</b> : ventral blood island; <b>nkx3.2</b> : mouth primordium
	37 & 38	free swimming tadpole	gut almost same length as tail; eye's <b>choroid fissure</b> closing ventrally but remain open; <b>mouth</b> invagination deep, round-shaped; heart contractions obvious, blood flow visible; <b>proctodeum</b> at obtuse angle (~140 degrees) to <b>tail somites</b> ; 40 post-otic somites; <b>melanophores</b> extend over tail	paired <b>lymph hearts</b> ; entire <b>pronephric kidney</b> functioning; <b>ventral bud of pancreas</b> formed;		<b>tnn3</b> : heart; <b>sftp</b> : lung buds; <b>myh6</b> : lymph heart; <b>atp1a1</b> : pronephric kidney and pronephric duct; <b>nkx2.1</b> : thyroid and lungs; <b>foxe3</b> : thyroid and lens; <b>ptfa1</b> , <b>pdia2</b> : pancreatic buds
	39	free swimming tadpole	gut equal in length to tail somites; <b>melanophores</b> around <b>nasal pits</b> & along ventral edge of <b>tail somites</b> ; ventral <b>choroid fissure</b> nearly closed; <b>proctodeum</b> at ~125 degree angle to tail somites; 43 post-otic somites	retinal ganglion cell axons reach <b>optic tectum</b> ; <b>mesonephric kidney</b> begins to form		<b>bmp4</b> , <b>hoxa13</b> : proctodeum; <b>map2</b> , <b>pou4f1</b> : retinal ganglion cell layer; <b>insm1</b> , <b>nos1</b> : optic tectum
	40	free swimming tadpole	<b>tail</b> now longer than the abdomen; optic choroid fissure completely closed; <b>mouth opening</b> 'breaks through'; <b>stomach</b> and <b>pancreas</b> visible on the left side of gut; <b>proctodeum</b> at 90 degree angle to <b>tail somites</b> (lateral view), ~45 post-otic somites	<b>gall bladder primordium</b> formed and sometimes visible (iridescent on ventral view); blood circulation in gills visible	embryos begin taking gulps of air from the surface	<b>cel1.2</b> : pancreas; <b>sftp5</b> , <b>kif5</b> : stomach; <b>oncet</b> , <b>hhex</b> , <b>sox7</b> : gall bladder; <b>hhex</b> , <b>nr1h5</b> : liver
	41	free swimming tadpole	conical shaped <b>proctodeum</b> formed, at angle of ~ 60 degrees to <b>tail somites</b>	<b>myocardium</b> thickens and develops <b>trabeculae</b> , <b>atrium</b> posterior to <b>ventricle</b> ; torsion of gut starts; <b>post-anal gut disappears</b> ; the <b>pancreas</b> , now visible in ventral view, posterior to left-sided <b>stomach</b>		<b>clcnkb</b> : pronephros; <b>ins</b> : pancreas; <b>myod1</b> : tail somites; <b>s1pr1</b> : brain segments; <b>nkx2.5</b> , <b>tpm1</b> : myocardium; <b>hoxa13</b> : proctodeum
	42	free swimming tadpole	opercular fold first visible; <b>head somites I and II</b> disappeared	<b>trachea</b> and <b>esophagus</b> separate		<b>sox2</b> : esophagus and stomach; <b>nkx2.1</b> : trachea and lung buds; <b>sftp</b> : lung buds
	43	free swimming tadpole	<b>cement gland</b> starts to lose pigmentation; <b>lateral line</b> pits visible	stomach has lengthened further; <b>pancreas</b> shifted to right side [5]; <b>duodenum</b> formed by 1st gut coil constriction to anterior-right; <b>midgut</b> and <b>hindgut</b> form hairpin curve, visible on left side (will become the 'apex' of future <b>intestinal coil</b> )		<b>foxq1</b> , <b>spdef</b> , <b>bmpr1a</b> , <b>clap161</b> : stomach; <b>cel1.2</b> : pancreas; <b>aldh1a2</b> : duodenum;
GUT-COILING	44	free swimming tadpole	<b>heart</b> fully formed and clearly visible; <b>barbels/tentacles</b> start to grow; <b>gills/branchial basket</b> shrinking	septum begins to form in <b>cardiac atrium</b> which is slightly anterior to <b>ventricle</b> ; <b>midgut</b> and <b>hindgut</b> lengthened more; the <b>intestinal apex</b> visible in ventral view as a "U" shape in the upper left quadrant of the gut cavity	visual avoidance behavior begins	<b>tnn3</b> , <b>frzb</b> , <b>sox9</b> , <b>nkx2.5</b> : heart; <b>cdx2</b> : midgut-hindgut
	45	feeding tadpole [3]	operculum partly covers the gills, <b>hindlimb bud</b> not visible	<b>midgut</b> and <b>hindgut</b> continue to lengthen; the intestinal apex begins to rotate inward in a counterclockwise trajectory; <b>spleen</b> forms; mesonephric kidney	tadpoles to start swim continuously and begin feeding	<b>darmin</b> , <b>a2m</b> : midgut, hindgut and liver; <b>nkx2.5</b> : spleen primordium
	46	feeding tadpole	crescent-shaped <b>hindlimb bud</b> first appears although is difficult to see; <b>pigment cells</b> appear on <b>eye</b> and around abdomen; trunk somite 1 disappeared	midgut and <b>hindgut</b> lengthen further- apex continues to rotate inward, forming multiple <b>coils of intestine</b> ; blood circulation to gills diminishing	food can be seen in intestine as now feeding	
	47	feeding tadpole	iridescent gold-coloured abdominal wall surrounds coiled gut; blood circulation visible from heart to gills, and through <b>paired dorsal aorta</b> ; cement gland starts to degenerate; <b>barbels/tentacles</b> longer	<b>retinal ganglion cells</b> have formed complex synapses with <b>optic tectum</b> neurons; <b>thyroid gland</b> begins to function; <b>thymus gland</b> detectable		<b>foxn1</b> : thymus
	48		<b>hindlimb bud</b> now clearly visible, with nearly semi-circular shape	<b>retinal ganglion cells-optic tectum</b> synapses more compact		<b>fgf8</b> , <b>spry1</b> , <b>sall4</b> : hindlimb bud
	49		<b>hindlimb bud</b> length equal to its width	<b>thyroid</b> follicles first appear		
	50		<b>hindlimb bud</b> slightly constricted at base; tiny oval <b>forelimb buds</b> just visible	<b>gonads</b> undifferentiated		<b>hoxd10</b> , <b>hoxa13</b> , <b>hoxa9</b> : hindlimb bud
	51		<b>hindlimb bud</b> is cone-shaped; <b>forelimb bud</b> is oval shaped (in lateral view)	resorption vacuoles in <b>thyroid</b> follicles first appear		<b>hoxd10</b> , <b>hoxa13</b> , <b>hoxa9</b> : hindlimb bud
	52		<b>hindlimb bud</b> with slight 'wrist' indent; <b>forelimb bud</b> slightly constricted at base	5 complete coils of the intestine ( <b>internal</b> and <b>external</b> coils)	regeneration competent [4]	<b>sox9</b> : hindlimb digits (cartilage elements)
PREMETAMORPHOSIS						

PROMETAMORPHOSIS	<a href="#">53</a>	<a href="#">hindlimb bud</a> paddle-like, with wrist constriction, <a href="#">hindlimb digits</a> not discernable; <a href="#">forelimb bud</a> with slight wrist constriction	onset of sexual differentiation of <a href="#">gonads</a>	regeneration competent; thyroid animals have arrested development
	<a href="#">54</a>	<a href="#">hindlimb bud</a> length (not including foot) 2x the width; <a href="#">foot</a> paddle splayed with 5 digits and thinner <a href="#">inter-digital webbing</a> ; forelimb paddle with 4 digits and thinner inter-digital membranes	<a href="#">pronephric kidney</a> begins to atrophy	thyroid hormone detectable in blood <a href="#">tbx4</a> , <a href="#">sall4</a> : <a href="#">interdigital mesenchyme</a>
	<a href="#">55</a>	<a href="#">hindlimb</a> length (not including foot) 3x width; forelimb <a href="#">hand</a> rotates 90 degrees, free parts of fingers as long as they are wide	all major muscles of <a href="#">hindlimb</a> developed	regeneration restricted [4]
	<a href="#">56</a>	<a href="#">hindlimbs</a> visible from above as they can rotate away from body; hindlimb length = ~ 5 tail somites; <a href="#">larval pigmentation</a> pattern established	sexual differentiation of <a href="#">gonads</a> into <a href="#">ovary</a> or <a href="#">testis</a> ; <a href="#">hindlimb skeleton</a> completely chondrified	regeneration restricted [4]
	<a href="#">57</a>	<a href="#">hindlimb</a> length = ~ 9 tail somites; <a href="#">forelimb</a> remains enclosed in operculum; lip folds form		
	<a href="#">58</a>	<a href="#">hindlimb</a> length = 11-12 tail somites; claws form on toes 1-3 (mostly always still white); <a href="#">forelimb</a> emerges from operculum, elbows first; <a href="#">tail tip</a> begins to atrophy	melanin/pigment deposited in under skin especially in tail	regeneration incompetent [4]
	<a href="#">59</a>	hindlimb muscular, <a href="#">claws</a> start to harden and turn black, shortest <a href="#">toes</a> first; <a href="#">finger tips</a> reach base of hindlimb when <a href="#">forelimb</a> is positioned along the abdomen; tentacles/barbels regress	melanin/pigment surrounds intersomitic <a href="#">blood vessels</a> and between <a href="#">fibres of somites</a> ; forelimb muscles differentiated; pronephros no longer functional	regeneration incompetent [4]
	<a href="#">60</a>	<a href="#">gill chamber</a> opening still wide; fingertip reach beyond base of hindlimb (almost to 'knee') when <a href="#">forelimb</a> /arm is positioned along side of the abdomen; forelimb held posterior to heart; <a href="#">tail fins</a> greatly reduced	pigmentation across body increases	regeneration incompetent [4]; animal switches from tail to leg swimming
	<a href="#">61</a>	first sign of gill resorption, openings to <a href="#">gill chamber</a> much narrower; <a href="#">hindlimb</a> and <a href="#">forelimb</a> fully formed; forelimb at level of posterior half of heart	lateral finger-like protrusions from <a href="#">olfactory organ</a>	cessation of feeding (due to oral and <a href="#">intestinal remodeling</a> )
	<a href="#">62</a>	tailed froglet <a href="#">head</a> slightly broader than abdomen; corner of mouth still in front of eye; <a href="#">forelimb</a> reaches middle of heart; <a href="#">ventral tail fin</a> gone from abdomen; adult skin on <a href="#">hindlimbs</a> ; only tiny nubs of <a href="#">barbels/tentacles</a> remain.	tiny ' <a href="#">stirnorgan</a> ' (light detecting cells/part of pineal gland) appears; notochord atrophies along length of tail	peak levels of thyroid hormone in plasma
	<a href="#">63</a>	tailed froglet <a href="#">head</a> narrower than abdomen; <a href="#">barbels/tentacles</a> (most often) completely gone; <a href="#">forelimb</a> at level of anterior half of heart; tail shortens as <a href="#">tail somites</a> are rapidly resorbed, <a href="#">tail</a> still slightly longer than body		
	<a href="#">64</a>	corner of mouth behind eye; tail length is about 1/3 of body length, at level of ankle when legs are in typical neutral position; body completely covered in adult <a href="#">skin</a> , but 'border lines' clearly visible	<a href="#">thymus gland</a> ventral-lateral to otic capsule	
	<a href="#">65</a>	tail length a few millimeters, all tail somites have disappeared; body completely covered in adult skin, but 'border lines' still visible in some areas		feeding resumes
	<a href="#">66</a>	froglet tail very nearly gone, not visible from ventral view; adult skin 'border lines' have disappeared, froglet body ~ 10mm long	<a href="#">skin</a> remodelled with underlying <a href="#">dermis</a> and secretory glands	thyroid hormone in plasma returns to prometamorphic levels

## FOOT NOTES

- 1 Dorsal-ventral pigment variation only occurs in some batches of embryos. Select 2-4-cell embryos with clear pigment variation - otherwise only accurate about 70% of time.  
 2 In the Normal Table and other texts, organ primordia are often called 'anlage'; they are visualized by as a thickening of specific cells via histology or by molecular markers. Search specific XAO terms on Xenbase for more molecular markers.  
 3 NF stages 41-66 are not referred to by specific 'stage names' by Nieuwkoop and Faber  
 4 Regeneration classes from Azeitekin et al 2021 PMID:34105722  
 5 Left and right sides refer to that of the tadpole/embryo, and not the viewer.