Develop	omental	NF stage	NF stage name	LANDMARKS		
Proc	ess	number	otago name	EXTERNAL	INTERNAL	MOLECULAR MARKERS [gene: anatomy]
ATION		stage V oocyte	oocyte	1 cell; ovulated unfertilized egg; animal hemisphere dark, vegetal hemisphere light; animal-vegetal axis in random orientation; soft texture	large nucleus, 'germinal vesicle' in animal hemisphere	nanos1: mitochondrial cloud; vegt: vegetal cortex
FERTILL		1	1-cell, fertilized egg	1 cell; vitelline membrane swells; eggs rotate with dark animal hemisphere up, light vegetal hemisphere down; firm texture; sperm entry point indictated by pigment condensation; germinal vesicle breakdown leaving pale spot in animal hemisphere	cleavage has not begun; germinal vesicle breaks down and pronuclei fuse; visible cortical layer thickest on animal and dorsal sides.	
	CLEAVAGE	2-		first cleavage begins as a top to down furrow in animal hemisphere	cleavage furrow has not yet reached the vegetative pole	
		2	2-cell	2 cells; 1st cleavage plane is meridional along the dorso-ventral plane dividing right and left halfs		atp4a: animal hemisphere; vegt: vegetal hemisphere
		3	4-cell	4 cells; 2nd cleavage is meridional, perpendicular to the 1st cleavage; in many embryos, the 2 dorsal blastomeres are smaller and lighter (on animal surface) than 2 larger darker ventral blastomeres [1]	cleavage cavity present	
CLEAVAGE		4	8-cell	8 cells; 3rd cleavage plane is equatorial giving 4 smaller animal blastomeres (micromeres) and 4 larger vegetal blastomeres (macromeres); animal dorsal cells are lighter and ventral cells are darker in some embryos		nanos1: germ plasm
		5	16-cell	16 cells; 4th cleavage is again meridional; animal blastomeres smaller than vegetal; dorsal blastomeres lighter than ventral.		
		6	32-cell	32 cells; 5th cleavage equatorial, giving 4 rows each with 8 blastomeres; animal pole with smallest rosette of microblastomeres, 2 middle rows of irregular shaped cels, larger vegetal rosette of macromeres		
		6.5	morula	64 cells; 6th deavage; deavages becomming asynchronous; animal/dorsal blastomeres divide before vegetal blastomeres		gdf1 : vegetal hemisphere; atp5f1a , h3-3a , shroom1 , shroom2 : animal hemisphere
		7	early blastula	128-512 cells; 7th-9th cleavages, no longer possible to count cells reliably; size of animal cells can distinguish embryo stage	pre-gastrualtion cell movement begins; internal blastocoel cavity forms	nr5 : earliest zygotic transcription
ASTULA		8	middle blastula	~1000-4000 cells; 10-12+ clevages; cell number no longer a stage guide; animal hemisphere with small dark pigmented cells refered to as 'animal cap'; animal surface looks 'pebbly'	at stage 8.5, after 12th cell divisions the mid-blastula transition (MBT) occurs; asynchronous internal cell division; zygotic genome activation	gs17, nr1: major initiation of zygotic transcription
	5	9	late blastula	animal hemisphere still darkly pigmented; animal surface more like 'grains of sand' than 'pebbles' of NF stage 8	blastocoel now maximum size; 3 germ layers becoming distinct; ectoderm in the animal 1/3 of the embryo, a ring of mesoderm in the middle and yolky endoderm on the vegetal 1/3	sox17: endoderm; tbxt: mesoderm
		10	initial gastrula	darker pigment from bottle cells on the dorsal vegetal surface indicate dorsal blastopore lip formation, between '11 & 1 o'clock'; blastopore groove where cell ingress, is not yet visible	initial formation of Spemann-Mangold Organizer at the dorsal blasopre lip	gsc: dorsal blastomore lip; tbxt: marginal zone
		10.25	early gastrula	blastopore lip extends laterally ~1/4 around the circumference, from ~ '10 to 2 o'clock' ; indentation of blastopore visible as a groove	marginal zone involutes on dorsal side; internal dorsal mesendoderm begins to migrate anteriorly along bastoceal surface; vegetal rotation of endoderm mass	
NO		10.5	crescent-shaped blastopore	arch of pigmented blastopre lip extends laterally from doral to lateral sides ~1/4 to 1/2 around the circumference; yolk plug almost round	medial-lateral intercalation of dorsal mesoderm; Brachet's cleft' forms between internal leading edge mesendoderm and the dorsal mesoderm; neural induction begins, dorsal-ventral patterning	chrd.1: dorsal mesoderm; ventx2, wnt8a: ventro-lateral mesendoderm; hhex: anterior mesendoderm; ihx1: involuted dorsal mesoderm
ASTRULATI		11	horse-shoe shaped blastopore	pigmented blastopore lip ~ half the circumference, pigmented bottle cells extend to the ventral side; yolk plug ~1/2 diameter of embryo, is slightly elongated in the dorsal-ventral direction	Brachet's cleft begins to open forming the archenteron; ectoderm, mesoderm and endoderm germ layers specified; blastoceol becoming smaller, mesodermal mantel undergoes convergent extension and endoderm is internalized	sox17: endoderm; tbxt: mesoderm; sox2: neurectoderm; krt12.4: non-neural ectoderm
đ		11.5	large yolk plug	blastopore lip extends all the way around yolk plug; ventral blastopore lip more darkly pigmented; yolk plug not quite round, ~1/3 of embryo diameter elongated in the dorsal-ventral direction	blastoceol displaced to the ventral side; involuting mesendoderm mantel 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; neurectoderm transforming into the discernible neural plate	blastoceol beging to close as the archenteron expands	sox2, sox3: neural plate
		12.5	small yolk plug	darker pigment lines on dorsal surface indicate future median groove and neural plate; blastopore slit is slightly open, diameter decreasing and oval shaped	clear delineation of the 3 primary germ layer tissues; notochord forms from mediolateral convergence of dorsal axial mesoderm under the neural ectoderm	chrd.1, nog, shh: axial mesoderm, notochord
		13	slit blastopore	blastopore completely closed to a 'slit'; neural plate on dorsal side clearly outlined	neural crest form at the edge of the neural plate, i.e., the boundary between the neural and non-neural ectoderm	sox2, sox3: neural plate; dlx3, msx1: neural plate border; snail2; neural crest; tbx2: cement gland primordium; myf5: paraxial medoderm; rax: optic field
	URULATION	13.5	initial neural plate	sharp demarcation of anterior neural plate; anterior aspect of neural plate bent down; yolk plug internalized		tubb2b: differentating neural progenitors; foxI1c: anterior neural plate border
		14	neural plate	neural plate obvious; dorsal midline thin with neural folds thickening anteriorly and laterally; neural folds begin to elevate; convergence extension begins to narrow neural plate posteriorly	blastoceol continues to close in the ventral foregut region as archenteron expands on the internal dorsal side	pax6: eye field, neural plate, lens placode; pax2: between anterior and posterior neural plate
		15	early neural fold	neural folds distinct; anterior neural fold round; demarcation of neural plate clear caudally, narrowing in middle and caudal regions; neural groove deepens; pigmented cement gland primordium faintly visible at the ventral-anterior border of neural plate, cells stand out from epithelial layer.	physical segregation of neural crest from anterior neural plate; blastoceol closes in the ventral foregut	pax3: neural fold/neural crest; pax8: intermediate mesoderm; rax, otx2, eya1, six1: optic field; hhex: foregut endoderm
	NE	16	mid-neural fold	eye primordia [2] become discernable as indentations near the lateral edges of the anterior neural plate; anterior neural plate rectangular' in shape; neural plate sharply constricted in the middle.	right and left cardiac mesoderm migrates to anterior-ventral midline; foregut diverticulum forms	pax6: optic field, anterior neural plate, lens placode; nkx2-5: cardiac progenitors; krt12.4: ectoderm, non-neural ectoderm, epidermis

		17	late neural fold	anterior neural plate oblong, triangular, angles formed by eye primordia [2]; clear neurenteric canal along midline, posterior end of which continues over closed blastopore slit; neural folds closing in trunk region	delineation of neural crest lateral to the anterior neural plate; 1st indication presomitic mesoderm	on of somite segregation from	<pre>sox2, sox3: neural plate/neurenteric canal, optic field; eya1, six1: eye field; lhx1: intermediate mesoderm</pre>
OGENESIS		18	neural groove	anterior part of neural plate narrow, club shaped; parallel neural folds very close but not touching	neural crest segregation begins; 3-4 anterior somites segregate from pa	raxial mesoderm	myod1: early somites and presomitic mesoderm; casz: early somites; snai1, snai2, twist1: neural crest
		19	initial neural tube	neural folds mostly closed forming a neural tube, except for an open slit in the anterior neural plate; neurentic canal deepening; dark pigmented cement gland primordium immediately ventral to anterior neural plate; laterla view of embryo convex oval, not elongated	migration of 4 neural crest streams begins; 4-6 anterior somites segrega	ted	mybpc3: somiles; actc1: heart and somiles; ag1, agr2: cement gland
		20	fused neural tube	neural tube fused anteriorly; no pharyngeal bulge visible; dark oval cement gland primordium below border of anterior neural plate; embryo starts to elongate; optic cup (eye primordium) forms; oral evagination (mouth) visible	neural crest extends to front to eye; paired glomus primordia present [2]; anterior 6-7 somites; thickening of cardiac mesoderm		rax, pax6, otx2, six3: optic vesicle; wt1, cndp: glomus; nkx2-5: cardiac progenitors; ogr2: rhombomeres R3, R5, neural crest; on2: midbrain-inidrain boundary; snai2: neural crest; chrd.1, nog, ssh: notochord
		21	neural tube embryo has a dorsal curvature, with flat ventral surface; one pharyngeal arch bulge; neural tube completely closed; optic vesicle ('eyes') begin protuding, forming 2 oblique 'oval spots'; multi-ciliate cells form on the surface of the epidermis				pax8: olic placode: pax8, <i>lim1</i> : pronephric mesoderm; pax6, sox2: optic vesicle, neural tube; pax3: hatching gland; tekt3, fox/1: multi-oilated cells
		22	early tailbud	embryo begins to elongate (convergent extension); ventral surface slightly concaved; two pharyngeal arch bulges; distinct eye protrusion; anal opening displaced to ventral side	9-10 somites; ventral blood island forms; segreation of forebrain, midbrian and hindbrain		gsx1, sox3: brain segments: gata1, hba3, tal1: ventral blood island: nodal1: lateral plate mesoderm on the left side only; tbx6, foxd411.1: early tailbud
RGAN				EXTERNAL	INTERNAL	BEHAVIOR & PHYSIOLOGY	MOLECULAR MARKERS
EARLY OF		23	early tailbud	ventral surface concave giving embryo a 'coffee bean' look; two pharyngeal arch bulges; olfactory placodes thicken between eyes; slight depression of otic placode; jaw and gills separated by groove; "inverted Y shaped" hatching gland between eyes to cement gland	12 somites; forebrain regions telencephalon and diencephalon distinguishable		cxc/14, ast/3a.1, pax3: hatching gland; myod1: somites and presomitic mesoderm; six1: olfactory placode
		24	early tailbud	noticable elongation of the embryo and tail bud outgrowth; in dorsal view, eyes protruding out laterally less than gills; gill primordium area smooth (ungrooved)	15 somites; primary germ cells detecable in cell trunk endoderm; primary and secondary heart fields indictated	initial motor reactions to external stimuli	hoxa13, Imo2, apInr: tail bud; Inni3, nkx2-5: primary heart field; bmp4: secondary heart field; pgc, pgat: primordial germ cells; pcdh8: otic vesicle
		25	early tailbud	embryo still convex dorsally and concave ventrally; eyes protruding out laterally equal to or more than gills, gills now grooved; otic vesicle pigmented	16 somites; head somite 1 diminished; brain flexure ~90o	multi-cilliated cells in the epidermis become active	aldh1a3, agr2: otic vesicle; ag1: cement gland
		26	tailbud	If liberated from the vitelline membrane the embryo is straight, not convex dorsally; if the embryo remains in the vitelline membrane it is curved laterally; tail bud obvious; otic (ear) vesicle protruding	17 somites; head somite I disintegrated; pronephros distinct; myotomes distinct	spontaneous movements begin	pax8, atp1b1, slc26a1: pronephric mesenchyme
		27	tailbud	tail bud defined in lateral view; fin translucent; lens begins to form, eyes flatten laterally; otic vesicle closes	heart fields merge forming a triangular-shape at ventral midline, behind the cement gland and anterior to liver diverticulum; 19 somites		pax6, sox3, prox1, foxe3, nrl: lens; fox11c, sox3: epibranchial placodes
		28	tailbud	tail bud elongates distally and extends downward to cloaca; fin divided into outer transparent and inner translucent bands; black cement gland fully formed; otic vesicle separates from epidermis	heart primordium and pericardial cavity discernible; pronephric nephrostomes form; 20-22 somites; epibranchial placodes first segregate	embryos liberated from the vitelline membrane glide around due to multi-cilliated cell fluid flow	hand2, actc1: endocardial tube; d/x2, sox9, sox10: cranial neural crest; pax2, lhx1: nephrostomes; neurog2, eya1: epibranchial placodes; fgf8, sox9: otic veside; fgf8, frxb1: mouth primordium
		29 & 30	late tailbud	tail bud distinct; outer fin edge transparent over entire length; gray disc of the eye cup now visible	23-25 somites segregated to end of tail; lumen in pronephric kidney collecting duct; appearance of glomus and thyroid gland		<pre>runx1: olfactory placode; gsx1, lhx9: brain segments; nphs1, wt1: glomus; nkx2-1: thyroid primordium</pre>
		31	late tailbud	tail bud equal in length and height; nasal/olfactory pits first indicated	heart primordium extends ventrally and bends slightly to right; 22-23 post-otic somites; midbrain-hindbrain boundary distinct		hoxd10, myod1, actc1: somites; en2, pax2, fgf8: midbrain- hindbrain boundary
		32	late tailbud	talibud ~1.5x longer than height; eye cup distinct, U-shaped (open); mouth primordium not visible; 26 post-otic somites	heart a linear tube with anterior outflow tract, left ventricle, atrioventricular canal and atrium; nephrostomes form; lung buds visible		pax2, vax1, vax2: optic stalk; cfap161: nephrostomes and multiciliated epidermal cells; nkx2-1: lung and thyroid progenitors
		33 & 34	late tailbud	tailbud -2x longer than height; gut -3 x longer than tail; eye cup open C-shape with darker pigmentation dorsally; mouth primordium a shallow vertical groove; 32 post-otic somites; pigmented melanophores first appear on head (near hindbrain) and anterior trunk (near pronephric kidney)	heart looping begins; foregut begin to constrict at trachea-eophagus boundary; cranial nerves distinct; thyroid promorium discernible [2]		dab2: pronephric sinus, posterior cardinal vein; sox2: dorsal foregut; tubb2b: cranial nerves; myl2, bves: heart; pax2, lhx1: pronephric nephrostomes [2]
		35 & 36	free swimming tadpole [3]	talibud -3x longer than height; gut -2 x longer than tail; outline of the proctodeum still curved; optic vesicle/retina completely black, choroidal fissure open; cardiac mesoderm starts to spontaneously contract; mouth invagination not quite round; 2 gill lobes; 36 post-olic somites; melanophores extend over top of head and along dorsal trunk	heart S-shaped, with distinct chambers; vasculature to head and tail developing; liver bud visible posterior to heart; pronephric duct fused with rectal diverticulum, pronephric nephrostomes obvious	embryos naturally hatch from vitelline membrane to become free swimming	hbz: blood vessels and heart; onecut, nr1h5, hbex: liver; pax2, lbx1: pronephric nephrostomes; aldh1a1: choroidal fissure; hba3: ventral blood island; hesx1: mouth primordium
	VESIS	37 & 38	free swimming tadpole	gut almost same length as tail; eye's chorold fissure closing ventrally but remain open; mouth invagination deep, round-shaped; heart contractions obvious, blood flow visible; proctodeum at obtuse angle (~140 degrees) to tail somites; 40 post-otic somites; melanophores extend over tail	paired lymph hearts; entire pronephric kidney functioning; ventral bud of pancreas formed		tnni3: heart; sftpc: lung buds; myh6: lymph heart; atp1a1: pronephric kidney and pronephric duct; pdia2: pancreatic buds; foxe4, nkx2-1: thyroid and lungs
	ORGANOGE	39	free swimming tadpole	gut equal in length to tail somites; melanophores around nasal pits & along ventral edge of tail somites; retinal ventral choroid fissure nearly closed; proctodeum at ~125 degree angle to tail somites; 43 post-otic somites	retinal ganglion cell axons reach optic tectum		foxi1, hoxa13: proctodeum; Imnb2: retinal ganglion cell
	Ũ	40	free swimming tadpole	tail now longer than the abdomin; optic choroid fissure completely closed; mouth opening "breaks through"; stomach and pancreas visible on the left side of gut; proctodeum at 90 degree angle to tail somites (lateral view), ~45 post-otic somites	gall bladder primordium formed and sometimes visible (irridescent on ventral view); blood circulation in gills visible	embryos begin taking gulps of air from the surface	ins, cela1.2, foxa1: pancreas; klf5: stomach; onecut, hhex: liver gall baldder; sox17a: gall bladder
		41	free swimming tadpole	conical shaped proctodeum formed, at angle of \sim 60 degrees to tail somites	myocardium thickens and develops trabeculae, atrium posterior to ventride; torsion of gut starts; post-anal gut disappears; the pancreas, now visible in ventral view, posterior to left-sided stomach		adamst9 : pronephros; pdia2 : pancreas buds
1							

GUT-COILING		42	free swimming tadpole	opercular fold first visible; head somites I and II disappeared	trachea and esophagus seperate		<pre>sox2: esophagus and stomach; nkx2.1: trachea and lung buds; sftpc: lung buds</pre>
		43	free swimming tadpole	cement gland starts to lose pigmentation; lateral line pits visible	stomach has lengthened further; pancreas shifted to right side; duodenum formed by 1st gut coil constriction to anterior-right; midgut + hindgut form harpin curve, visible on left side (will become the 'apex' of future intestinal coil)		foxi1, spdef, bmpr1a, cfap161: stomach
		44	free swimming tadpole	heart fully formed and clearly visible; tentacles start to grow; gills shrinking	septum begins to form in heart atrium which is slightly anterior to ventrice; midgut and hindgut lengthened more; the intestinal apex visible in ventral view as a "U" shape in the upper left quadrant of the gut cavity	visual avoidance behavior begins	tnni3, frzb, sox9, nkx2-5, actc1, celf1: heart; apoe, cdx2, gpd2, jund: midgut-hindgut
		45	feeding tadpole [3]	operculum party covers the gills, hindlimb bud not visible	midgut and hindgut continue to lengthen; the intestinal apex begins to rotate inward in a counterclockwise trajectory; spleen forms; mesonephric kidney	tadpoles to start swim continuously and begin feeding	<i>pdlim5, darmin</i> : hindgut; a2m: midgut, hindgut, liver
		46	feeding tadpole	crescent-shaped hindlimb bud first appears; pigment cells appear on eye and around abdomen; trunk somite 1 disappeared	midgut and hindgut lengthen further- apex continues to rotate inward, forming multiple coils of intestine; blood circulation to gills diminishing	food can be seen in intestine; now feeding	
		47	feeding tadpole	irredescent gold-coloured abdominal wall surrounds colled gut; blood circulation visible from heart to gills, and through paired dorsal aorta; cement gland starts to degenerate; tentacles longer	retinal ganglion cells have formed complex synapses with optic tectum neurons; thyroid gland begins to function; thymus primordium detectable		foxn1: thymus primordia
		48		hindlimb bud shape nearly semi-circular	retinal ganglion cells-optic tectum synapses more compact		fgf8, fgf2, sall4: hindlimb bud
	SISOF	49		hindlimb bud length equal to it's width	thyroid follicles first appear		foxe3, nkx2-1, pax2: thyroid
	NORP	50		hindlimb bud slightly constricted at base: tiny oval forelimb buds just visible	oonads undifferentiated		faf2: forelimb bud: sprv4: hindlimb bud
	IETAI						
	PREN	51		hindlimb is cone-shaped; forelimb bud is oval shaped (in lateral view)	resorption vacuoles in thyroid follicles first appear		
		52		hindlimb bud with slight 'wrist' indent; forelimb bud slightly constricted at base	5 complete coils of the intestine	regeneration competent [4]	sox9: hindlimb digits/cartilage elements
		53		hindlimb paddle-like, with wrist constriction, digits not discernable; forelimb limb bud with slight wrist constriction	onset of sexual differentiation of gonads	regeneration competent; athyroid animals have arrested development	
		54		hindlimb length (not including foot) 2x the width; foot paddle splayed with 5 digits and thinner inter-digital webbing; forelimb paddle with 4 digits and thinner inter-digital membranes	pronephric kidney begins to atrophy	thyroid hormone detectable in blood	<i>tbx4, sall4</i> : interdigital mesenchyme
		55		hindlimb length (not including foot) 3x width; forelimb hand rotates 90 degress, free parts of fingers as long as they are wide	all major muscles of hindlimb developed	regeneration restricted [4]	
		56		hindlimbs visible from above as they can rotate away from body; hindlimb length = \sim 5 tail somites	sexual differentiation of gonads; hindlimb skeleton completely chondrified	regeneration restricted	
<u>0</u>		57		hindlimb length = ~ 9 tail somites; forelimb remains enclosed in operculum; lip folds form			
PROMET AMORPHOSI		58		hindlimb length = 11-12 tail somites; claws form on toes 1-3 (mostly always still white); forelimb emerges from operculum, elbows first; tail tip begins to atrophy	melanin/pigment deposited under skin especially in tail	regeneration incompetent [4]	
		59		hindlimb muscular, claws start to harden and turn black, shortest toes first; fingertips reach base of hindlimb when forelimb is positioned along the abdomen; tentacles/barbels regress	melanin/pigment surrounds blood vessels and between fibres of somites; forelimb muscles differentiated; pronephros no longer functional	regeneration incompetent	
		60		gill chamber opening still wide; fingertips reach beyond base of hindlimb (almost to 'knee') when forearm is positioned along side of the abdomen; forelimb held posterior to heart; tail fins greatly reduced	pigmentation across body increases	regeneration incompetent; animal switches from tail to leg swimming	
I MAX OF METAMORPHOSIS		61		first sign of gill resorption, openings to gill chamber much narrower; hindlimb and forelimb fully formed; forelimb at level of posterior half of heart	lateral finger-like protrusions from olfactory organ	cessation of feeding (due to oral and intestinal remodeling)	
		62	tailed froglet	head slightly broader than abdomen; corner of mouth still infront of eye; forelimb reaches middle of heart; ventral fin gone from abdomen; adult skin on hindlimbs; only tiny nubs of tentacles remain.	tiny 'stirnorgan' (light detecting cells/part of pineal gland) appears; notochord atrophies along length of tail	peak levels of thyroid hormone in plasma	
		63	tailed froglet	head narrower than abdomen; tentacles (most often) completely gone; forelimb at level of anterior half of heart; tail shortens as somites are rapidly resorbed, tail still slightly longer than body			
		64		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 skin, but 'border lines' clearly visible	thymus gland ventral-lateral to otic capsule		
		65		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	
C		66	froglet	tail very nearly gone, not visible from ventral view; adult skin 'border lines' have disappeared, froglet body ~ 10mm long	skin remodelled with underlying dermis and secretory glands	thyroid hormone in plasma returns to prometamorphic levels	
	F	OOTNOTES		Dorsal-ventrial pigment variation only occurs in come batches of embryos. Select 2-4-cell embryos with clear pigment variation - otherwise In the Normal Table and other twst, organ primordia are othen called antiget; they are visualized by as a thickening of specific cells via h NF stages 41-66 are not referred to as having specific 'stage names' by Nieuwkoop and Faber Regeneration classes from Aztekin et al 2021 PMID:341 (05722.	e only accurate about 70% of time. Isistology or by molecular markers. Search specific XAO terms on Xenbase for more m	olecular markers	