

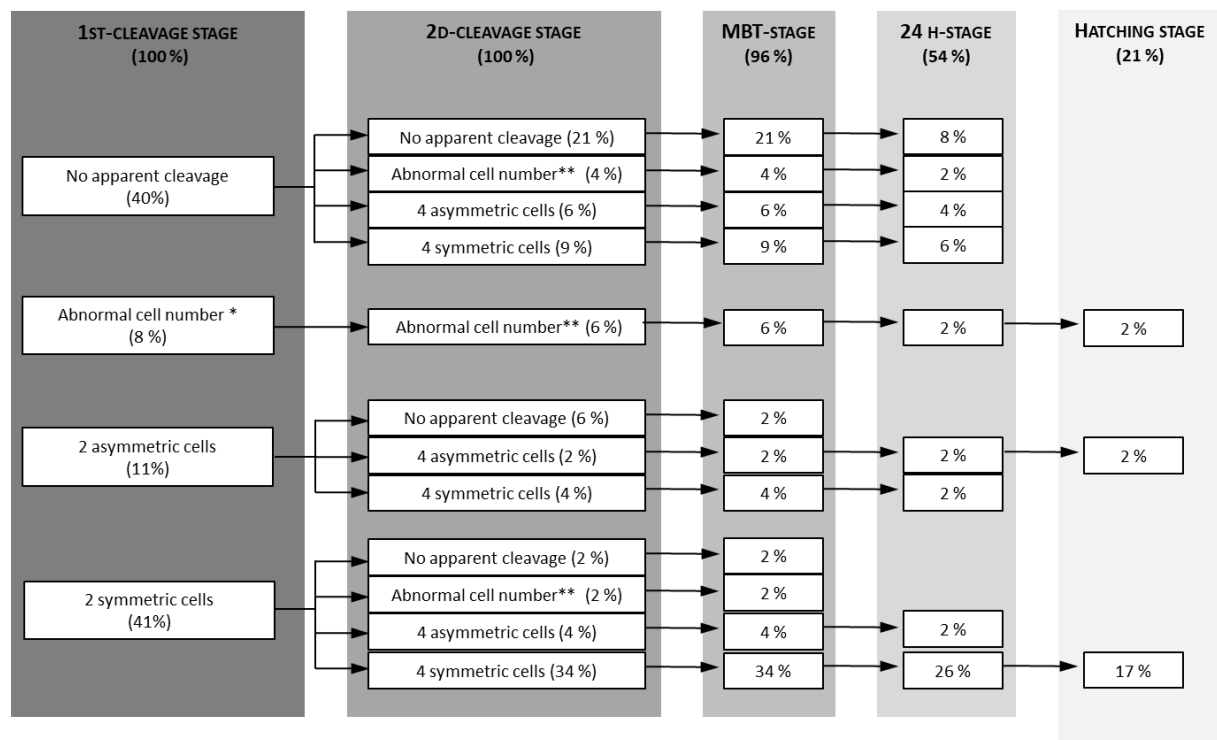
Supplementary data

Embryonic fate after somatic cell nuclear transfer in non-enucleated goldfish oocytes is determined by first cleavages and DNA methylation patterns

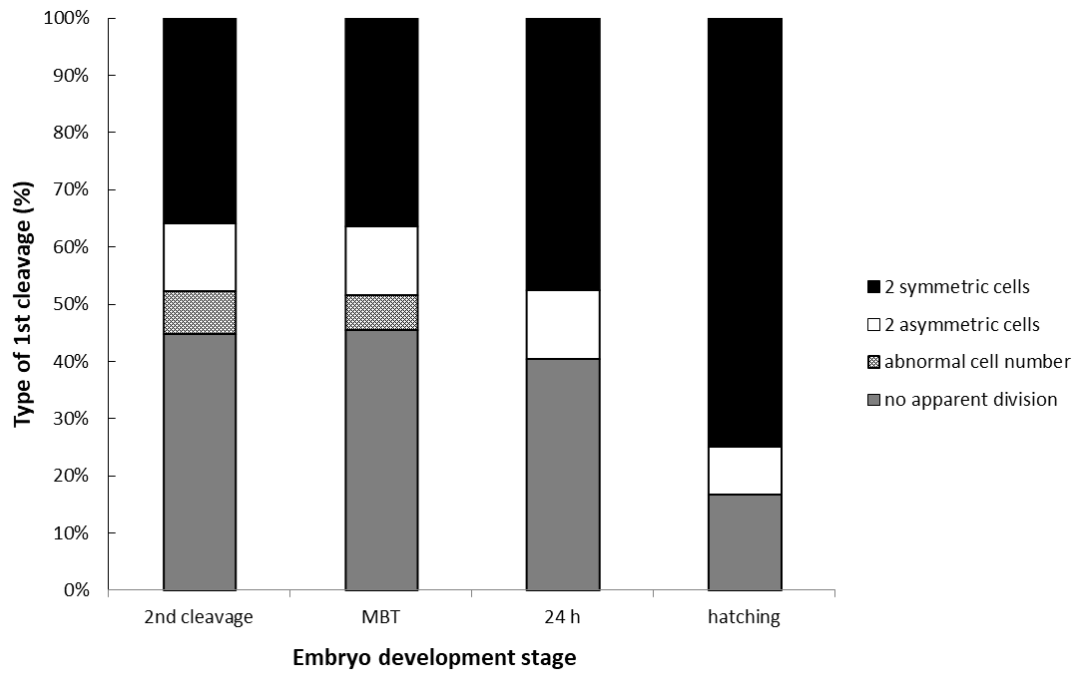
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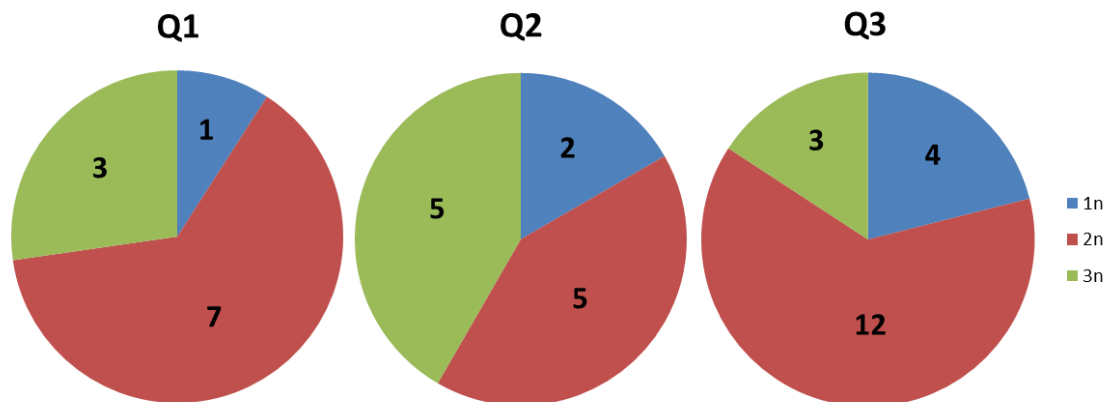
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Supplementary figure S1. Clone fate up to hatching in relation to the morphology of the first cleavage. Proportions are expressed as a percentage of the n=67 clones undergoing development (see table 1). MBT: Mid Blastula Transition. * = 3 or 4 cells, ** = 3, 5 or 6 cells



Supplementary figure S2. Fate of the clones at 4 development stages depending on their first-cleavage pattern. Within each bar are represented the different first-cleavage types the embryos are coming from. Values are expressed as a percentage of the number of embryos still alive at the stage considered (given in table 1).



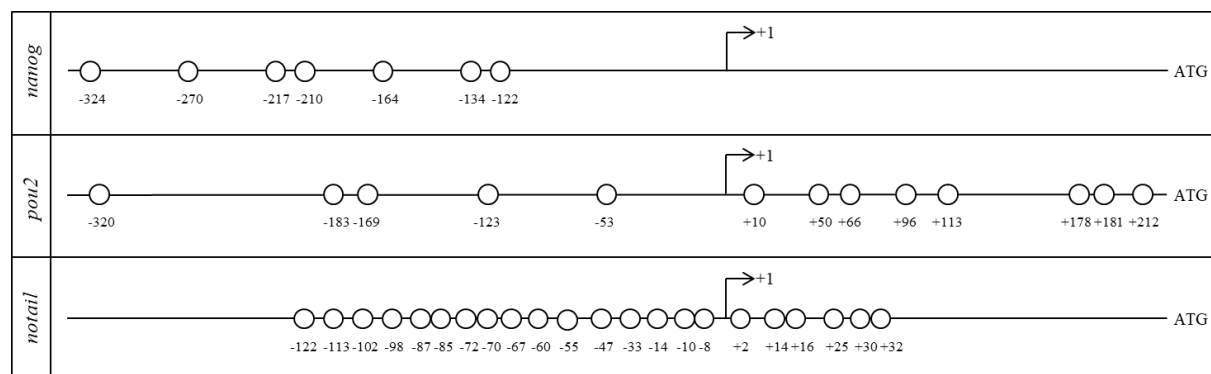
Supplementary figure S3. Distribution of haploid (1n), diploid (2n) and triploid (3n) clones within each morphological types at 24 h-stage. Q1, Q2, Q3: three types of morphology (from the better to the worse) as described fig. 6. Numbers in the sectors: number of clones.

Experiment 1		Experiment 2			Odds to obtain real clones at 24 h
Operated clones	Reaching 24 h-stage	Reaching 24 h-stage	Diploids	Diploids from donor origin	
100 % (132)	32 % (42)	100 % (55)	44 % (24)	24 % (13)	8 %

Supplementary Table S4. Summary of the clone developments at 24 h-stage (experiment 1), ploidy and genetic origin (experiment 2), and odds to obtain authentic clones (diploid from donor genetic origin). Data are summarized from Table 1 and Fig. 4. Numbers between brackets: number of clones. Odds to obtain real clones at 24 h was calculated from development rates at 24 h and from percentage of diploid clones from donor genetic origin.

Locus	Repeat motif	Accession No.	Primer F sequence (5'-3')	Primer R sequence (5'-3')	AT (°C)	Amplicon size (bp)	No. of alleles
<i>Ca02</i>	(TA) ₁₉	AB040746 (GeneBank)	TTGTTACAGCATTGCAAGTATGT	CACTGTTGGAAGAGTACGAACTG	50	181-203	6
<i>Ca03</i>	(TG) ₄ TT(TG) ₆	AF001406 (GeneBank)	AATAGCCGCTTTTCCTGATGG	CCCCGGGTGTTCTCCTTTTC	55	196-214	8
<i>YJ0010</i>	(TG) ₁₁	Aurora (GCGD)	GATGGTTGTGCTGTGAGCT	GAGTTCGTTTACATCTGGAC	53	150-202	11
<i>YJ0020</i>	(GT) ₁₀	3-1473 (GCGD)	CGAATCGATGTCAACCAATG	TCCACGAGTCTCAGGCAGC	55-50	140-170	8
<i>YJ0039</i>	(TG) ₁₉	EF532925 (GeneBank)	GAAGAATACTTTATGACTGAGG	GACCAAGACAGACAGCCCAG	50	126-156	8
<i>YJ0042</i>	(AC) ₂ A(AC) ₃ AA(CA) ₁₂	EF532928 (GeneBank)	GGCCACCTACAGTATATGC	GAAAACCAGGACCGACATG	55-50	90-120	11
<i>HLJYJ018</i>	(AGAC) ₉ (AGAT) ₂₄	FJ827513 (GeneBank)	TCTGCCCAGTGACATAATTTTC	TGCAAGGAGAGTCTCAGCAA	58	192-248	12
<i>HLJYJ029</i>	(AGAT) ₁₉ (AGAC) ₁₀	FJ827517 (GeneBank)	CACCGAAATACTGAGACAGACAG	GCGCTTTCTTGACTGAGAC	60	140-232	13
<i>HLJYJ032</i>	(GATG) ₁₉ (GATA) ₇	FJ827520 (GeneBank)	GACTGGCAAGTGGGAACTC	GCTGCCAAAGCTTTATGGTC	60	153-368	13
<i>HLJYJ033</i>	(AGAT) ₇	FJ827521 (GeneBank)	ACAGCTTTCAGTGACGGTGA	AGGCAGATTGCATTATGAGG	60	226-316	10
<i>HLJYJ038</i>	(ATCT) ₁₂	FJ827523 (GeneBank)	GTTATCTGCGTGGTGGGACT	GCAATGACTGTGAAAAATGG	60	166-254	10
<i>HLJYJ122</i>	(TGA) ₆	FJ827560 (GeneBank)	AGTCCACAGCACATTCATGC	CTGCAGCAGAACATCACCAT	59	122-321	19

Supplementary table S5. Microsatellite markers and the corresponding primers used for genotyping of the clones at 24 h-stage. bp: base pairs, AT: Annealing Temperature



Supplementary table S6. Promoter regions and CpG location in the 3 marker genes used to assess DNA methylation pattern. Numbers indicate the location of the CpG sites (depicted by circle) with respect to the putative transcription start site (arrow and +1 symbol).

Gene		Primer sequence (5' to 3')	Target region	CpG number	Amplicon size
<i>nanog</i>	Forward-outer	TTTGTTTAAATTTGATGTTTGA	-590 to -42	7	549 bp
	Reverse-outer	CCCTTAACATAACCTAAAAAA			
	Forward-nested	ATTAGATAAGTTGAGTAAATTA	-362 to -44		319 bp
	Reverse-nested-β	CTTAACATAACCTAAAAAAAC			
<i>pou2</i>	Forward-outer	GATTAAATAGTTTTATTAAATATTTG	-604 to +274		877 bp
	Reverse-outer	CATCTTCTCACAACAAAATTATATAAA			
	Forward-nested 1	AATGTGGTATGAATATAAAA	-384 to -85	4	299 bp
	Reverse-nested 1-β	CTCCTATTAATCACCTAAAA			
	Forward-nested 2	GTTGTGGTTTATTGTTTtaggt	-118 to +235	9	352 bp
	Reverse-nested 2-β	AAAAAACTTCAAAAAAAAAAAAA			
<i>notail</i>	Forward-outer	AATAAAAGTGAGAAATATTTT	-315 to +80		398 bp
	Reverse-outer	CCGATCAAAAACCTAATATAAATCC			
	Forward-nested	TTTTGTAATGGATTTTGTGTAAGT	-160 to +64	22	228 bp
	Reverse-nested-β	ATAAATCCGAATACACTAAATCCTA			

Supplementary table S7. Primers for amplification of *nanog*, *pou2* and *notail* bisulfite converted promoter regions. β: biotinylated reverse primer, bp: base pairs.

Gene	Primer sequence (5' to 3')	Sequence to analyse (5' to 3')	Position of CpGs
<i>nanog</i> pyro 1	GAGTAAATTATTTAATTAATT	TTAG YG TATTTTA	-324
<i>nanog</i> pyro 2	TTTAATTTATTTTATTATTG	TTTAATTTTTT YG TTA	-270
<i>nanog</i> pyro 3	TATATAATTGTGTAGATAAAATG	TAAAATTTAA AY GTTAT YGA AT	-217, -210
<i>nanog</i> pyro 4	GGTAAATGTGGAATGGGGGTGTG	GTT GY GTTTTTAAGAAGATGTATTGATTGGTT Y GAGTATTTTT Y GTA	-164, -134, -122
<i>pou2</i> pyro c	GTGTTATTGTATTG	TAAATATATAAT Y GTA	-320
<i>pou2</i> pyro d	GGAAAATATAAATATATATAG	ATATATTT Y GATTTTTAAAT Y GTT	-183, -169
<i>pou2</i> pyro e	TTTAATGAGAAATTAAGT	TTATTTATAAATTTAT Y GTTA	-123
<i>pou2</i> pyro 1	GGAGATGGTTAAGATTTTAG	TTTTAATTT Y GATTG	-53
<i>pou2</i> pyro 2	GATTGGGAAGAGTTGG	T GY GGTGAGT	+10
<i>pou2</i> pyro 3	GAAGGTTTATTGATGG	Y GTTATATAAGGATT GY GTA	+50, +66
<i>pou2</i> pyro 4	TAGGTTGATATTGGG	Y GTTATATAAGGATT GY GTA	+96, +113
<i>pou2</i> pyro 5	TGGTATTGAGATAAT	Y G TY G T GTAT	+178, +181
<i>pou2</i> pyro 6	TATTAAATTAATAGTTTTA	AY GTTTTTTTTTTGA	+212
<i>notail</i> pyro 1	TTTGTGTAAGTTAAAAATTT	TTTT Y GTTTGTGG Y GATAGATTAT Y GGG Y GTTA	-122, -113, -102, -98
<i>notail</i> pyro 2	GATTATYGGGYGTTATTAGT	GY GY GATGGGTTTGGTT TY GY GTTT Y GGGTT AY GTTT Y GTA	-87, -85, -72, -70, -67, -60, -55
<i>notail</i> pyro 3	YGTTTYGGGTTAYGTTTYGTGAT	AG Y GGGAGTTTAGTAT Y GGGTTTTTAAAAAGATTT Y G	-47, -22, -14
<i>notail</i> pyro 4	GGGTTTTTAAAAAGATTTYG	GG Y GY GTTGAAAG Y GTAGATAGAG Y GY GTTGTTAA Y GAT Y GY GAT	-10, -8, +2, +14, +16, +25, +30, +32

Supplementary table S8. Pyrosequencing primers and the target sequences in *nanog*, *pou2*, and *notail* promoter region. Y: C or T