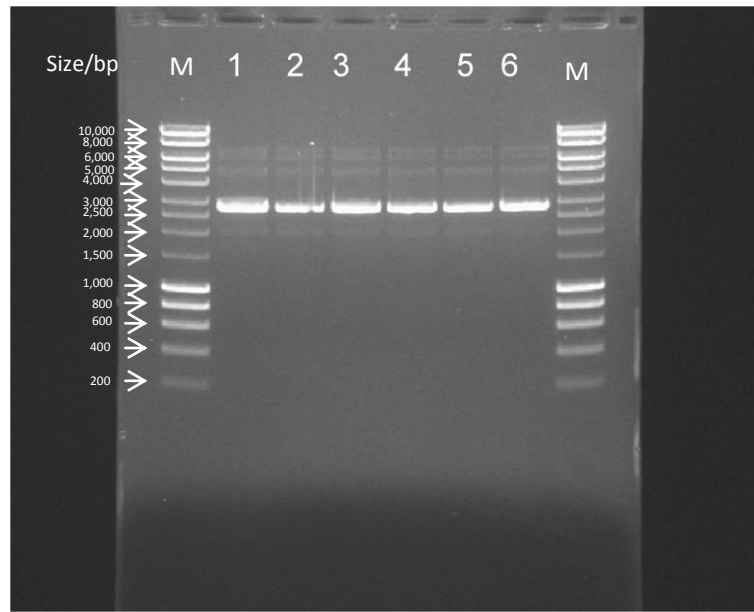# Results Appendix

Construction of hsnanog-pCS2+

Figure S1.hsnanog- pCS2+

1-6, Uncut hsnanog-pCS2+ constructs 1-6. M, Marker Hyperladder I. In lanes 1-6 are bands of uncut hsnanog-pCS2+ plasmids. The pattern of bands in each lane is the same. There are four bands in each lane, representing distribution of plasmid conformations. The brightest band representing supercoiled plasmid is near where 3 kb marker DNA is. Nicked and linear plasmids run slower than supercoiled, located near 5 kb and 7 kb marker DNA, respectively. Denatured supercoiled plasmid runs faster than supercoiled, almost as fast as 2 kb marker DNA.
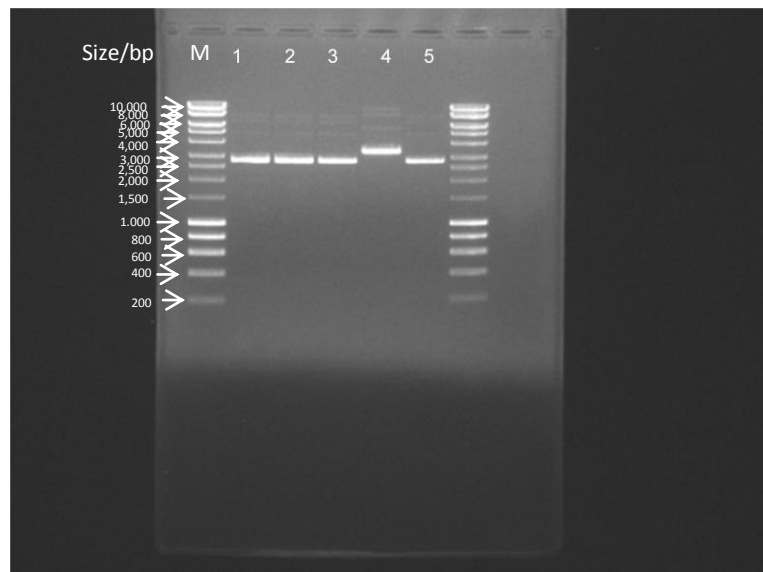
Construction of mnanog-pcs2+

Figure S2. mnanog-pCS2+ 1-5

M, Hyperladder I. 1-5, mnanog-pCS2+ 1-5. In lanes 1-5 are bands of mnanog-pCS2+ plasmids. There are about four bands in each lane. The pattern of the bands in all these lanes is the same except that of the lane 4, which run slower. In lanes 1-3 and 5, the brightest band representing supercoiled plasmid is near 3 kb marker DNA. Nicked and linear plasmid runs almost as fast as 5 kb and 7 kb marker respectively. The slowest band near 8 kb marker DNA may represent dimers and multimers of plasmid. In lane 4, the supercoiled plasmid is near 4 kb marker DNA. The other three slower bands are near 6 kb, 8 kb and 9 kb marker DNA respectively.
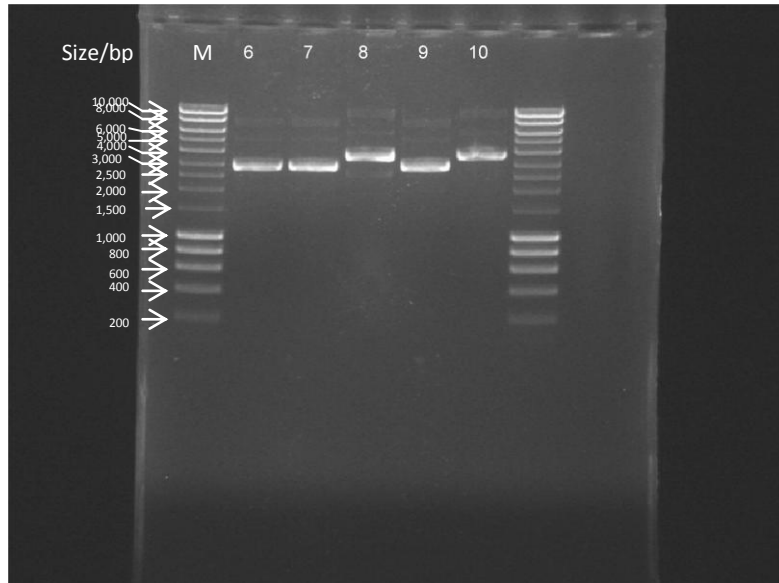
Figure S3. mnanog-pCS2+ 6-10

M, Marker Hyperladder I. 6-10, mnanog-pCS2+ 6-10. In lanes 6-10 are bands of mnanog-pCS2+ plasmids. Lanes 6, 7 and 9 have the same band pattern while the other two lanes 8 and 10 share another type of bands which run slower. In lanes 6, 7 and 10, the brightest band representing supercoiled plasmid is near 3 kb marker DNA. Nicked and linear plasmids run slower and are near 5 kb and 8 kb marker DNA. In lanes 8 and 10, the brightest supercoiled band runs almost as fast as 4 kb marker DNA does. The other two bands, representing nicked and linear plasmid, are near 6 kb and 10 kb marker DNA.

Quantitative RT-PCR

Standard curves

 Standard curves yielded by a plot of log 10 of quantities of *chordin*, *squint*, *goosecoid* ,*wnt8a*, *bmp2b* and *bactin* against Ct in a series of diluted cDNA mix (undiluted, 1 in4, 1 in 16, 1 in 64, 1in 256, and 1 in 1024) of three replicates of uninjected control embryos
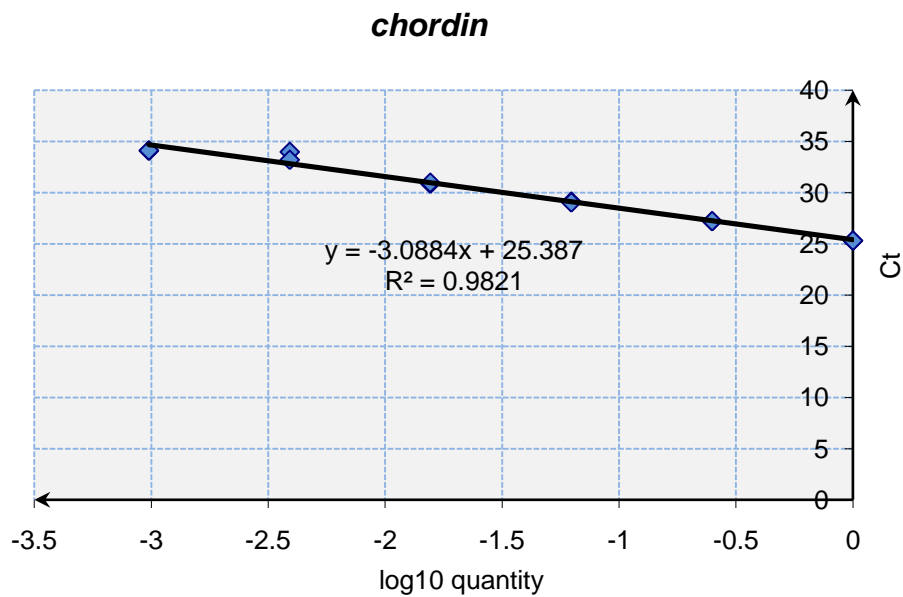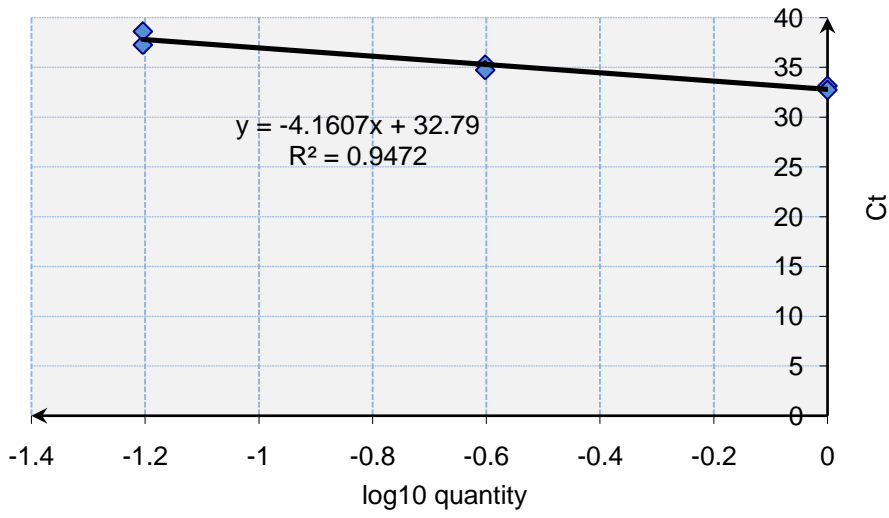
chordin

$y = -3.0884x + 25.387$
$R^2 = 0.9821$

squint

y = -4.1607x + 32.79
R² = 0.9472

log10 quantity

Ct

goosecoid

y = -3.0827x + 27.324
R² = 0.985

log 10 Quantity

Ct

wnt8a

$$y = -3.1813x + 29.332$$
$$R^2 = 0.9794$$

Ct

log10 quantity

bmp2b

$$y = -3.5352x + 24.367$$
$$R^2 = 0.9658$$

Ct

log 10 Quantity

bactin

$$y = -3.6745x + 21.18$$
$$R^2 = 0.9892$$
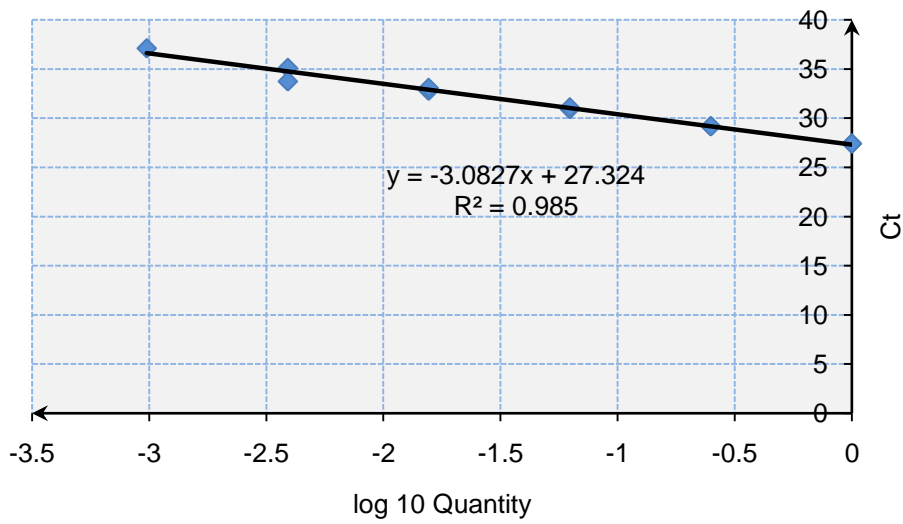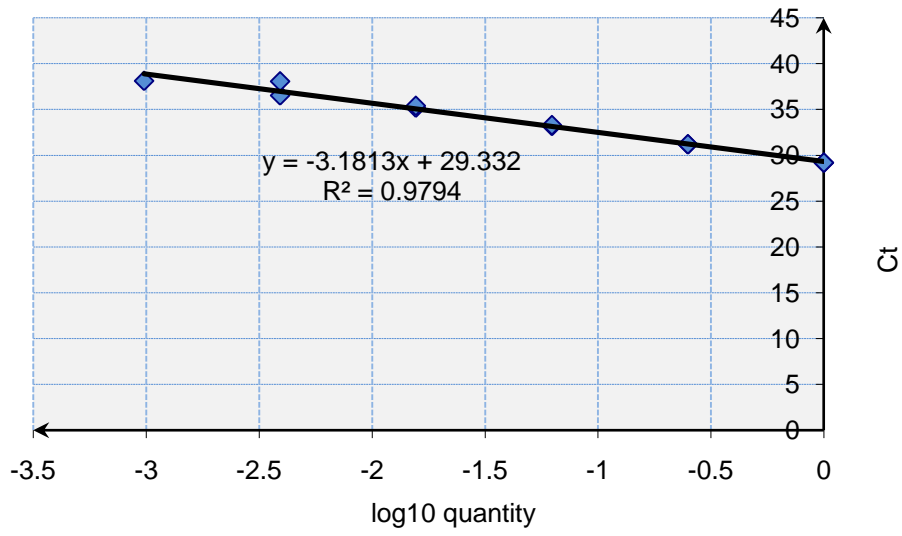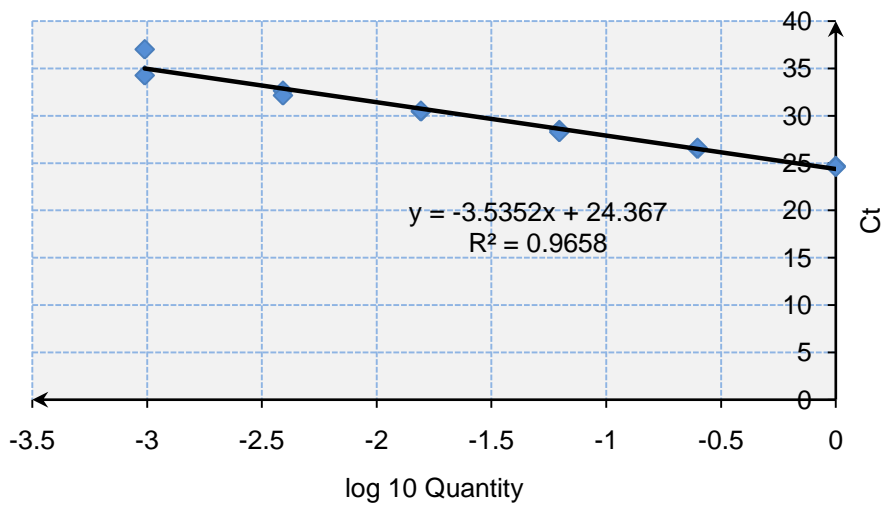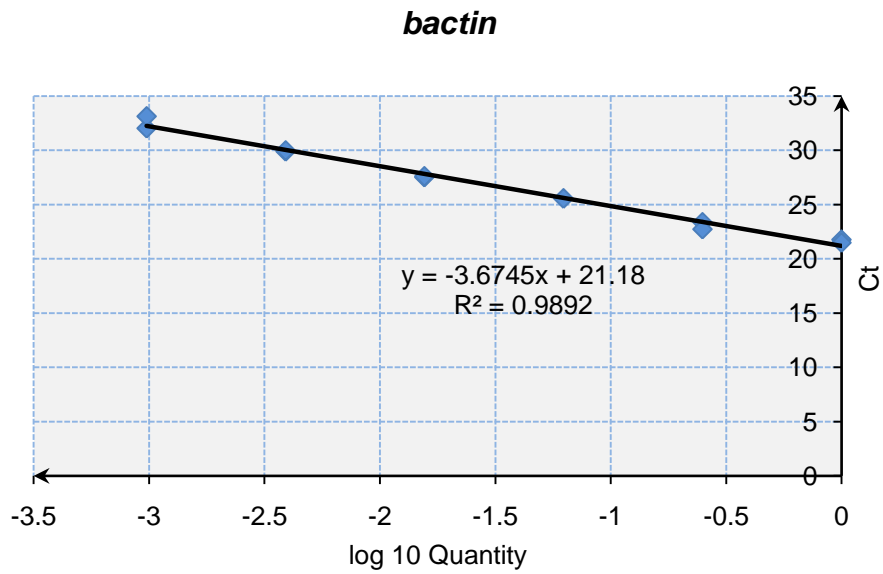
Ct and Quantities of *chordin, squint, goosecoid, wnt8a, bmp2b* and *beta-actin*

Ct and Quantities of *chordin,squint, goosecoid ,wnt8a, bmp2b* and *beta-actin* in *NANOG/Nanog-*injected embryos and uninjected control embryos. un1, un2 and un3 represent three replicates of uninjected control embryos. inj1, inj2 and inj3 are replicates of embryos injected with human *NANOG*, while inj4, inj5 and inj6 are replicates injected with mouse *Nanog*.

chordin

		Ct	Quantity
un1	zf chd	24.88	1.46
un1	zf chd	24.71	1.66
un2	zf chd	25.09	1.25
un2	zf chd	25.14	1.21
un3	zf chd	25.38	1.01
un3	zf chd	25.33	1.04
inj1	zf chd	24.27	2.31
inj1	zf chd	24.23	2.38
inj2	zf chd	24.81	1.54
inj2	zf chd	24.84	1.5
inj3	zf chd	25.13	1.21
inj3	zf chd	25.05	1.28
inj4	zf chd	26.21	5.43E-01
inj4	zf chd	26.2	5.45E-01
inj5	zf chd	26.43	4.59E-01
inj5	zf chd	26.43	4.61E-01
inj6	zf chd	25.82	7.26E-01
inj6	zf chd	25.83	7.21E-01

squint

		Ct	Quantity
un1	sf sqtutr	31.94	1.6
un1	sf sqtutr	31.8	1.73
un2	sf sqtutr	32.72	1.04
un2	sf sqtutr	32.37	1.26
un3	sf sqtutr	32.85	9.63E-01
un3	sf sqtutr	33.44	6.96E-01
inj1	sf sqtutr	32.28	1.33
inj1	sf sqtutr	32.53	1.15
inj2	sf sqtutr	32.25	1.35
inj2	sf sqtutr	33.01	8.84E-01
inj3	sf sqtutr	32.22	1.37
inj3	sf sqtutr	32.16	1.41
inj4	sf sqtutr	32.76	1.01
inj4	sf sqtutr	33.22	7.85E-01
inj5	sf sqtutr	33.99	5.15E-01
inj5	sf sqtutr	33.48	6.82E-01
inj6	sf sqtutr	32.85	9.65E-01
inj6	sf sqtutr	33.16	8.15E-01

goosecoid

		Ct	Quantity
un1	Zf gsc	27.03	1.25
un1	Zf gsc	27.16	1.13
un2	Zf gsc	27.42	0.933
un2	Zf gsc	27.43	0.926
un3	Zf gsc	27.53	0.857
un3	Zf gsc	27.59	0.819
inj1	Zf gsc	25.57	3.72
inj1	Zf gsc	26.07	2.56
inj2	Zf gsc	26.15	2.41
inj2	Zf gsc	26.23	2.26
inj3	Zf gsc	26.33	2.1
inj3	Zf gsc	27.61	0.806
inj4	Zf gsc	28.61	0.383
inj4	Zf gsc	28.14	0.546
inj5	Zf gsc	27.99	0.607
inj5	Zf gsc	28.66	0.369
inj6	Zf gsc	26.99	1.29
inj6	Zf gsc	26.65	1.65

wnt8a

		Ct	Quantity
un1	Zfwnt8a	28.57	1.74
un1	Zfwnt8a	28.6	1.69
un2	Zfwnt8a	29.38	9.68E-01
un2	Zfwnt8a	29.42	9.41E-01
un3	Zfwnt8a	29.82	7.02E-01
un3	Zfwnt8a	29.89	6.69E-01
inj1	Zfwnt8a	29.92	6.55E-01
inj1	Zfwnt8a	29.96	6.37E-01
inj2	Zfwnt8a	31.03	2.94E-01
inj2	Zfwnt8a	30.87	3.29E-01
inj3	Zfwnt8a	30.89	3.23E-01
inj3	Zfwnt8a	31.15	2.68E-01
inj4	Zfwnt8a	31.29	2.43E-01
inj4	Zfwnt8a	31.19	2.60E-01
inj5	Zfwnt8a	32.55	9.73E-02
inj5	Zfwnt8a	32.77	8.29E-02
inj6	Zfwnt8a	32.16	1.29E-01
inj6	Zfwnt8a	32.13	1.32E-01

bmp2b

		Ct	Quantity
un1	Zfbmp2	23.96	1.3
un1	Zfbmp2	24.01	1.26
un2	Zfbmp2	24.22	1.1
un2	Zfbmp2	24.34	1.02
un3	Zfbmp2	24.66	0.824
un3	Zfbmp2	24.51	0.913
inj1	Zfbmp2	24.29	1.05
inj1	Zfbmp2	24.12	1.18
inj2	Zfbmp2	24.64	0.838
inj2	Zfbmp2	24.46	0.944
inj3	Zfbmp2	24.26	1.07
inj3	Zfbmp2	25	0.663
inj4	Zfbmp2	25.35	0.528
inj4	Zfbmp2	25.43	0.501
inj5	Zfbmp2	25.37	0.519
inj5	Zfbmp2	25.52	0.473
inj6	Zfbmp2	24.95	0.684

bactin

		Ct	Quantity
un1	zfbact	20.9	1.19
un1	zfbact	20.86	1.22
un2	zfbact	21.35	0.901
un2	zfbact	21.33	0.913
un3	zfbact	21.4	0.874
un3	zfbact	21.38	0.882
inj1	zfbact	20.59	1.45
inj1	zfbact	20.74	1.32
inj2	zfbact	21.32	0.915
inj2	zfbact	21.41	0.867
inj3	zfbact	21.71	0.72
inj3	zfbact	21.63	0.756
inj4	zfbact	22.65	0.398
inj4	zfbact	22.68	0.39
inj5	zfbact	22.8	0.363
inj5	zfbact	22.69	0.389
inj6	zfbact	21.92	0.631
inj6	zfbact	21.94	0.62

T-tests

T-tests with two samples assuming unequal variances were performed to compare *chordin*, *squint*, *goosecoid, wnt8a* and *bmp2b* expression in *NANOG/Nanog*-injected embryos with that in uninjected control embryos. UNINJ: uninjected control embryos; INJ: *NANOG/Nanog*-injected embryos

chordin

	UNINJ	INJ
Mean	1.272717	1.474478
Variance	0.009261	0.063877
Observations	3	6
Hypothesized Mean Difference	0	
df	7	
t Stat	-1.72169	
P(T<=t) one-tail	0.064399	
t Critical one-tail	1.894579	
P(T<=t) two-tail	0.128799	
t Critical two-tail	2.364624	

squint

	UNINJ	INJ
Mean	1.19814	1.55416
Variance	0.05139	0.235064
Observations	3	6
Hypothesized Mean Difference	0	
df	7	
t Stat	-1.50035	
P(T<=t) one-tail	0.088605	
t Critical one-tail	1.894579	
P(T<=t) two-tail	0.17721	
t Critical two-tail	2.364624	

goosecoid

	UNINJ	INJ
Mean	1	2.050471
Variance	0.001818	0.383778
Observations	3	6
Hypothesized Mean Difference	0	
df	5	
t Stat	-4.13402	
P(T<=t) one-tail	0.004525	
t Critical one-tail	2.015048	
P(T<=t) two-tail	0.009049	
t Critical two-tail	2.570582	

wnt8a

	UNINJ	INJ
Mean	1.085453	0.383838
Variance	0.104018	0.024905
Observations	3	6
Hypothesized Mean Difference	0	
df	2	
t Stat	3.560843	
P(T<=t) one-tail	0.035307	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.070615	
t Critical two-tail	4.302653	

bmp2b

	UNINJ	INJ
Mean	1	1.039978
Variance	0.007073	0.033214
Observations	3	6
Hypothesized Mean Difference	0	
df	7	
t Stat	-0.44998	
P(T<=t) one-tail	0.333164	
t Critical one-tail	1.894579	
P(T<=t) two-tail	0.666329	
t Critical two-tail	2.364624	