

Primers

- For RT

Nanog forward: 5'-CGCTGCTCCGCTCCATAACT-3'

Nanog reverse: 5'-GCGCATGGCTTTCCCTAGTG-3'

Nr0b1 forward: 5'-CTGGTGTGCAGCGTCTGA-3'

Nr0b1 reverse: 5'-GTGTTGGTCTCCGGATCTC-3'

Sall1 forward: 5'-AGTTCTCCCAGGAGGCGAGGTG-3'

Sall1 reverse: 5'-GGTTGGCAGATGTTTCGTAAAGT-3'

Hoxa1 forward: 5'-GGTCAACCCAACGCAGTG-3'

Hoxa1 reverse: 5'-TGCTTCATGCGGCGATT-3'

Otx2 forward: 5'-CCGGAAACAGCGAAGGGA-3'

Otx2 reverse: 5'-GCTGTTGGCGGCACTTAG-3'

Hand1 forward: 5'-ACCAGTTACATCGCCTACTTGA-3'

Hand1 reverse: 5'-CGCGACCACCATCCGTCTT-3'

Flk1 forward: 5'-GGATGGAGGCCTCTACACC-3'

Flk1 reverse: 5'-TGCCGACGAGGATAATGAC-3'

T forward: 5'-GTTCCCGGTGCTGAAGGTAAAT-3'

T reverse: 5'-GCGAGTCTGGGTGGATGTAGA-3'

Isl1 forward: 5'-GCAGCAGCAACCCAACGA-3'

Isl1 reverse: 5'-TTTGCAAGGCGAAGTCAC-3'

Sox17 forward: 5'-CTCTGCCCTGCCGGGATGG-3'

Sox17 reverse: 5'-AATGTCGGGGTAGTTGCAATA-3'

Pou5f1 forward: 5'-CCCTGGGCGTTCTCTTTGGAA-3'

Pou5f1 reverse: 5'-ACCAGGGTCTCCGATTTGCAT-3'

Hoxb8 forward: 5'-AAGCGGAGGATCGAGGTA-3'

Hoxb8 reverse: 5'-GCTCCTCCTGCTCGCATTTAC-3'

Dkk1 forward: 5'-ATCAATTCCAACGCGATCAAG-3'

Dkk1 reverse: 5'-CTTCAGCGCAAGGGTAGG-3'

Goosecoid forward: 5'-TGCTGCCCTACATGAACGTG-3'

Goosecoid reverse: 5'-CTCCAGGGCTTCGAGCTG-3'

Gata6 forward: 5'-GCCACTGTGGAGACGAGA-3'

Gata6 reverse: 5'-CATATAGAGCCCGCAAGCAT-3'

- For ChIP

Nanog prom forward: 5'-CTTACTAAGTAGCCCAGTC-3'

Nanog prom reverse: 5'-GTTTATACACGGTTCTTT-3'

Nanog enh forward, 5'-CCACGGAGTCAAGGCCAC-3'

Nanog enh reverse, 5'-CAAACCGCTTCCCTGGAT-3'

Pou5f1 forward, 5'-GGGTCCCTCTCGTCCTAGC-3'

Pou5f1 reverse, 5'-CCTTCTACCACATGGGTCCAA-3'

Nrob1 forward, 5'-CGGGAGTCTTTAGCTCAAC-3'

Nrob1 reverse, 5'-TGTGAAACAGGGTCAAGGC-3'

Sall4 forward, 5'-GGCCTTCAGATCCTGATCCT-3'

Sall4 reverse, 5'-CTTGCCTTCGACTCTTCCGTC-3'

Sox2 forward, 5'-CAAGGGCATTCTCCGAGACT-3'

Sox2 reverse, 5'-TGTTGTGGAGGTGCGGACT-3'

Otx2 forward, 5'-CCCCTGCTCCCTCAACATAA-3'

Otx2 reverse, 5'-CCTGTAGGTCGCAAGAGTCCC-3'

Pax6 forward, 5'-GCTCTGGAATGCGGGAGT-3'

Pax6 reverse, 5'-TGCCTTGGAGGGGGTCATA-3'

Hand1 forward, 5'-GGCCACACCCTGCTACA-3'

Hand1 reverse, 5'-AGTCTGGAGAAGCAACCC-3'

Isl1 forward, 5'-GTGCCCTCCCAAACCTCTAGCC-3'

Isl1 reverse, 5'-AATTCATATCTGCCGGTGTG-3'

Hoxb13 forward, 5'-CCGGTGGGTCTAACACGAG-3'

Hoxb13 reverse, 5'-GGAAGAAGTGCCTAGAAC-3'

T forward, 5'-CTGCCTTCTTGTTCTTTATCAGC-3'

T reverse, 5'-CCCAGGTGTACCGACCACT-3'

β -globin forward, 5'-TGGCCTTGAATCACTTGGA-3'

β -globin reverse, 5'-AGGAGGTGCCCATCAGAC-3'

Spry2 forward: 5'-CAGCCCACCTGAGGTAGTAA-3'

Spry2 reverse: 5'-TGAAGCCACTCCCAATTCC-3'

Supporting figure legends

Figure S1: Specificity of Sall1 and Nanog antibodies. Comparison of commercial (com) versus affinity-purified antibodies against Sall1 and Nanog. The antibodies were tested in western blotting on ES cell nuclear extracts.

Figure S2: Selected targets of Sall1 and Nanog as viewed in Integrated Genome Browser (IGB) during microarray analysis. IGB images showing Sall1, Nanog and a non-specific factor (Fras) binding on 13 selected gene loci that were validated in Fig.S3.

Figure S3: Validation of ChIP-on-chip data. Data for 13 selected genes were tested by chromatin immunoprecipitation and qPCR.

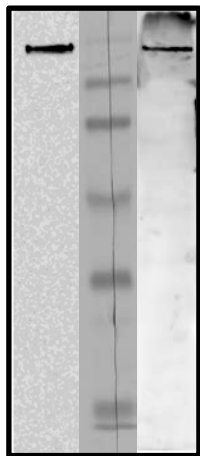
Figure S4: Determination of best binding sequences for Sall1 and Nanog using the Weeder algorithm.

Figure S5: mRNA levels of pluripotency and differentiation genes during EB differentiation in wt ES and clones stably overexpressing Sall1. Oct4 and Nrobl (pluripotency genes) and Sox17 and Gata6 (endodermal differentiation genes) are similarly regulated during EB formation in wt ES (◆) and in clones stably expressing Sall1 (▲). Data were obtained from 3 independent clones.

Supplement figure 1

com

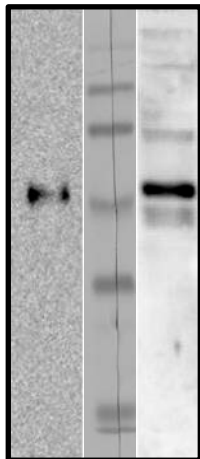
pure



W.B.: α -Sall1

com

pure



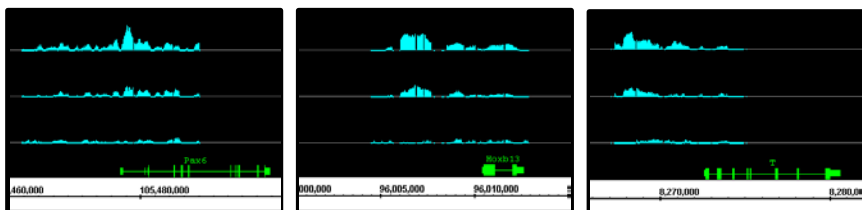
W.B.: α -Nanog

Supplement figure 2

α -Nanog

α -Sall1

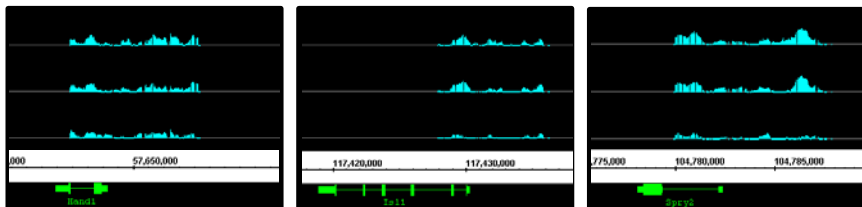
α -Fras



α -Nanog

α -Sall1

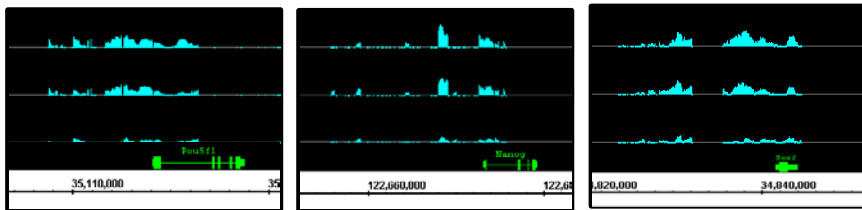
α -Fras



α -Nanog

α -Sall1

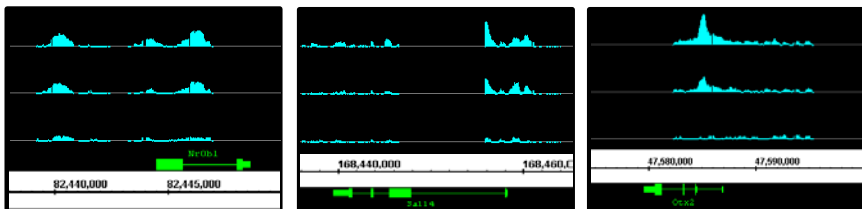
α -Fras



α -Nanog

α -Sall1

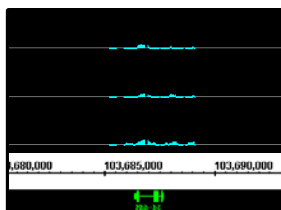
α -Fras



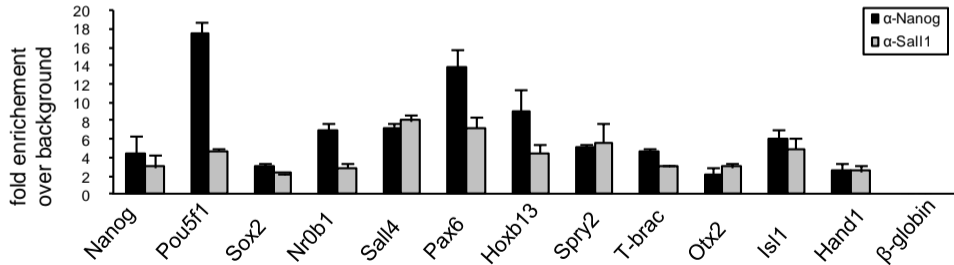
α -Nanog

α -Sall1

α -Fras



Supplement figure 3



Supplement figure 4

Nanog	All occurrences				Best occurrences			
	A	C	G	T	A	C	G	T
1	18	132	9	15	4	27	0	4
2	143	15	2	14	31	3	0	1
3	7	10	8	149	0	0	0	1
4	10	15	5	144	0	1	0	34
5	11	6	7	150	0	0	0	35
6	20	19	117	18	4	3	27	1
7	25	122	12	15	3	31	0	1
8	138	13	7	16	33	0	0	2
Sall1	All occurrences				Best occurrences			
	A	C	G	T	A	C	G	T
1	81	6	4	5	28	0	0	1
2	4	10	2	80	0	1	0	28
3	7	3	0	86	0	0	0	29
4	5	81	5	5	0	29	0	0
5	11	67	1	17	4	17	0	8
6	22	23	40	11	10	10	7	2
7	80	6	1	9	26	0	0	3
8	8	74	6	8	1	23	1	4

Supplement figure 5

