

Supplementary Material

WNT3A signalling pathway in buffalo (*Bubalus bubalis*) embryonic stem cells

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Table S1. Primary antibodies used for immunocytochemistry

Pluripotency marker	Clone	Description
SSEA-1	MC480	Mouse anti-SSEA1, IgM
SSEA-4	MC813-70	mouse anti-SSEA4, IgG
TRA-1-60	TRA-1-60	mouse anti-TRA-1-60, IgM
TRA-1-81	TRA-1-81	mouse anti-TRA-1-81, IgM
OCT-4	9E3	mouse anti-OCT-4, IgG
SOX-2	6F1.2	mouse anti-SOX-2 IgG
NANOG	NNG-811	mouse anti-Nanog, IgG

Table S2. Primers for real-time PCR

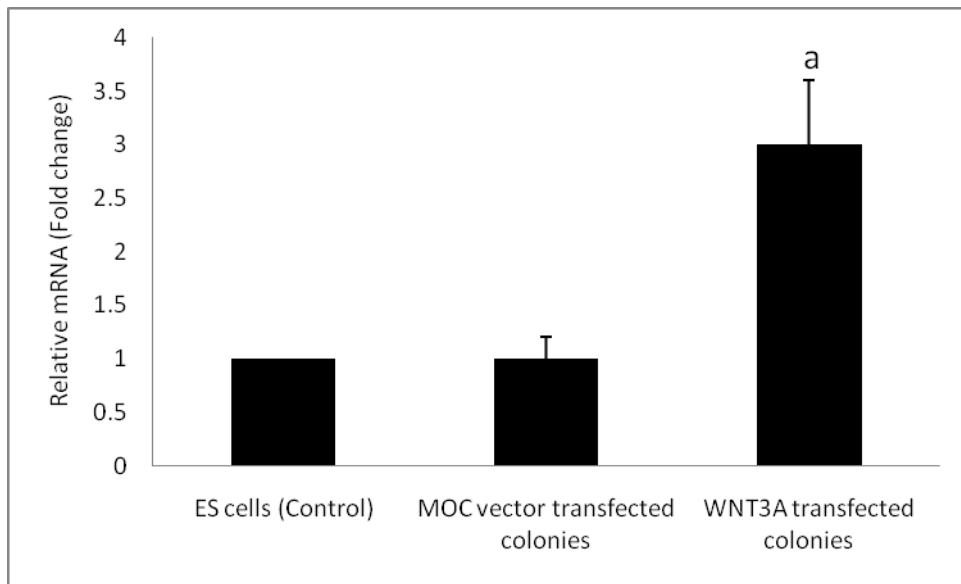
Gene	Sequence	Annealing temperature	Base pairs	Acc. No
<i>SOX2</i>	F-5'CGTGGTTACCTCTTCTTCC3'	60	139	GQ85388
	R-5'CTGGTAGTGCTGGGACAT3'			
<i>OCT3/4</i>	F-5'TTGCAGCTCAGTTTCAAG3'	54	75	EU926737
	R-5'GTTGTTGTCAGCTTCCTC3'			
<i>NANOG</i>	F-5'CCGAAGCATCCA ACTCTAGG3'	60	100	NM001025344 .1
	R-5'GAGACAGTGTC CGTGTCGAG3'			
<i>C-MYC</i>	F-5'CTCCTCACAGCCCGTTAGTC3'	53	156	GU296437.1
	R-5'ATTTGCGGTTGTTGCCTATC3'			
<i>β-CATENIN</i>	F-5'ACAGAAAAGCAGCCGTCAGT3'	56	191	NM001076141 .1
	R-5'AGAAAACCCCTGTTCCCACT3'			
<i>GAPDH</i>	F-5'TCAAGAAGGTGGTGAAGCAG3'	57	121	GU324291.1
	R-5'CCCAGCATCGAAGGTAGAAG3'			

Table S3. WNT3A signaling pathway specific primers

Gene	Sequence	Annealing temperature	Base pairs	Acc. No
<i>WNT3A</i>	F-5'CCGCAATTACATCGAAATCA3' R-5'AGCCACAGATGGTGGAGGTA3	60	245	XM002696056.1
<i>APC</i>	F-5'ATGAGGACCACAGGCAAATC3' R-5'CCACAATGCTTCCTGGTCTT3'	60	152	NM001075986.2
<i>AXIN</i>	F-5'GTCCCGAGGCTACTCAGAGA3' R-5'TTCTGGTTCTTCTCCGCATC3'	60	166	AF009674.1
<i>CBP</i>	F-5'GCAAGATGGGAATGACTGGT3' R-5'TGGTGACTGAAGCGTTCTTG3'	60	168	NM001164022.1
<i>DAB2</i>	F-5'AGAGAACAGAACGGCTTCCA3' R5'CTGAGATGGGAGGAGCAAAG3'	59	202	XM879138.3
<i>DSH</i>	F-5'GATGTGGTGGACTGGCTGTA3' R-5'CCTGATCAGAAGCCCCACTA3'	59	214	gb BC154382.1
<i>FZD1</i>	F-5'CCCGAGTTCTGGACTAGCAA3' R-5'CCCCAGGAAGTGGTAGTTGA3'	59	175	NM001101048.1
<i>GSK3β</i>	F-5'TTGCACTTTGTAGCCGTCTG3' R-5'CCGAGCATGAGGAGGAATAA3'	60	209	NM001101310.1
<i>LEF1</i>	F-5'GCATCCAGATGGAGGTCTCT3' R-5'TCGCTGTACGTGATGAGAGG3'	60	201	XM615475.4

<i>LRP6</i>	F-5'GTACACAAACGAAGCGCAGA3' R-5'AGGAAAGCCTCTGGGACAAT3'	60	230	XM002687783.1
<i>NRCAM</i>	F-5'ACCCTCTCGTCACCATGAAG3' R-5'ATAACGGTGACCTGGATGGA3'	60	165	XM614286.3
<i>SFRP2</i>	F-5'AGGACAACGACCTTTGCATC3' R-5'TTGCTCTTGGTCTCCAGGAT3'	60	217	NM001034393.1
<i>P53</i>	F-5'ATTTACGCGCGGAGTATTTG'3' R-5'CCAGTGTGATGATGGTGAGG3'	60	166	NM174201.2

(A)



(B)

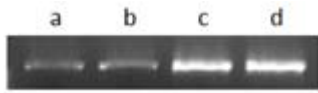


Fig. S1. Transfection of buffalo ES cells with WNT3A. (A) The expression of WNT3A mRNA of ES cells, MOC vector transfected colonies and WNT3A transfected colonies. Different letters indicate significant difference ($P < 0.05$). (B) Agarose gel electrophoresis for WNT3A expression. Lane a: ES cells as Control. Lane b: MOC vector transfected colonies. Lane c and d: *WNT3A* transfected colonies.