

PRD-class homeobox genes in bovine early embryos: function, evolution and overlapping roles

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Affiliations:

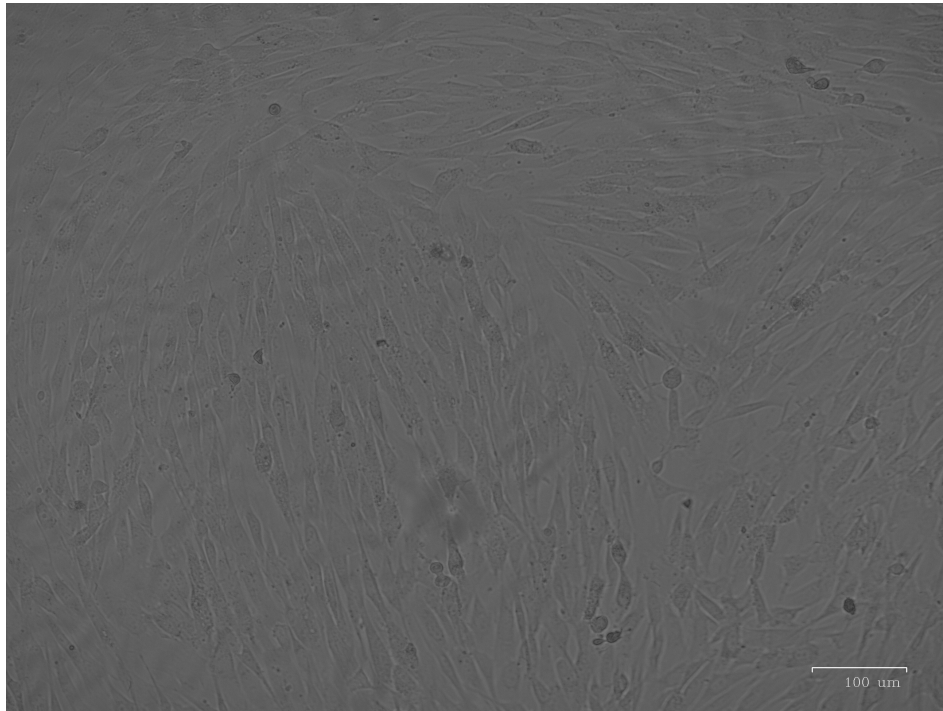
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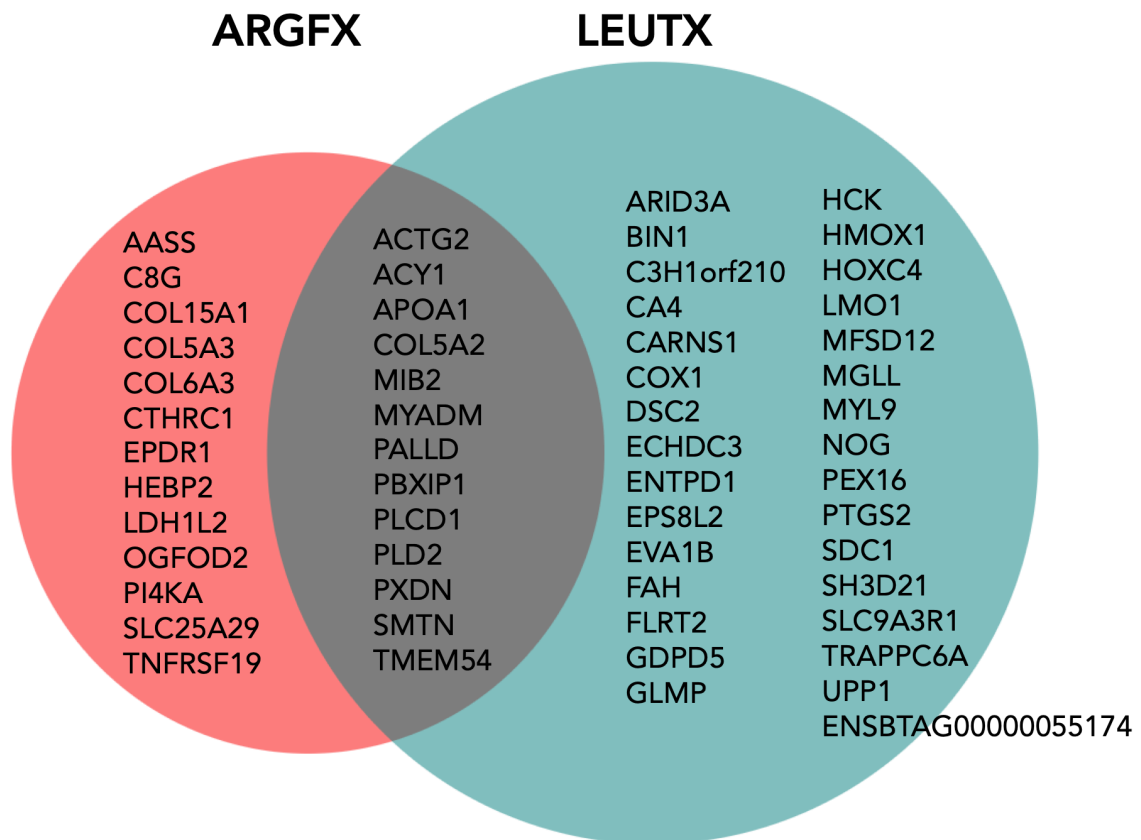
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Supplementary Figures S1 – S5



Supplementary Fig. S1 Image of primary bovine foetal fibroblast (BFF) cells at passage 3 post-isolation. Image captured with ZOE Fluorescent Cell Imager (Bio-Rad).



Supplementary Fig. S2 Blastocyst genes (profile 48) that are upregulated by *ARGFX*, *LEUTX* or both genes.

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>BFF COI gene
TATGGCGATAGGCACCGGACTCTGCTCGGAGACGACCAAATCTACAACGTAGTNGTTNCGCA
CACGCATTTGTAATAATCTTCTTCATAGTAATACCAATCATAATTGGAGGATTCGGTAACTG
ACTTGTTCCCCTAATAATTGGTGCTCCCGATATAGCATTTCCCGAATAAATAATATAAGCT
TCTGACTCCTCCCTCCCTCATTCCCTACTACTCCTCGCATCCTCTATAGTTGAAGCTGGGGCA
GGAACAGGCTGAACCGTGTACCCTCCCTTAGCAGGCAACCTAGCCCATGCAGGAGCTTCAGT
AGATCTAACCATTTTCTCTTTACACTTAGCAGGAGTTTCCTCAATTTTAGGAGCCATCAACT
TCATTACAACAATTATCAACATAAAGCCCCCGCAATGTCACAATACCAAACCCCTCTGTTC
GTATGATCCGTAATAATTACCGCCGTACTACTACTACTCTCGCTCCCTGTATTAGCAGCCGG
CATCACAATGCTATTAACAGACCGGAACCTAAATACAACCTCNTTCCGAACCGGAA
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Supplementary Fig. S3 *Cytochrome c oxidase subunit I (COI)* gene of the cultured bovine foetal fibroblasts, sequenced by Sanger sequencing.

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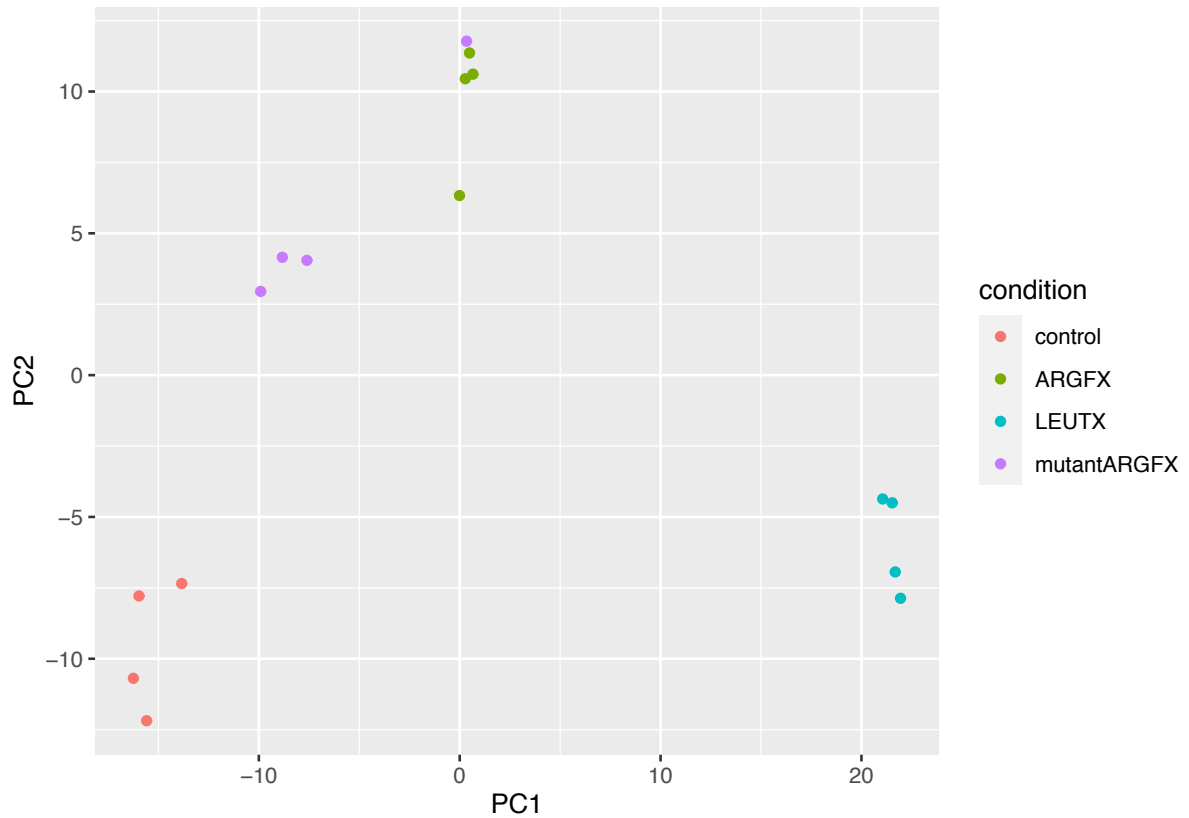
>LEUTX
ATGGGCAGAAAAACAAAATTCAACTCGGCGATTCCGCACACGCTTTAATGGAGAACAGCTAGG
AGCACTAAGAGATGTATTTGAAAGGACCAGGTACCCACATTGTTTCCTCATAAGAACACTTG
CTTCAACTATTTCATCTTGATGCGTCAGTTATAAAGACTTGTTTAAAAACCAACGTGTCAA
AGGAGGAGGGAGGAGAATCAGACTCAGCAAAATCTGTACCAGGAGACCCGCGCCGGGTGT
CTCAGTGAAGGAGGAAGAGATGCCCTTACCGGGCACTTCCGGAAGCACTCATCCCACGAGTC
TCAGCCTTGCAGGTGATTCTCATCACGAGCTACCTGAGCTTTCTTGTGCTGAGCAGTGTGAA
GGGGCTGCTGCCACTCCATGCCCTTCATCCTGCAATTTCTGACTGCTCTAAGTCTCAGAGA
CACTGATCTTCTTGGGCCTCCAGTCCTTATGACATGGATCAACTTATACAGTTATACGACT
TACCTGGGGATGATGACCCCAGCAGTCTGGATCAGTACCTCTTCCCAGAGTGCTCCAGCTGG
GGGACTGTGGCCGGCACTGATCACCATCATGAGGACGAACACAGCTACAATCCTGAGAAAGT
TCCGGGCGGGGGGGCTCCGGAGGAGGTGGCAGCGACTACAAAGACCATGACGGTGATTATA
AAGATCATGACATCGATTACAAGGATGACGATGACAAGTGA

>ARGFX
ATGGGCACTCCAAGGGAATTCATGACAGATTTCCCTTGCCAGACATCAGCACCCCTGATGAA
CAAAAGAGTCCAAGAGAATCCCCTGCCAGACCCTTTTGTCTACATGGATGATTCTAGCACGA
ACTTCCAGGATGATTCAAAGATACCAGCTGGTGCCAGTCTTTTTTTCATCACGGCCTACGACG
TGTGGAATCTTAACTCCCCAACCAGGGATCAAACCCGGGCCTTCGACAGTGAAAGCAAAATG
GAAGAAGAGTCATGAACGCACCTCATTCACCCACACACAGTACAAGGAGTTGGAGGCTCTGT
TTAGCTGCAACATGTTTCCAGATAAAAACCTCCAGAGAGAACTTGCTTTAAACTCAATCTA
CCAGAGTCAACAGTAAAGATTTGGTTTCAGGAACCGGCGGTTCAAATGAAGAAGCAGCAGCG
GGAGCAAGAGCAGCAATCACTAAAGCCACCAAGCCAGGTCCTTCCAGCCAAGGATGTGCCCCA
CAGTATCAACCAGCCCTCATTCTTTTCTCCTTGCAATTTTCAGATTCCTATAACTCCCTCTCA
CCTCAGCCCTTAGACACTTTCCCCTGGGCAGGGGACTCTATGATCATGGAGATTCTTACAAG
TGATGTCCAAACGCAAGATCCTCAACTGGAGAGGCTAGTGGCCTCAGTTCCTGCTTTGTACT
CTGATGCATTTGACATCACCCAAATCATGGAAGTGTACAGTGTTCTGATGAGGATGACATC
ACCAACTCTTCTTCTATTCTCTATATCAGTATCTCTCACCGACAAGGCCAGTGGCGGGGG
GGGCTCCGGAGGAGGTGGCAGCGACTACAAAGACCATGACGGTGATTATAAAGATCATGACA
TCGATTACAAGGATGACGATGACAAGTGA

>ARGFX mutant
ATGGGCACTCCAAGGGAATTCATGACAGATTTCCCTTGCCAGACATCAGCACCCCTGATGAA
CAAAAGAGTCCAAGAGAATCCCCTGCCAGACCCTATGATTCTAGCACGAAGTTCAGGATGA
TTCAAAGATACCAGCTGGTGCCAGTCTTTTTTTCATCACGGCCTACGACGTGTGGAATCTGGC
GGGGGGGGCTCCGGAGGAGGTGGCAGCGACTACAAAGACCATGACGGTGATTATAAAGATCA
TGACATCGATTACAAGGATGACGATGACAAGTGA

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Supplementary Fig. S4 Sequences of ectopically expressed ETCHbox genes, including GGGGSGGGGS linkers (blue) and 3xFLAG (red).



Supplementary Fig. S5 Principle Component Analysis (PCA) to test for outliers in the RNA-seq replicates. Figure shows PCA biplot including control, *LEUTX*, WT *ARGFX* and mutant *ARGFX* samples. *LEUTX*, WT *ARGFX* and mutant *ARGFX* samples are well separated by PCA, except for one *ARGFX* mutant sample, which is an outlier, clustering with WT *ARGFX*. This sample was removed before differential expression analysis.