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Supplementary Material

Role of linker histone H1c during the reprogramming of Chinese swamp buffalo (*Bubalus Bubalis*) embryos produced by somatic cell nuclear transfer

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1. Use the 5′ RACE System for Rapid Amplification of cDNA Ends,Version 2.0 to obtain 5′end

R551-1(GSP1): GCAACCTTGGGCTTAG

R551-2(GSP2): GGCTTAACCGCTTTCGCTGC

R551-3(GSP3): GGCTCTTCGCCACTTTCTTG

protocol：

1.1 Total RNA extraction

1.2 Use SUPERSCRIPT II RT and primer GSP-1for the first cDNA. Use RNase Mix to degrade RNA which mix in cDNA.

1.2.1 Add the following to a 0.5-ml microcentrifuge tube (or thin-walled PCR tube if using a thermal cycler):

Component	**Amount**
GSP1	2.5 pmoles (~10 to 25 ng)
sample RNA	1–5 µg
DEPC-treated water (or sterile, distilled water)	sufficient for a final volume of 15.5 µL

1.2.2 Incubate the mixture 10 min at 70° C to denature RNA. Chill 1 min on ice. Collect the contents of the tube by brief centrifugation and add the following in the order given:

Component	**Volume (µL)**
10X PCR buffer	2.5
25 mM $MgCl_2$	2.5
10 mM dNTP mix	1
0.1 M DTT	2.5
final volume	8.5

The final volume of step 1 and 2 is 24 µL.

1.2.3 Mix gently, and collect the reaction by brief centrifugation. Incubate for 1 min at 42°C.

1.2.4 Add 1 µL of SUPERSCRIPT II RT. Mix gently and incubate for 50 min at 42°C.

1.2.5 Incubate at 70°C for 15 min to terminate the reaction.

1.2.6 Centrifuge 10 to 20 s and place the reaction at 37°C.

1.2.7 Add 1 µL of RNase mix, mix gently but thoroughly, and incubate for 30 min at 37°C.

1.2.8 Collect the reaction by brief centrifugation and place on ice.

1.3 Use DNA Purification System: GLASSMAX DNA isolation spin cartridges for cDNA purification.

1.3.1 Equilibrate the binding solution to room temperature.

1.3.2 For each sample to be purified, equilibrate ~100 µL of sterilized, distilled water at 65°C for use in step 9.

1.3.3 Add 120 µL of binding solution (6 M NaI) to the first strand reaction.

1.3.4 Transfer the cDNA/NaI solution to a GLASSMAX spin cartridge. Centrifuge at 13,000 x g for 20 s.

1.3.5 Remove the cartridge insert from the tube and transfer the flowthrough to a microcentrifuge tube. Save the solution until recovery of the cDNA is confirmed. Place the cartridge insert back into the tube.

1.3.6 Add 0.4 mL of **COLD** (4°C) 1X wash buffer to the spin cartridge. Centrifuge at 13,000 x g for 20 s. Discard the flowthrough. Repeat this wash step **three** additional times.

1.3.7 Wash the cartridge two times with 400 µL of **COLD** (4°C) 70% ethanol as described in step 6.

1.3.8 After removing the final 70% ethanol wash from the tube, centrifuge at 13,000 x g for 1 min.

1.3.9 Transfer the spin cartridge insert into a fresh sample recovery tube. Add 50 µL of sterilized, distilled, water (preheated to 65°C) to the spin cartridge. Centrifuge at 13,000 x g for 20 s to elute the cDNA.

1.4 Add Poly C

1.4.1 Add the following components to each tube and mix gently:

Component	Volume (µL)
DEPC-treated water	6.5
5X tailing buffer	5.0
2 mM dCTP	2.5
GLASSMAX purified cDNA sample	10.0
final volume	24.0

1.4.2 Incubate for 2 to 3 min at 94°C. Chill 1 min on ice. Collect the contents of the tube by brief centrifugation and place on ice.

1.4.3 Add 1 µL TdT, mix gently, and incubate for 10 min at 37°C.

1.4.4 Heat inactivate the TdT for 10 min at 65°C. Collect the contents of the reaction by brief centrifugation and place on ice.

1.5 Use primer GSP-2 for PCR

1.5.1 Equilibrate the thermal cycler block to 94°C. In most cases, the “good start” procedure gives specific amplification products. For some target and primer sets, “hot start” has been reported to improve the specificity of the reaction (44, 45).

1.5.2 Add the following to a 0.2 or 0.5-ml thin-wall PCR tube sitting on ice:

Component	Volume (µL)
sterilized, distilled water	31.5
10X PCR buffer [200 mM Tris-HCl (pH 8.4), 500 mM KCl]	5.0
25 mM $MgCl_2$	3.0
10 mM dNTP mix	1.0
nested GSP2 (prepared as 10 µM solution)	2.0
Abridged Anchor Primer (10 µM)	2.0
dC-tailed cDNA	5.0
final volume	49.5

1.5.3 Add 0.5 µL of Taq DNA polymerase (5 units/µL) immediately before mixing.

1.5.4 Mix the contents of the tube (Taq DNA polymerase is added immediately before going into the thermal cycler) and overlay with 50 to 100 µL of mineral oil (if necessary).

1.5.5 Transfer tubes directly from ice to the thermal cycler preequilibrated to the initial denaturation temperature (94°C).

1.5.6 Perform 30 to 35 cycles of PCR. A typical cycling protocol for cDNA with <1 kb amplified region is:

PAD: 94°C for 1–2 min

Cycle:

Denaturation: 94°C for 0.5–1 min

Annealing of primers: 55°C for 0.5–1 min

Primer extension: 72°C for 1–2 min

Followed by:

Final extension: 72°C, 5–7 min

Indefinite hold: 5°C, until samples are removed.

1.6 Use primer GSP-3 for PCR

1.6.1 Dilute a 5 µL aliquot of the primary PCR into 495 µL TE buffer [10 mM Tris-HCl, (pH 8.0), 1 mM EDTA].

1.6.2 Equilibrate the thermal cycler block to 94°C.

1.6.3 Add the following to a 0.2 or 0.5-mL thin-wall PCR tube sitting on ice.

Component	**Volume (µL)**
sterilized, distilled water	33.5
10X PCR buffer [200 mM Tris-HCl (pH 8.4), 500 mM KCl]	5.0
25 mM $MgCl_2$	3.0
10 mM dNTP mix	1.0
nested GSP (prepared as 10 µM solution)	1.0
AUAP or UAP (10 µM)	1.0
dilution of primary PCR product	5.0
final volume	49.5

1.6.4 Add 0.5 µL of Taq DNA polymerase (5 units/µL) immediately before mixing.

1.6.5 Mix the contents of the tube (Taq DNA polymerase is added immediately before going into the thermal cycler) and overlay with 50 to 100 µL of mineral oil (if necessary).

1.7 Gel analysis of products.

Marker: DL2000

1.8 Sequencing

>R551-72_M13F_B04，AG12090273_R556-29_M13-47

GGCCACGCGTCGACTAGTACGTGTGGGGGTGGGGGGG(adapter)ATCGGCGTTTTGCCACTTGTACCCGA
GTTCTACATTTTCACCATGTCGGAGACTGCTCCTGCTGCTCCCGCTGCCGCACCTCCTGCGGAGAAAACCCC
AGTCAAGAAGAAGGCTGCCAAAAAGCCGGCTGGGGCGCG*CCGGAAGGCCTCTGGGCC*CCCGGTGTCCGA
GCTCATCACCAAGGCTGTTGCCGCCTCCAAGGAGCGCAGCGGCGTGTCTCTGGCTGCGCTCAAGAAGGCA
CTGGCGGCCGCCGGCTACGATGTGGAGAAGAACAACAGCCGTATCAAGCTGGGTCTCAAGAGCTTGGTGA
GCAAGGGCACCCTGGTGCAGACCAAGGGCACCAGGGCTTCTGGTTCTTTCAAGCTCAACAAGAAGGCAGC
CACCGGGGAGGCCAAGCCCAAGGCGAAGAAGGCCGGCGCGGCCAAGCCCAAGAAGGCTGCAGGGGCG
GCTAAGAAGACCAAGAAAGCCACGGGCACGGCCACTCCAAAGAAAACTGCTAAAAAGACCCCGAAGAAA
GCGAAGAAGCCGGCCGCAGCTGCTGTGACCAAGAAAGTGGCGAAGAGCC

2. Amplification of cDNA 3'Ends

Primers: 3'304-1: GCTCATTGCTCCAGCCCGTGATTTTTC

3'304-2: AACTTGCTGTCGTGGGAGCGTTGGTGT

protocol：

2.1 RT-PCR was performed using SMARTScribe™ Reverse Transcriptase and primer 3' CDS primer

2.2 Then performed the 1th PCR amplification by using primers 3'304-1 and UPM.

2.3 The production was diluted 50 times and the use the primers 3'304-2 and UPM for 2th PCR amplification Kit

Marker: DL2000

2.4 Sequencing

>3'304-42_rv-m

AACTTGCTGTCGTGGGAGCGTTGGTGTTCAACCCAGGCAAGAGGGTGGGTACTGACTCCGTTGCCTCA
CATTCTTAATTCCTGTTTACTCGGGCAGCAAAGGAACATTTGAAAAATGCTACAGCGGTGGCAGTGATT
CTTCCACCAGAAGCAAAGCTGGACCAGGGATCTTTCTGTTCCTTGCACTTTCTAACTTTATAAGCAAATG
TGTGATGATTTGACAGCATCAAAAACAACTTTTAGTCGGCCATTTTGTCCTGGCCGGCTAGTCAAGATC
CTCCTTTTCGGGAACCTCAACATCTGAAAATCGTAGATGCTTTAAACGCCTTTTTGTGTAATCCAAATGT
TTCTACGCTAAGTAGAAAAAAATGTATACTGGTAAAGTTTTAAAACGTGTGTATATGGGATGGGGTGGC
GTTTTTAATAAAGGACTTTTTTTTTTCACTAAAAAAAAAAAAAAAAAAAAACTCTGCGTTGATACCACT
GCTTGCCCTATAGTGAGTCGTATTAAA(adapter)

3. Full-length sequence and ORF Forecast

ATCGGCGTTTTGCCACTTGTACCCGAGTTCTACATTTTCACCATGTCGGAGACTGCTCCTGCTGCTCCCG
CTGCCGCACCTCCTGCGGAGAAAACCCCAGTCAAGAAGAAGGCTGCCAAAAAGCCGGCTGGGGCGC
GCCGGAAGGCCTCTGGGCCCCCGGTGTCCGAGCTCATCACCAAGGCTGTTGCCGCCTCCAAGGAGCG
CAGCGGCGTGTCTCTGGCTGCGCTCAAGAAGGCACTGGCGGCCGCCGGCTACGATGTGGAGAAGAAC
AACAGCCGTATCAAGCTGGGTCTCAAGAGCTTGGTGAGCAAGGGCACCCTGGTGCAGACCAAGGGCA
CCAGGGCTTCTGGTTCTTTCAAGCTCAACAAGAAGGCAGCCACCGGGGAGGCCAAGCCCAAGGCGA
AGAAGGCCGGCGCGGCCAAGCCCAAGAAGGCTGCAGGGGCGGCTAAGAAGACCAAGAAAGCCACG
GGCACGGCCACTCCAAAGAAAACTGCTAAAAAGACCCCGAAGAAAGCGAAGAAGCCGGCCGCAGCT
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AAGGGTAGGCTTACGCGGGAGTTGTTGCAGATGGCTGGCACTACCCAAGGAACAGCAGTGAGAGCA
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GTGTCTTCTGTCAGCCTTCTGGTCAGCAGTTGCAAGCGCTTTCCGCAGGCTTGCGTACTTGGGATTTCC
GCCGGCCCCGCCCCACTCCTGTGAAGAGCAAAAACAACCAGCACTAAACCCAGCCAGTTGCTCAGTCT
TACGGGATATTCCGATTGGGCTTAAAAACTCATTCAAAAGTCCCGCCTTGCTCGCCGGTTGGCTCATTGC
TCCAGCCCGTGATTTTTCACCCTGTTTTTAATTGGATGGTGACTGTCTTCCATCTGTGGTCTCTTAATGTT
TTGTTTTCTTTCTGTTGGGTTAGCCCTCTTTGCTTGATAGAGAAATGTGTCGTATTTCGGACGTTTTCCGC
GTTTCCAAAAGTCAACTTGCTGTCGTGGGAGCGTTGGTGTTCAACCCAGGCAAGAGGGTGGGTACTG
ACTCCGTTGCCTCACATTCTTAATTCCTGTTTACTCGGGCAGCAAAGGAACATTTGAAAAATGCTACAGC
GGTGGCAGTGATTCTTCCACCAGAAGCAAAGCTGGACCAGGGATCTTTCTGTTCCTTGCACTTTCTAAC
TTTATAAGCAAATGTGTGATGATTTGACAGCATCAAAAACAACTTTTAGTCGGCCATTTTGTCCTGGCCG
GCTAGTCAAGATCCTCCTTTTCGGGAACCTCAACATCTGAAAATCGTAGATGCTTTAAACGCCTTTTTGT
GTAATCCAAATGTTTCTACGCTAAGTAGAAAAAAATGTATACTGGTAAAGTTTTAAAACGTGTGTATATG
GGATGGGGTGGCGTTTTTAATAAAGGACTTTTTTTTTTCACTAAAAAAAAAAAAAAAAAAAAAA