

## Laser Captured Microdissection (I): RT-PCR RNA

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### Analysis of the Gene Expression by Laser Captured Microdissection (I): Minimum Conditions Required for the RNA Extraction from Oocytes and Amplification for RT-PCR

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**Objective:** Recently, microdissection of tissue sections has been used increasingly for the isolation of morphologically identified homogeneous cell populations, thus overcoming the obstacle of tissue complexity for the analysis cell-specific expression of macromolecules. The aim of the present study was to establish the minimal conditions required for the RNA extraction and amplification from the cells captured by the laser captured microdissection.

**Methods:** Mouse ovaries were fixed and cut into serial sections (7  $\mu$ m thickness). Oocytes were captured by laser captured microdissection (LCM) method by using PixCell II<sup>TM</sup> system. The frozen sections were fixed in 70% ethanol and stained with hematoxylin and eosin, while the paraffin sections were stained with Multiple stain. Sections were dehydrated in graded alcohols followed by xylene and air-dried for 20 min prior to LCM. All reactions were performed in ribonuclease free solutions to prevent RNA degradation. After LCM, total RNA extraction from the captured oocytes was performed using the guanidinium isothiocyanate (GITC) solution, and subsequently evaluated by reverse transcriptase-polymerase chain reaction (RT-PCR) for glyceraldehyde-3-phosphate-dehydrogenase (GAPDH).

**Results:** With the frozen sections, detection of the GAPDH mRNA expression in the number of captured 25 oocytes were not repeatable, but the expression was always detectable from 50 oocytes. With 25 oocytes, at least 27 PCR cycles were required, whereas with 50 oocytes, 21 cycles were enough to detect GAPDH expression. Amount of the primary cDNA required for RT-PCR was reduced down to at least 0.25  $\mu$ l with 50 oocytes, thus the resting 19.75  $\mu$ l cDNA can be used for the testing other interested gene expression. Tissue-to-slide, tissue-to-tissue forces were very high in the paraffin sections, thus the greater number of cell procurement was required than the frozen sections.

**Conclusion:** We have described a method for analyzing gene expression at the RNA level with the

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(2000-2-20500-001-2)

homogeneously microdissected cells from the small amount of tissues with complexity. We found that LCM coupled with RT-PCR could detect housekeeping gene expression in 50 oocytes captured. This technique can be easily applied for the study of gene expression with the small amount of tissues.

**Key Words:** Laser captured microdissection, Mouse ovary, Gene expression, RT-PCR

가  
가  
가 RNA 가  
Northern blot analysis, RNase protection assays, in situ hybridization mRNA LCM  
mRNA (frozen section)  
가 mRNA (paraffin section)  
LCM RT-PCR  
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1 laser captured microdissection (LCM)  
LCM RT-PCR  
DNA, RNA, LCM,<sup>2-6</sup> RT-PCR, cDNA, PCR cycle  
LCM mRNA  
housekeeping glyceraldehyde-3-phosphate-dehydrogenase (GAPDH)  
laser  
beam (homogeneous tissue)  
PixCell II™ LCM system transfer 1.  
film laser pulse 3 ICR pregnant mare serum gonadotropin (PMSG, Folligon, Intervet, Boxmeer, Holland) 5 IU 48  
, transfer film film  
. 7 transfer film compound OCT cryostat (Shandon, Cheshire, England, UK) 7 ì m  
DNA, RNA, digestion buffer .<sup>10</sup> -70  
LCM 70% EtOH  
, -20 LCM

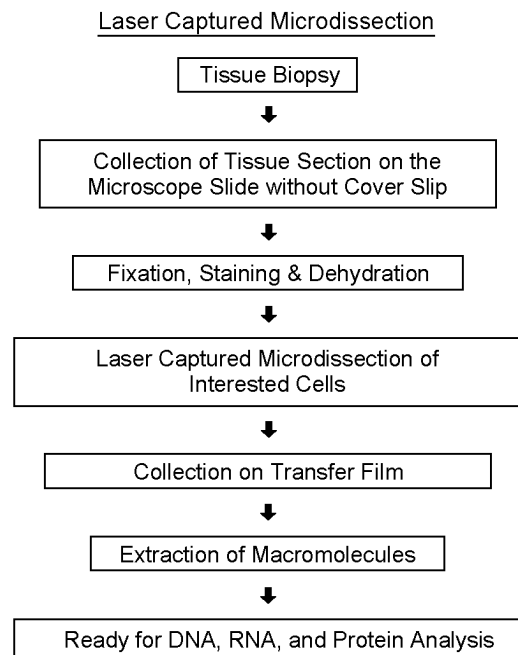
## 2. LCM

30 , 70% EtOH 30 DEPC-dH<sub>2</sub>O , Hematoxylin  
 DEPC-dH<sub>2</sub>O bluing  
 70% EtOH 90% EtOH  
 Eosin , 70% EtOH,  
 95% EtOH, 100% EtOH 30  
 , xylene 5 2  
 20 air dry  
 , xylene  
 ,  
 , Multiple stain  
 가 70% EtOH, 95% EtOH, 100% EtOH  
 30 , xylene 5  
 2 20 air dry

## 3. LCM

1) (Procure)  
 PixCell II™ system (Arcturus Engineering, Inc.,  
 Mountain View, USA)  
 가 400 μm antrum  
 (Figure 1). 5, 25,  
 50, 100, 200 5 laser beam  
 7.5~30 μm  
 diameter spot size  
 15 μm laser , power  
 20~40 mW  
 2) Total RNA extraction  
 가 transfer film (CapSure TF-  
 100; Arcturus Engineering, Inc., Mountain View, USA)  
 200 μl GITC digestion buffer 가 (Brin-  
 kmann Inc, Westbury, NY) cap  
 buffer 42  
 incubation 3~4 inversion  
 lysis 2, 5, 15, 30  
 . Incubation  
 spin down , RNA extraction

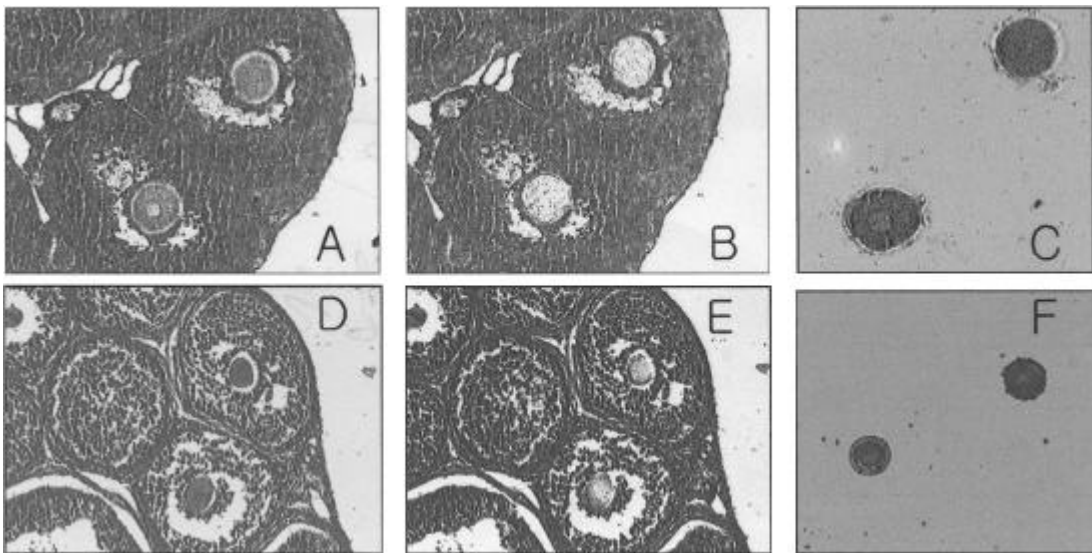
1.5 ml 2 M sodium acetate (pH 4.0) 20 μl  
 water saturated phenol 220 μl, chloroform-isoamyl alco-  
 hol (23:1) 60 μl 가 15  
 4 14000 rpm 30  
 tube  
 15 μg/μl Glycoblue (Ambion, Inc., Austin, Tx) 3 μl  
 isopropanol 200 μl 가 -70 1  
 4 14000 rpm 30  
 RNA  
 75% EtOH 4 5  
 14000 rpm washing . RNA  
 DEPC-dH<sub>2</sub>O  
 -70  
 3) (RT - PCR)  
 (reverse transcription) RNA  
 suspension 5 μl Superscript II RTase (Gi-



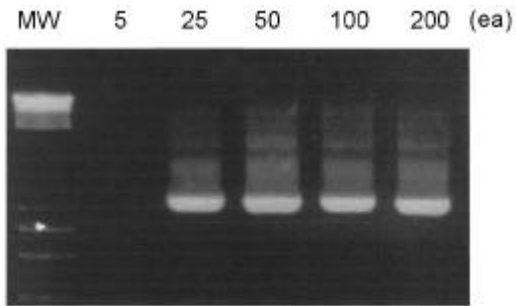
**Figure 1.** Schematic diagram describing procedures for LCM. After tissue fixation, staining, dehydration as described in the materials and methods, cells of interest are located and CapSure™, optically transparent device, is placed on the tissue. A laser pulse releases the cell from surrounding structures transferring it to the thermo-plastic film. The cells bound to the CapSure device is lifted and placed onto a standard microcentrifuge tube for subsequent extraction and analysis of macromolecules.

bcoBRL, Rockville, MD) 42 60  
 cDNA 20  
 Superscript II RTase 200 U, 10 mM dNTP,  
 oligo (dT) primer 0.5 µg, 25 mM MgCl<sub>2</sub>  
 cDNA (PCR)  
 GAPDH primer forward primer 5'-ACCAC-  
 AGTCCATGCCATCAC-3', reverse primer 5'-TCCA-  
 CCACCCTGTTGCTGTA-3'  
 PCR 446 bp . PCR 75 mM Tris -  
 HCl (pH 8.8), 20 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1.5 mM MgCl<sub>2</sub>,  
 0.01% Tween 20, 0.2 mM dATP, dCTP, dGTP, dTTP,  
 0.625 UNIT Taq DNA polymerase 7 PCR mas-  
 ter mixture (ABgene, Survey, UK) 25 µl  
 . 94 5 predenatura-  
 tion 94 40 denaturation, 60  
 40 annealing, 72 1 extension  
 postelongation 72 10  
 PCR product 1.5% aga-  
 rose gel ethidium bromide  
 Image Analyze (Vilber Lourmat, France)

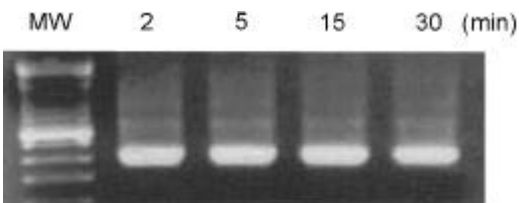
Figure 1 LCM RNA,  
 DNA,  
 flow chart  
 LCM  
 , Pixcell II™ LCM system  
 transfer film  
 transfer film  
 RNA housekeeping  
 Figure 2  
 (A, D) (B, E) antrum  
 type 7 type 8  
 . Figure 2C, 2F  
 transfer film  
 (D, E, F)  
 Figure 2E 가



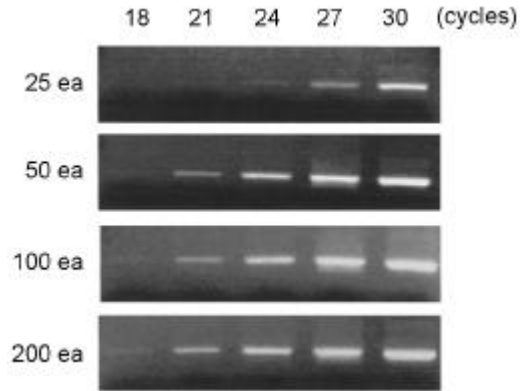
**Figure 2.** Microphotographs of sections of preovulatory ovarian tissues for LCM. Sections were fixed and stained. Panels show the section before (A, D) and after (B, E) removal of a oocytes by LCM. The archived image of the oocytes captured on the CapSure™ transfer film is shown in C and F. Panels A, B, C: frozen sections (×400), Panels D, E, F: paraffin-embedded sections (×200).



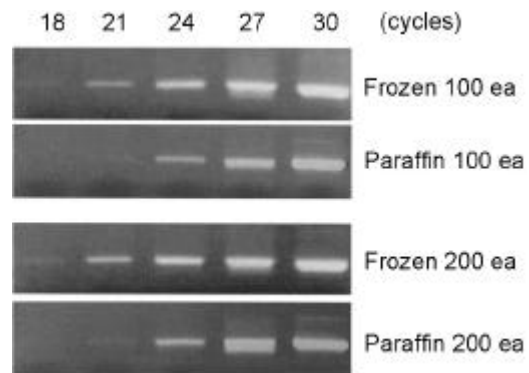
**Figure 3.** Expression of mouse GAPDH according to the number of oocytes captured by LCM. Lane 1: 1 kb plus ladder, Lane 2: 5 oocytes, Lane 3: 25 oocytes, Lane 4: 50 oocytes, Lane 5: 100 oocytes, and Lane 6: 200 oocytes.



**Figure 4.** Expression of mouse GAPDH according to the duration for the lysis buffer treatment. Total RNA derived from 50 laser captured microdissected oocytes was extracted for 2 min (lane 2), 5 min (lane 3), 15 min (lane 4), and 30 min (lane 5). Lane 1 depicts 1 kb plus ladder.



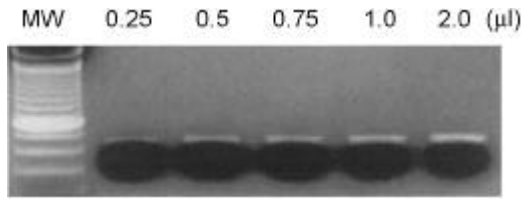
**Figure 5.** Expression of mouse GAPDH according to the number of cells procured and that of PCR cycles in the case of frozen sections. Total RNA derived from each number (25, 50, 100, 200) oocytes was amplified for cDNA synthesis, and the 20 ì l primary cDNA was used for PCR. PCR was performed to the number of 18, 21, 24, 27, and 30 PCR cycles, then analyzed on the electrophoresis gel.



**Figure 6.** Expression of mouse GAPDH according to the number of cells procured and that of PCR cycles in the case of paraffin-embedded sections. Total RNA derived from 100 and 200 oocytes were amplified for cDNA synthesis, and the 20 ì l primary cDNA was used for PCR. PCR was performed to the number of 18, 21, 24, 27, and 30 PCR cycles, and analyzed on the electrophoresis gel.

transfer rate가  
 Figure 3 5, 25, 50, 100, 200  
 GAPDH  
 5 GAPDH  
 25, 50, 100, 200  
 , 25  
 band  
 50 band  
 Figure 4 50 transfer film  
 42 lysis buffer  
 GAPDH  
 2 30 band  
 42  
 30

Figure 5 25, 50, 100, 200  
 PCR cycle GAPDH  
 25, 50,  
 100, 200, total RNA 20 ì l  
 cDNA 4 ì l PCR . Lane 1  
 5 18, 21, 24, 27, 30 cycles PCR



**Figure 7.** Expression of mouse GAPDH according to the amount of cDNA used for PCR. Total RNA derived from 50 oocytes (frozen) was amplified for cDNA synthesis and various amounts of cDNA was used for PCR. Lane 1: 1 kb plus ladder, Lane 2: 0.25  $\mu$ l, Lane 3: 0.5  $\mu$ l, Lane 4: 0.75  $\mu$ l, Lane 5: 1  $\mu$ l, Lane 6: 2  $\mu$ l cDNA.

25  
24 cycle  
27 cycle  
50  
18  
cycle  
21 cycle  
21 cycle  
PCR cycle  
sample  
transfer rate가  
cDNA PCR cycle 가  
100 200  
PCR  
band  
PCR 3 cycle 가  
cDNA  
50 , PCR cDNA  
GAPDH  
(Figure 7). cDNA 0.25  $\mu$ l, 0.5  $\mu$ l, 0.75  $\mu$ l, 1  $\mu$ l, 2  $\mu$ l  
PCR 21 cycle , cDNA 0.25  
 $\mu$ l PCR 21 cycle

cell line culture  
( ), xenograft ( ), flow cytometry ( )  
8

가  
가  
microdissec-  
LCM  
tion . laser  
가  
11,12  
cumulus cell ,  
cell, theca interna, theca externa granulosa  
가  
LCM  
Figure 2  
가  
mRNA  
LCM  
GAPDH  
50 0.25  $\mu$ l cDNA  
21 cycle PCR , GAPDH  
5 GAPDH  
, 25

, 50  
 transcripts  
 가  
 , GAPDH  
 cDNA  
 ,  
 (marker)  
 , internal control  
 cDNA  
 LCM RNA RNA  
 (transfer rate)  
 (fresh-frozen section)  
 ,  
 LCM cover slip  
 ,  
 RNA RNA  
 RT-PCR 가  
 , RNA en-  
 dogeneous nuclease  
 ,  
 DNA  
 100~500 ,<sup>13-15</sup> RNA  
 500~1000 <sup>1,16-18</sup>  
 10 , 20 DNA  
 가 (data not shown), RNA  
 50 , 100  
 가  
 RNA  
 ,  
 5000 가 <sup>6</sup>

NANO  
 ,  
 ,  
 ,  
 LCM  
 cDNA  
 RT-PCR  
 Microarray subtractive hybridization<sup>19</sup>  
 LCM  
 LCM  
 가

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