Applied Biosystems® Arcturus$^{XT™}$ Microdissection Systems: Optimized Protocol for Laser Microdissection of Living *In Vitro* Cells

Introduction

Laser capture microdissection (LCM) is a proven technique for the isolation of pure cell populations for downstream molecular analysis. The combined use of UV laser cutting with LCM using an infrared (IR) laser permits rapid and precise isolation of larger numbers of cells while maintaining cellular and nucleic acid integrity necessary for downstream analysis. In this application note, it is shown that these established techniques can also be used for the isolation of living cells, avoiding other more laborious methods of cell selection and enabling a wide range of research applications.

This application note describes a simple and effective protocol for the isolation of living adherent cells and the successful subsequent recultivation of homogeneous subpopulations. To demonstrate the success of this technique, three cell lines were used: TM3 (normal mouse testis), SKBR3 (human breast cancer), and 3T3 (embryonic mouse fibroblasts).

Materials

- Arcturus$^{XT™}$ or Veritas™ microdissection instrument (#ARCTURUSXT)
- PEN membrane frame slide for live-cell microdissection (#LCM0530 or LCM0531)
- CapSure® Macro LCM Caps (#LCM0211 or LCM0212)
- Trypsin-EDTA (0.25% trypsin, 1 mM EDTA, #25200-056)
- Hanks' Balanced Salt Solution (#14170-161)
- Petri dish, sterile, 100 mm diameter (Falcon Cat. #351005)
- Cover glass, 24 x 60 mm, No. 1 thickness (VWR Cat. #48393-106)
- RNase AWAY® (#10328-011)
- RNase*Zap*® (#AM9780)
- 100% ethanol (VWR Cat. #34172-00)
- CO_2 incubator Major Lab Supplier (MLS)
- Pipettor and sterile pipette tips (MLS)
- Kimwipes® Delicate Task Laboratory Wipers (Kimberly-Clark Kimtech)
- 2-well chamber slides (VWR Cat. #62407-325)
- NanoDrop® ND-1000 UV/VIS Spectrophotometer (NanoDrop Technologies)
- Agilent® 2100 Bioanalyzer (Agilent Technologies Cat. #G2940CA)
- SKBR3 cell culture medium
  - McCoy's Modified 5A Medium (#16600-082)
  - 10% fetal bovine serum (#16600-044)
  - 1 mM sodium pyruvate (#11360-070)
  - 10 U penicillin/10 µg streptomycin (#17-60ZE)
- TM3 cell culture medium
  - Dulbecco's Modified Eagle's Medium (ATCC Cat. #30-2002)/ Ham's F-12 Medium (#117650-054), 1:1
  - 5% horse serum (#16050-122)
  - 2.5% fetal bovine serum (#16600-044)
  - 10 U penicillin/10 µg streptomycin (BioWhittaker Cat. #17-60ZE)
- 3T3 cell culture medium
  - Dulbecco's Modified Eagle's Medium (ATCC Cat. #30-2002)
  - 10% bovine calf serum (ATCC Cat. #30-2030)

Methods

Specimen preparation

1. Thoroughly rinse the metal frame PEN membrane slide with 100% ethanol and air-dry prior to use. Keep slide in a sterile environment.
   Note: Ensure the slide is completely dry prior to use.
2. Trypsinize adherent cells from growth vessel (plate, flask) using standard protocol.
3. Deactivate trypsin with medium using standard protocol.
4. Resuspend 1–2 mL of trypsinized cells in 10 mL of fresh medium.
5. Place a metal frame membrane slide with chamber facing up into a sterile Petri dish. Transfer 1 mL of the cell suspension into the chamber of the frame membrane slide. If necessary, gently rock the slide in the Petri dish to completely cover the chamber area with medium.
6. Place lid on the Petri dish and incubate using appropriate culturing conditions for the cells until desired cell confluency is achieved.
   Note: Replace with fresh medium as needed.

Laser microdissection slide preparation

1. Thoroughly clean the instrument and work area, including pipettors, pipette tip box, etc., with 100% ethanol and RNase AWAY® or RNase*Zap*®.
2. Thoroughly rinse a cover glass with 100% ethanol and air-dry prior to use. Keep cover glass in a sterile environment.
   Note: Ensure the cover glass is completely dry prior to use.
3. When cells have reached the desired confluency, remove the medium from the chamber using a sterile pipette tip.
4. Add 950–1,000 µL of fresh medium to the chamber.
5. Carefully place a cover glass over the chamber side of the frame slide to create a mini-environment for the cell culture, enabling extended survival and reducing the possibility of the cells drying out (Figure 1).
   Note: Take care to reduce the amount of air bubbles formed when applying the cover glass.
6. Using a Kimwipe, carefully blot any excess medium that has seeped outside the cover glass.
7. Transport the slide in the Petri dish to the Veritas™ or Arcturus$^{XT™}$ system.
8. Remove the slide from the Petri dish and use a Kimwipe soaked in 100% ethanol to clean the flat side of the frame slide. Dry the slide completely.
   Note: Make sure not to rupture the membrane.
9. Insert the frame slide with the chamber and cover glass facing down (flat side up) onto the Veritas™ or Arcturus$^{XT™}$ instrument and proceed to laser microdissection (Figure 2).

Laser microdissection protocol

The following settings were used for protocol validation and should be used as a guideline for the microdissection of live cells. Optimization of settings may be required, depending on the individual cell preparation.

Important:

- Use CapSure® Macro LCM Caps.
- Perform cut and capture at 10x or 20x.
- Always capture first, then cut.
- Turn the visualizer off (Veritas™ system) and take the diffuser out (Arcturus$^{XT™}$ system).

Cutting (UV) laser settings:

- UV laser power: Veritas system: 20–25 Arcturus$^{XT™}$ system (all ND filters out)
- UV spacing: Veritas system = 5000 µm Arcturus$^{XT™}$ system = 5000 µm
- Tab size/length: Veritas system = 1 Arcturus$^{XT™}$ system = 0
- Automatic LCM spots: Veritas system = 0 Arcturus$^{XT™}$ system = 0
- UV cut speed: Veritas system = N/A Arcturus$^{XT™}$ system = 525

Capture (IR) laser settings:

- IR laser power: Veritas system = 80 Arcturus$^{XT™}$ system = 65 mW
- Pulse/Duration: Veritas = system 4000 ms Arcturus$^{XT™}$ system = 22 ms
- LCM spot overlap: Veritas = system 40% Arcturus$^{XT™}$ system = 60%

1. Locate cells of interest to capture.
2. Use the Cut Line feature to draw around cells.
3. Use the Single Point Capture feature to apply LCM spots that will fuse LCM membrane to PEN membrane.
   Note: Apply an adequate number of LCM spots for the given region. Try to avoid placing spots directly onto areas containing cells.
4. Place the CapSure® Macro LCM Cap onto the area of the slide containing cells of interest.
5. Locate LCM laser and fire a test LCM shot. If necessary, adjust laser settings.
   Note: Confirm that LCM film has made contact with PEN film. LCM spot will be dark.
6. Locate UV cutting laser.
7. Activate LCM laser first and then UV cutting laser.
8. Move Macro LCM Cap to QC station.
9. Confirm presence of cells on LCM Cap (Figure 3).
10. Move cap to offload station.

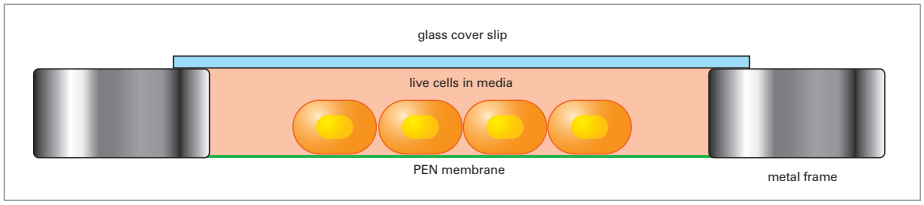

Figure 1. Laser microdissection cell culture chamber. Cells cultured in "well" of a metal frame membrane slide. The live-cell culture is covered by a large cover glass prior to microdissection.

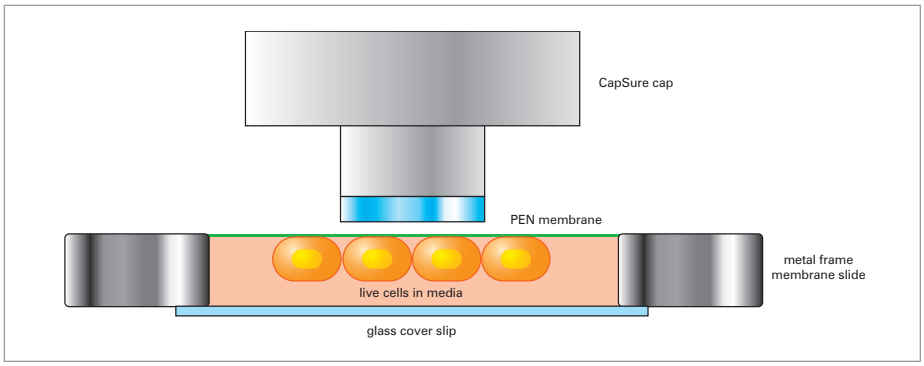

Figure 2. Inverted laser microdissection cell culture chamber with CapSure® LCM Cap. The CapSure® Cap sits atop the inverted chamber in contact only with the PEN membrane during microdissection.

Reculturing captured live cells

1. Remove Macro LCM Cap from the offload station and invert. Place cap with isolated cells facing up into a clean Petri dish.
2. Pipette 50 µL of Hanks' solution onto the Macro LCM cap film surface. Pipette up and down 2–3 times, and dispose of the solution.
3. Pipette 50 µL of trypsin-EDTA directly onto the captured cells on the cap and incubate for 5 minutes at room temperature. Cover with the Petri dish lid during this incubation.
4. After incubation, pipette the trypsin-EDTA up and down several times to ensure a single-cell suspension, then transfer the cell suspension into a well of a sterile chamber slide (or alternate desired growth vessel) containing 1–2 mL of appropriate cell medium.
5. Place the chamber slide in the incubator under appropriate conditions. Wait 2–3 days for detection of cell growth. Monitor cell growth using standard culture technique, changing medium as needed.
6. Recultured cells may be used as desired for further experiments.

Summary

Using two different cell lines, TM3 and SKBR3, crop circles were captured using the above techniques, each onto a separate CapSure® LCM Cap. Captured areas were designated as "large" (2–3 mm diameter) or "small" (500–700 µm diameter) and contained varied numbers of cells. Subsequent to laser microdissection, some caps containing live cells were recultured following the above steps for protocol validation.

Alternately, microdissected cells were lysed and RNA was extracted to evaluate if there was any negative effect on the nucleic acid as a result of the microdissection protocol. These caps, containing captured live cells, were removed from the microdissection instrument and were immediately placed into tubes containing PicoPure® RNA extraction buffer. Total RNA was isolated following the standard PicoPure protocol and eluted in 30 µL of elution buffer.

Total RNA yield for each sample was assessed using the NanoDrop® ND-1000 (Table 1). The total RNA yields varied between replicates, as the cell counts within the captured areas were not monitored. However, the larger crop circles did result in higher yields than the smaller areas, as expected, and the RNA yield for each captured area, regardless of size, yielded sufficient RNA for downstream molecular analysis. The amount of RNA obtained from live cells subsequent to laser microdissection also indicates that the isolation protocol did not have a negative effect on the nucleic acids.

RNA profiles were obtained using the Agilent 2100 Bioanalyzer (Figure 4). Clear 18S and 28S peaks are seen in each profile, further confirming that the microdissection process preserves RNA integrity.
Note: 1.5 µL of each total RNA sample was measured using the NanoDrop® ND-1000 UV/Vis spectrophotometer.

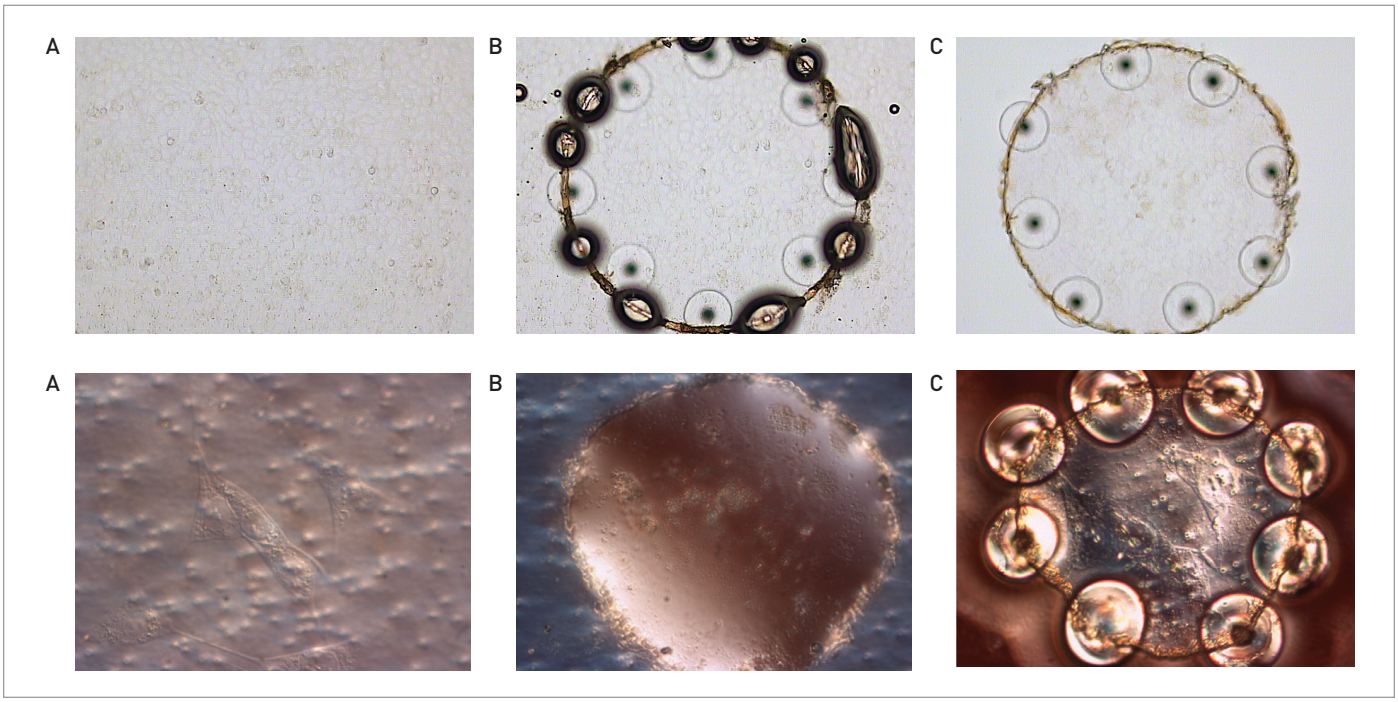

Figure 3. Microdissection of living *in vitro* cells. (A) Live cells in medium before laser microdissection. **(B)** Cell culture chamber after microdissection. **(C)** Microdissected live cells on the CapSure® Macro LCM Cap. **Top row:** Veritas™ microdissection instrument, cultured SKBR3 cells. **Bottom row:** Arcturus^{XT}™ microdissection instrument, cultured 3T3 cells under DIC, all cells microdissected using IR-LCM and UV laser cutting.

Sample ID	Cell Type	RNA Conc. (ng/µL)	Total RNA Yield (ng)	Average Yield (ng)
Large Crop 1	SKBR3	38.73	1161.90	964.65
Large Crop 2	SKBR3	25.58	767.40	
Small Crop 1	SKBR3	4.35	130.50	131.64
Small Crop 2	SKBR3	6.73	201.90	
Small Crop 3	SKBR3	3.77	113.10	
Small Crop 4	SKBR3	4.44	133.20	
Small Crop 5	SKBR3	2.65	79.50	
Small Crop 1	TM3	4.34	130.20	184.74
Small Crop 2	TM3	1.13	33.90	
Small Crop 3	TM3	7.78	233.40	
Small Crop 4	TM3	7.14	214.20	
Small Crop 5	TM3	10.40	312.00	

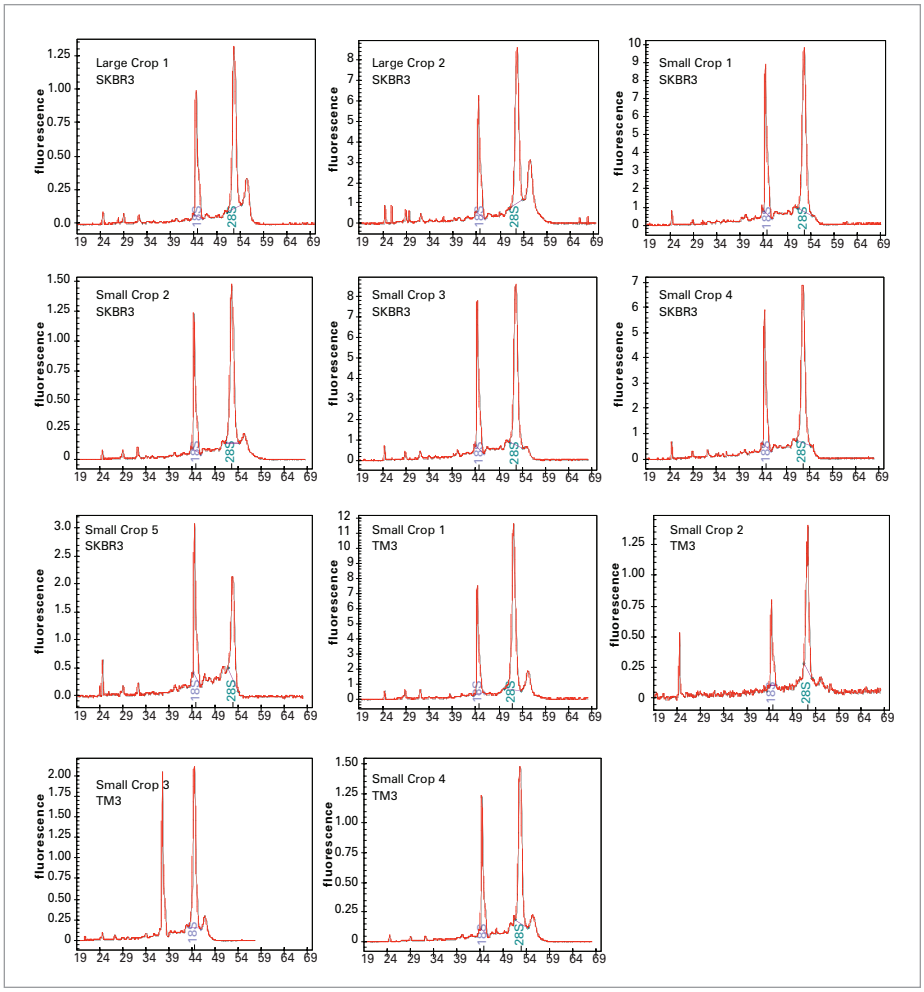

Figure 4. Bioanalyzer profiles of total RNA isolated from microdissected live cells. 1 µL of each sample was loaded onto the bioanalyzer and run per the manufacturer's protocol. **Note:** TM3 Small Crop #5 is not present due to sample loading error.

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Headquarters
850 Lincoln Centre Drive | Foster City, CA 94404 USA
Phone 650.638.5800 | Toll Free 800.327.3002
www.appliedbiosystems.com

International Sales
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