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Laser Capture Microdissection for Analysis of Macrophage Gene Expression from Atherosclerotic Lesions

Jonathan E. Feig and Edward A. Fisher

Abstract

Coronary artery disease, resulting from atherosclerosis, is the leading cause of death in the Western world. Most previous studies have subjected atherosclerotic arteries, a tissue of mixed cellular composition, to homogenization in order to identify the factors in plaque development, thereby obscuring information relevant to specific cell types. Because macrophage foam cells are critical mediators in atherosclerotic plaque advancement, we reasoned that performing gene analysis on those cells would provide specific insight in novel regulatory factors and potential therapeutic targets. We demonstrated for the first time in vascular biology that foam cell-specific RNA can be isolated by laser capture microdissection (LCM) of plaques. As expected, compared to whole tissue, a significant enrichment in foam cell-specific RNA transcripts was observed. Furthermore, because regression of atherosclerosis is a tantalizing clinical goal, we developed and reported a transplantation-based mouse model. This involved allowing plaques to form in apoE^{−/−} mice and then changing the plaque's plasma environment from hyperlipidemia to normolipidemia. Under those conditions, rapid regression ensued in a process involving emigration of plaque foam cells to regional and systemic lymph nodes. Using LCM, we were able to show that under regression conditions, there was decreased expression in foam cells of inflammatory genes, but an up-regulation of cholesterol efflux genes. Interestingly, we also found that increased expression of chemokine receptor CCR7, a known factor in dendritic cell migration, was required for regression. In conclusion, the LCM methods described in this chapter, which have already lead to a number of striking findings, will likely further facilitate the study of cell type-specific gene expression in animal and human plaques during various stages of atherosclerosis, and after genetic, pharmacologic, and environmental perturbations.

Keywords

apoE; Atherosclerosis; Gene expression; LCM; CCR7

1 Introduction

Macrophage foam cells are critical in the development of atherosclerosis [1, 2]. Therefore, a better understanding of the gene expression changes in foam cells during disease progression and regression has become an important goal in order to develop potential therapies and interventions [3–5]. The study of macrophage foam cell gene expression in arterial lesions, however, is hampered by the cellular heterogeneity of arterial tissue, which

besides macrophages, also contain lymphocytes, smooth muscle cells, endothelial cells, and fibroblasts. To overcome these technical obstacles, we describe here a method for the use of laser capture microdissection (LCM) to selectively procure macrophage foam cells from arterial lesions (as identified by the macrophage-specific marker CD68/Macrosialin) [6–9]. RNA extracted from the microdissected foam cells exhibited a 30-fold enrichment in CD68 mRNA levels as compared to homogenization of the whole artery [10], suggesting that LCM provides a method to identify gene expression changes specific to a particular cell type and that may be regulated by disease stage (e.g., progression vs. regression) or by different genetic and pharmacologic manipulations.

We previously developed a transplantation-based mouse model of atherosclerosis regression by allowing plaques to form in apoE^{−/−} mice. Then, by grafting their aortic segments into a wild-type recipient, the plaque's plasma environment is changed from hyperlipidemia to normolipidemia [11, 12]. As a control, transplants are performed into apoE^{−/−} recipients. At various time points after transplantation, LCM was used to isolate the foam cells from the grafts and RNA was extracted. Using real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR), we were able to show that gene expression levels of inflammation markers such as MCP-1 and VCAM-1 were significantly down-regulated, and cholesterol efflux genes such as LXRA, ABCA1, and SR-BI levels were increased. Notably, CCR7 (a migratory factor for a number of leukocyte types, including dendritic cells) was up-regulated (Fig. 1) [13]. This was a crucial finding, as this gene turned out to be functionally required for the depletion of foam cells observed during regression [13]. In short, LCM makes possible the quantitative analysis of gene expression in macrophage foam cells and adds a powerful dimension to the study of atherosclerosis. It is important to note that while not described here, a similar approach can be taken for the molecular analysis of vascular smooth muscle cells, except that the identifying marker would be smooth muscle- α actin instead of CD68.

2 Materials

2.1 Animals and Tissue Processing

1. apoE^{−/−} mice (The Jackson Laboratory, Bar Harbor, ME).
2. Ketamine/Xylazine anesthesia working solution (Sigma-Aldrich, St. Louis, MO).
3. 1× Phosphate-buffered saline (PBS, store at room temperature).
4. 1× PBS containing 10 % Sucrose (w/v) and an RNase inhibitor (store at room temperature). The RNase inhibitor should be added fresh before use.
5. Protect RNA RNase Inhibitor 500× concentrate (Sigma-Aldrich).
6. OCT cryoembedding medium (VWR, San Diego, CA).
7. Superfrost Plus slides 75 × 25 mm (Fisher Scientific, Pittsburgh, PA).
8. RNase Away reagent (Molecular Bioproducts, San Diego, CA).
9. Diethyl pyrocarbonate (DEPC, Sigma-Aldrich): working solution contains 0.1 % v/v DEPC in distilled water (*see* Note 1).

2.2 Histological Detection of Macrophage Foam Cells for LCM

1. Acetone (Fisher Scientific).
2. Normal rabbit serum (Vector Laboratories, Burlingame, CA).
3. Rat anti-mouse CD68 (Serotec, Kidlington, UK).
4. Biotinylated rabbit anti-rat IgG mouse-adsorbed secondary antibody (Vector Laboratories).
5. Vectastain ABC alkaline phosphatase (AP) enzymatic detection antibody (Vector Laboratories).
6. Tris Buffer—100 mM Tris-HCl, pH 8–8.4.
 - a. Combine:
 - 6.3 g Tris-HCl
 - 7.4 g Tris base
 - 900 ml distilled H₂O.
 - b. Stir until dissolved.
 - c. Adjust pH to 8–8.4.
 - d. Add distilled H₂O to a final volume to 1.0l.
7. Vector Red substrate (Vector Laboratories).
8. Mayer's hematoxylin (Sigma-Aldrich, *see* Note 2).
9. Ammonium Hydroxide, Reagent ACS (Fisher Scientific, *see* Note 3).
10. Eosin Y (Harleco, Gibbstown, NJ).
11. 100 % Ethanol, Anhydrous, ACS/USP grade.
12. Xylenes (Fisher Scientific).
13. Permount Mounting Medium (Fisher Scientific).
14. Slide rack.
15. Staining dish.

2.3 Laser Capture Microdissection and RNA Isolation

1. Arcturus PixCell II LCM System (Arcturus, Mountain View, CA).
2. CapSure Macro LCM caps (Molecular Devices, Sunnyvale, CA).

¹To prepare DEPC water, combine 0.1 % (v/v) DEPC with distilled water in an autoclave-safe container. Shake vigorously and leave for 24 h at room temperature. Autoclave and leave it for another 24 h at room temperature. DEPC-treated water is now ready to use. DEPC treatment inactivates RNases.

²Mayer's hematoxylin must be filtered before use. Solution is toxic if swallowed and is also a known irritant to eyes, skin, and the respiratory system.

³Ammonium hydroxide causes eye and skin burns. It also causes digestive and respiratory tract burns.

3. PicoPure RNA isolation kit (Molecular Devices).
4. RNase-free DNase set (Qiagen, Valencia, CA).

2.4 Real-Time Quantitative RT-PCR

1. 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA).
2. MicroAmp Fast Optical 96-Well Reaction Plate (Applied Biosystems).
3. The MicroAmp Optical Adhesive Film (Applied Biosystems).
4. Gene-specific Taqman primers and probes (Table 1).
5. iScript One-Step RT-PCR Kit for Probes (Bio-Rad, Hercules, CA).

3 Methods

3.1 Animals and Tissue Processing

All reagents were maintained under RNase-free, sterile conditions. Transplantation was performed as previously described [12]. In brief, apoE^{-/-} mice, a standard model of human atherosclerosis [14], were fed a high-fat diet for 16 weeks. Mice were then sacrificed via exsanguination (under general anesthesia with ketamine/xylazine) by intravascular perfusion with PBS. The thorax was opened and a 21-gauge cannula inserted into the left ventricle. The right atrium was incised to allow efflux of blood. Perfusion was at physiological pressure (100 mmHg) with PBS. The aortic arch (graft) was removed and transplanted into the descending aorta of a recipient mouse in the region just above the iliac bifurcation [12]. After 3 days, the recipient mouse was sacrificed, perfused with a PBS/sucrose/RNA inhibitor solution and the graft was harvested. The tissue (which was cut in half cross-sectionally) was positioned in a semi-filled (with OCT) embedding mold to ultimately generate cross sections using a cryostat. The embedding mold was then fully filled with OCT and frozen on dry ice. The specimens were stored at -80 °C until sectioning, immunostaining, and LCM were performed. Eventually, the tissue was cryo-sectioned at a thickness of 6 µm, collected on Superfrost Plus slides, and maintained cold at all times after collection. All procedures were approved by the Institutional Animal Care and Use Committee of NYU School of Medicine.

3.2 CD68 Immunodetection of Macrophages

Using immunohistochemistry, we stained every fifth slide to identify the macrophage-specific marker CD68/Macrosialin. This served as a guide slide for the remaining non-immunostained slides. The following protocol assumes the staining of 15–20 slides.

1. Use 250 ml ice-cold acetone to fix cells on slides in slide rack (10 min).
2. Rinse in 250 ml, 1× PBS for 2 min (2×).
3. Block in 4 % normal rabbit serum diluted in 1× PBS for 10 min.
4. Primary antibody: Dilute the rat anti-mouse CD68 antibody 1:250 in the 4 % rabbit serum. Incubate slides in primary anti-body for 1 h.

5. 45 min into the primary antibody incubation, prepare the VECTASTAIN ABC-AP by adding two drops of reagent A and two drops of reagent B into 5 ml, 1× PBS, making sure to vortex well after each drop. Allow this solution to incubate at room temperature until ready for use.
6. Rinse slides in 1× PBS (3 dips).
7. Secondary antibody: Dilute the biotinylated rabbit anti-rat antibody 1:200 in the 4 % rabbit serum. Incubate slides in secondary antibody for 10 min.
8. Rinse slides in 250 ml 1× PBS (3 dips).
9. Incubate slides for 5 min with the VECTASTAIN ABC-AP solution.
10. During the 5 min incubation, prepare the Vector Red AP substrate. To 5 ml Tris buffer add two drops of each reagent, making sure to vortex well after adding each reagent.
11. Rinse in 250 ml 1× PBS (3 dips).
12. Pipette enough Vector Red solution to cover each tissue section on the slide. Develop in the dark until desired stain intensity develops. Monitor intensity under the microscope.
13. Place slides in 250 ml distilled water to stop staining reaction.
14. Counterstain with Mayer's hematoxylin for 1 min.
15. Place slides in 250 ml distilled water for 30 s.
16. Place slides in a bluing solution for 1 min. Bluing solution is prepared by adding 500 µl of ammonium hydroxide to 300 ml of distilled water.
17. Place slides in 250 ml 70 % ethanol for 30 s.
18. Place slides in 250 ml 80 % ethanol for 30 s.
19. Place slides in 250 ml 95 % ethanol for 1 min.
20. Place slides in 250 ml 100 % ethanol for 1 min.
21. Place slides in 250 ml xylene for 3 min.
22. Allow to air dry in hood until xylenes have evaporated (5 min or less depending on air flow and number of slides).
23. Once dry, mount with a resin mounting medium and coverslip the slides.

3.3 Hematoxylin and Eosin Y (H&E) Stain

These instructions follow a traditional H&E stain, with several modifications to significantly reduce the effects of RNases on the integrity and yield of RNA (*see* Note 4). H&E staining

⁴Eosin Y may be fatal or cause blindness if swallowed. It may cause damage to liver, kidneys, GI tract, and cardiovascular system. Wear eye protection and gloves. In addition, it is important to prepare all alcohol and xylene solutions fresh for the dehydration steps in order to achieve efficient capture during the LCM procedure.

is conducted on the remaining slides that are not immunostained and are to be used for laser capture microdissection. The following protocol assumes the staining of 15–20 slides.

1. Take slides out of the -80°C freezer and leave at room temperature for 2 min.
2. Place in staining tray containing 250 ml 70 % ethanol for 30 s.
3. Place in 250 ml RNase inhibitor-treated DEPC-treated water (DEPC water with Protect RNA RNase Inhibitor diluted to $1\times$ final concentration) for 1 min.
4. Place in 250 ml RNase inhibitor-treated Mayer's hematoxylin (Mayer's hematoxylin with Protect RNA RNase Inhibitor diluted to $1\times$ final concentration) for 1 min.
5. Place in 250 ml DEPC-treated water for 15 s.
6. Place in 250 ml PBS for 15 s.
7. Place in 250 ml RNase inhibitor-treated DEPC water for 15 s.
8. Place in 250 ml 70 % ethanol for 30 s.
9. Place in 250 ml 95 % ethanol for 30 s.
10. Dip in 250 ml eosin Y twice.
11. Place in 250 ml, 95 % EtOH for 15 s.
12. Place in separate 250 ml, 95 % EtOH for 30 s.
13. Place in 250 ml 100 % ethanol for 30 s.
14. Place in 250 ml xylenes for 30 s.
15. Place in separate 250 ml xylenes for 3 min.
16. Allow to air dry in hood until xylenes have evaporated (5 min or less depending on air flow and number of slides).
17. Proceed directly to LCM.

3.4 Laser Capture Microdissection

The following instructions assume the use of an Arcturus, PixCell II LCM System but common to all instruments, there is direct visualization of the sections with subsequent capture of the desired cells. Identification of foam cells from the atherosclerotic plaque was guided by the staining of CD68 (Fig. 2). These slides were used as “guide slides” for the preceding and subsequent slides, which were H&E stained. In the PixCell II System, the capture of cells is achieved by placing a specially made cap, lined with a thermolabile film, onto a thinly cut section of tissue, with “pickup” completed by activating the film with a near-infrared laser diode pulse. This melting of the thermoplastic film causes the cells of interest to adhere to the film, which are then isolated from the surrounding tissue when the cap is lifted away from the slide. The isolation of quality RNA was completed using the Arcturus PicoPure RNA isolation kit.

1. Set the PixCell II LCM System to the following parameters: 7.5 μ m laser spot size, 40 mW power, 3.0 ms duration, 100 mV target, 0.2 ms delay between pulses.
2. Place a CD68 positively stained slide onto tile microscope stage and locate the stained foam cells. Pressing the “Map” button, take several pictures at 4 $\times$, 10 $\times$, and 20 $\times$ magnifications to use as guides to help locate foam cells on the H&E slides that follow.
3. Replace the CD68 slide with an H&E stained slide and activate the vacuum to prevent the slide from shifting during capture.
4. Using the rotating cap arm, collect a cap from the loading area and carefully lay the cap over the tissue section.
5. Enable the laser and focus if necessary (Fig. 3).
6. Before collecting the macrophage foam cells, attempt a few pulses in a tissue-free area to ensure proper wetting of the cap. Adjust if necessary (Fig. 4, *see* Note 5).
7. When ready, bring the tissue into the microscope’s field of view and begin collecting foam cells. Continue collecting until the desired number of cells are obtained or until it is no longer possible to capture cells (*see* Note 6).
8. Once finished, lift and rotate the cap arm to bring the cap to the unloading area.
9. Into a 0.5 ml microfuge tube, included with the PicoPure kit, pipette 50 μ l of extraction buffer.
10. Place the cap into the tube and invert so that the buffer floods the captured tissue.
11. Incubate at 42 $^{\circ}$ C for 30 min.
12. Continue RNA isolation using manufacturer’s protocol, making sure to include the optional DNase treatment (*see* Note 7).

3.5 Analysis of Macrophage Foam Cell Gene Expression by Real-Time Quantitative RT-PCR

A very sensitive measurement method of low-abundance transcripts is qRT-PCR, and unlike Northern or RNase protection assays, it requires only a very small amount of total RNA (typically 100 pg to 1 ng). We have previously used qRT-PGR to demonstrate the selective enrichment from mouse plaques of lesional macrophage RNA by LCM [10]. The following instructions assume the use of a 7300 Real-Time PCR System from Applied Biosystems along with the iScript One-Step RT-PCR Kit for Probes from Bio-Rad (*see* Note 8). The

⁵Wetting of the cap refers to the melting of the thermolabile film. To obtain proper or optimal wetting, adjust the power and/or duration. Size and intensity of the pulse will vary depending on the parameters; however, keep in mind that high-power pulses will “burn” the film.

⁶When calculating the number of pulses needed for your application, remember that the typical yield of total RNA from a cell is approximately 10 pg. It has been observed that approximately 10,000 pulses of macrophage foam cells, using the parameters 40 mW, 10 ms, 7.5 μ l, and 100 mV (power, duration, size, and intensity), were enough to produce approximately 80 ng of total RNA. As the film becomes saturated with cells, it will become increasingly difficult to collect more, at which point it may be a sufficient amount of material to proceed to gene expression analyses.

⁷It is important to eliminate genomic DNA with the RNase-free DNase set, especially when your downstream applications include RT-PCR, PCR, Microarray, qPCR, or bioanalysis. Presence of genomic DNA may affect results.

protocol for the iScript kit has been slightly modified to reduce the amount of RNA sample needed for analysis. Keep reagents and samples on ice at all times.

1. Obtain an aliquot of each RNA sample and dilute in RNase-free water to a concentration of 20 pg/μl. Prepare enough sample to be used in 5 μl triplicates. Total RNA per reaction will be 100 pg.
2. Prepare a standard curve using an RNA sample (liver for all genes except CCR7, in which case we use spleen) of good quality and known concentration by making serial dilutions ranging from 20 to 0.0002 pg/μl.
3. Prepare the master mix for each gene of interest using the following setup, taking into account the necessary amount of wells per gene. Final total volume per reaction will be 25 μl. Always prepare an extra volume of master mix to account for any possible error in pipetting.
 - a. 2× RT-PCR Reaction Mix for Probes, 1× final concentration.
 - b. Forward primer, 300 nM final concentration.
 - c. Reverse primer, 300 nM final concentration.
 - d. Probe, 100 nM final concentration.
 - e. ROX reference dye, 1× final concentration.
 - f. iScript Reverse Transcriptase for One-Step RT-PCR, 0.5 μl per reaction.
 - g. Nuclease-free H₂O, to 25 μl final volume.
4. Into the 96-well plate, arrange and pipette 20 μl of the relevant master mix into its designated well.
5. Pipette 5 μl of the RNA sample into its corresponding well containing master mix. Mix well by pipetting.
6. When finished loading the wells, cover plate with the optical adhesive film and, place into the 7300 Real-Time PCR System.
7. Input the following settings:
 - a. cDNA synthesis: 10 min at 50 °C.
 - b. RT activation: 5 min at 95 °C.
 - c. PCR cycling and detection (40 cycles): 15 s at 95 °C, 30 s at 55 °C (data collection); for some mRNAs of interest, the annealing temperature setting may need adjustment, as for any PCR assay.

⁸The probe, labeled at the 5' and 3' ends with 6-carboxyfluorescein (6-FAM) reporter and tetramethyl-6-carboxyrhodamine (TAMRA) quencher, respectively, is hydrolyzed by the 5' exonuclease activity of Taq DNA polymerase, causing an increase in fluorescent signal that is measured in "real-time" after each cycle of PCR amplification. Standard curves were constructed by plotting log₁₀ RNA starting quantity vs. cycle threshold. On the basis of appropriate serially diluted standard RNA, the amount of input standard RNA yielding the same amount of PCR product measured from an unknown sample was calculated.

8. Based on the generated standard curve, values for relative amounts of the gene of interested are calculated by the software provided with tile 7300 Real-Time PCR System.

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References

1. Glass CK, Witztum JL. Atherosclerosis: the road ahead. *Cell*. 2001; 104:503–516. [PubMed: 11239408]
2. Smith JD, Trogan E, Ginsberg M, Gfigaux C, Tian J, Miyata M. Decreased atherosclerosis in mice deficient in both macrophage colony-stimulating factor (op) and apolipoprotein E. *Proc Natl Acad Sci USA*. 1995; 92:8264–8268. [PubMed: 7667279]
3. Chong PH, Bachenheimer BS. Current, new and future treatments in dyslipidaemia and atherosclerosis. *Drugs*. 2000; 60:55–93. [PubMed: 10929930]
4. Brewer HB Jr. The lipid-laden foam cell: an elusive target for therapeutic intervention. *J Clin Invest*. 2000; 105:703–705. [PubMed: 10727436]
5. Plutzky J. Atherosclerotic plaque rupture: emerging insights and opportunities. *Am J Cardiol*. 1999; 84:15J–20J.
6. Emmert-Buck MR, Bonner RF, Smith PD, Chuaqui RF, Zhuang Z, Goldstein SR, et al. Laser capture microdissection. *Science*. 1996; 274:998–1001. [PubMed: 8875945]
7. Bonner RF, Emmert-Buck M, Cole K, Pohida T, Chuaqui R, Goldstein S, Liotta LA. Laser capture microdissection: molecular analysis of tissue. *Science*. 1997; 278:1481–1483. [PubMed: 9411767]
8. Ramprasad MP, Fischer W, Witztum JL, Sambrano GR, Quehenberger O, Steinberg D. The 94- to 97-kDa mouse macrophage membrane protein that recognizes oxidized low density lipoprotein and phosphatidylserine-rich liposomes is identical to macrosialin, the mouse homologue of human CD68. *Proc Natl Acad Sci USA*. 1995; 92:9580–9584. [PubMed: 7568176]
9. Ramprasad MP, Terpstra V, Kondratenko N, Quehenberger O, Steinberg D. Cell surface expression of mouse macrosialin and human CD68 and their role as macrophage receptors for oxidized low density lipoprotein. *Proc Natl Acad Sci USA*. 1996; 93:14833–14888. [PubMed: 8962141]
10. Trogan E, Choudhury RP, Dansky HM, Rong JX, Breslow JL, Fisher EA. Laser capture microdissection analysis of gene expression in macrophages from atherosclerotic lesions of apolipoprotein E-deficient mice. *Proc Natl Acad Sci USA*. 2002; 99:2234–2239. [PubMed: 11842210]
11. Reis ED, Li J, Fayad ZA, Rong JX, Hansoty D, Aguinaldo JG, Fallon JT, Fisher EA. Dramatic remodeling of advanced atherosclerotic plaques of the apolipoprotein E-deficient mouse in a novel transplantation model. *J Vase Surg*. 2001; 34:541–547.
12. Cheresnev I, Trogan E, Omerhodzic S, Itskovich V, Aguinaldo JG, Fayad ZA, Fisher EA, Reis ED. Mouse model of heterotopic aortic arch transplantation. *J Surg Res*. 2003; 111:171–176. [PubMed: 12850459]
13. Trogan E, Feig JE, Dogan S, Rothblat GH, Angeli V, Tacke F, Randolph GJ, Fisher EA. Gene expression changes in foam cells and the role of chemokine receptor CCR7 during atherosclerosis regression in ApoE-deficient mice. *Proc Natl Acad Sci USA*. 2006; 103:3781–3786. [PubMed: 16537455]
14. Breslow JL. Mouse models of atherosclerosis. *Science*. 1996; 272:685–688. [PubMed: 8614828]

Fig. 1.
Changes in the expression of genes associated with cholesterol efflux, inflammation, and migration in foam cells after the reversal of dyslipidemia

Fig. 2. CD68 (*top panel*) and H&E staining (*lower panel: before LCM, bottom left; after LCM, bottom right*) of representative cross sections of an aortic arch containing atherosclerotic plaque. The *bottom right panel* shows microdissected H&E-stained tissue with the insert displaying the microdissected macrophage foam cells

Fig. 3.

Laser focusing. A focused laser will have an intensely *bright red* appearance with defined edges (*middle*). An unfocused laser will appear hazy (*left*) or as a ring containing a center line (*right*)

Fig. 4.

Pulse wetting. *Upper row, left to right:* Result of changing power from 20 to 40, 60, 80, 100 mW. Duration, size, and Intensity were kept constant at 10 ms, 7.5 μm , and 100 mV, respectively. At 100 mW, the thermoplastic film burned. *Bottom row, left to right:* Result of changing duration from 3.0 to 5.0, 10, 20, 30 ms. Power, size, and intensity were kept constant at 20 mW, 7.5 μm , and 100 mV, respectively. Note that the size of the pulse area does not become larger after 20 ms. Bar = 100 μm

Table 1

Oligonucleotide primer sequences for quantitative RT-PCR

<i>Peroxisomal proliferator activated receptor γ (PPARγ)</i>	
Fwd	5'-CATTCTGGCCACCAACTTC-3'
Rev	5'-AAGGAATGCGAGTGGTCT-3'
Probe	5'-FAM-TCAGCTCTGTGGACCTCTCCGTGATG - BHQ1-3'
<i>Liver-X-receptor α (LXRα)</i>	
Fwd	5'-GGAGGCAACACTTGCATCCT-3'
Rev	5'-AGGGCTGTAGGCTCTGCTGA-3'
Probe	5'-FAM-AGGGAGGAAGCCAGGATGCCCC-TAMRA-3'
<i>ATP-binding cassette transporter A1 (ABCA1)</i>	
Fwd	5'-ATTGCCAGACGGAGCCG-3'
Rev	5'-TGCCAAAGGGTGGACA-3'
Probe	5'-FAM-CCAGCTGTCTTTGTTGCATTGCCC-TAMRA-3'
<i>Scavenger receptor BI (SR-BI)</i>	
Fwd	5'-GATGATGACCTTGGCGCTG-3'
Rev	5'-TCACCAACTGTGCGGTTCATA-3'
Probe	5'-FAM-TCACCATGGGCCAGCGTGCTT-TAMRA-3'
<i>CCR7</i>	
Fwd	5'-CACGCTGAGATGCTCACTGG-3'
Rev	5'-ATCTGGGCCACTTGGATGG-3'
Probe	5'-FAM- CAGTGCCCAAGTGGAGGCCTTGATC-TAMRA-3'
<i>MCP-1</i>	
Fwd	5'-TTCCTCCACCACCATGCAG-3'
Rev	5'-CCAGCCGGCAACTGTGA-3'
Probe	5'-FAM-CCCTGTCATGCTTCTGGGCCTGC-TAMRA-3'
<i>VCAM-1</i>	
Fwd	5'-CCCCAAGGATCCAGAGATTCA-3'
Rev	5'-ACTTGACCGTGACCGGCTT-3'
Probe	5'-FAM-TTCAGTGGCCCCCTGGAGGTTG-TAMRA-3'

All primers were provided by Biosearch Technologies (Novato, CA)