

Cite as: Cold Spring Harb. Protoc.; 2008; doi:10.1101/pdb.prot5065



Protocol

Isolation of RNA and DNA from Biofilm Samples Obtained by Laser Capture Microdissection Microscopy

Ailyn C. Pérez-Osorio and Michael J. Franklin¹

Department of Microbiology, Montana State University, Bozeman, MT 59717, USA

¹Corresponding author (umbfm{at}montana.edu)

INTRODUCTION

The metabolic activities of bacteria growing in biofilms result in spatial gradients of oxygen, nutrients, and waste products. Because bacteria respond to local environmental conditions through changes in gene expression, mRNA levels of individual genes may vary spatially among bacteria within the biofilm. This article describes an approach to isolate RNA for quantification from cells at localized sites within biofilms. Biofilm thin sections are generated by embedding biofilms in cryoembedding resin, freezing the embedded biofilms on dry ice, and cutting with a cryomicrotome. The sections are placed on membrane-coated microscope slides and maintained on dry ice. Laser capture microdissection microscopy (LCMM) is used to dissect small subsets of cells at different regions within the biofilms, and RNA is extracted from the samples using either hot phenol or TRI reagent. A TRI reagent-based DNA extraction method is also presented.

RELATED INFORMATION

After RNA extraction, quantitative RT-PCR can be used to determine levels of RNA from cells at different zones within the biofilm (see [qRT-PCR of Microbial Biofilms](#) [Pérez-Osorio and Franklin 2008]).

MATERIALS

Reagents


Prepare all reagents used in this protocol with DEPC-treated H₂O.


 1-Bromo-3-chloropropane (BCP; Molecular Research Center) (for RNA extraction with TRI reagent, Step 35)

Biofilm cultivated using an appropriate system designed by the investigator (see Step 1)

Ethanol (75%, 100%)

HEPES (0.1 M)

 H₂O, diethyl pyrocarbonate (DEPC)-treated


 Isopropanol (for RNA extraction with TRI reagent, Step 37)


 LCMM lysis buffer

 NaOH (8 mM)

 O.C.T. compound (Tissue-Tek; Sakura Finetek USA)

Phase-Lock Gel tubes, 1.5 mL Heavy (5 PRIME) (for RNA extraction with hot phenol, Steps 12-29)

 Phenol, H₂O-equilibrated (Acros Organics) (for RNA extraction with hot phenol, Step 15)

 Phenol:chloroform:isoamyl alcohol (25:24:1) (for RNA extraction with hot phenol, Step 20)

Use H₂O-equilibrated phenol.


PolyAcryl carrier (Molecular Research Center) (optional; see Step 22; also for RNA extraction with TRI reagent, Steps 30 and 49)


Dilute the carrier 1:10 in DEPC-treated H₂O.

RNA, luciferase control (Promega)

Dilute the luciferase RNA to 1×10^4 molecules/ μ L.

Sodium acetate (3 M, pH 4.5) (for RNA extraction with hot phenol, Step 23)

 Sodium dodecyl sulfate (SDS; 2%, w/v) (for RNA extraction with hot phenol, Step 12)

 TRI reagent (MRC) (for RNA extraction with TRI reagent, Steps 30-42)

TURBO DNA-free DNase Treatment (includes TURBO DNase Buffer, TURBO DNase, and DNase Inactivation Reagent) (Ambion)

Equipment

 Cryomicrotome

 Dry ice

Ice

Microcentrifuges (room temperature and chilled to 4°C before use)

Microscope, laser capture (PALM MicroBeam System; Carl Zeiss MicroImaging)

The PALM MicroBeam System, which makes use of laser microdissection and pressure catapulting (LMPC) technology, is excellent for obtaining microdissected areas of biofilms. Other brands of laser capture microscopes are also available.

Slides, microscope (membrane-coated; Carl Zeiss MicroImaging)

Slides, microscope (stainless steel; BioSurface Technologies, # BFR25X75-316)

Tubes (microcentrifuge, 1.5 mL and 500 μ L)

Vacuum concentrator (e.g., SpeedVac, Thermo) (optional; see Step 28)

Vortexer

Water baths preset to 37°C and 65°C

METHOD

Cryoprocessing of Biofilms

Maintain samples in RNase-free zones on dry ice at all times.

1. Cultivate the biofilm using an appropriate system designed by the investigators, preferably on a heat-conducting surface or on a membrane that can be placed on a heat-conducting surface. If the biofilm has been cultivated on a membrane, place it on a stainless steel slide.
2. Cover the biofilm with O.C.T. compound at room temperature.
3. Freeze the biofilm and embedding resin by pressing the stainless steel slide onto a block of dry ice.
4. Once frozen, remove the biofilm and resin from the slide (by twisting the slide), invert the biofilm on the dry ice, and cover the opposite side of the biofilm with O.C.T. compound.
Keep track of the orientation of the biofilm.
5. Use a cryomicrotome to obtain thin sections (generally vertical transects) of the biofilm.
Thin sections may be any width, but sections ranging from 5 to 20 μm work well with the laser microdissection microscope.
All cryomicrotome surfaces should be clean and RNase free.
6. Place biofilm thin sections on membrane-coated microscope slides. Keep the microscope slides on a block of dry ice until examination and sampling.

Laser Capture Microdissection Microscopy (LCMM)

Because RNA half-life is short and the samples are at room temperature, all microscopic steps must be performed quickly. Generally, only one sample is obtained per microscope slide. A micrograph of a biofilm processed by cryosectioning and LCMM is shown in [Figure 1](#).

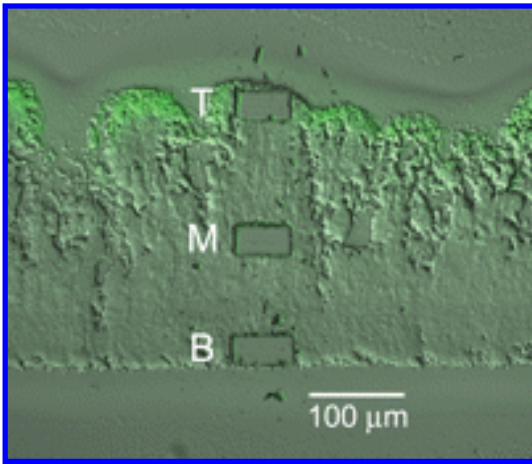


Figure 1. Combined light and epifluorescence micrograph of a *Pseudomonas aeruginosa* biofilm cryosectioned and processed by LCCM. The strain was engineered to express the green fluorescent protein along the top of the biofilm ([Lenz et al. 2008](#)). Following biofilm growth in a drip-flow reactor ([Xu et al. 1998](#)) on a stainless steel slide, the biofilm was cryoembedded and thin-sectioned as in Steps 2-6. The laser capture microdissection microscope was then used to obtain microdissected samples from the top (T), middle (M), and bottom (B) of the biofilm as in Steps 7-11.

View larger version (113K):

[\[in this window\]](#)

[\[in a new window\]](#)

7. Thaw microscope slides containing the O.C.T.-embedded biofilm sections for ~5 sec on the microscope stage.
8. Examine the sections using any of the objective lenses, depending upon the dissection area desired (generally 10X to 40X).
9. Use the dissecting laser to outline areas of the sections for sampling, generally ranging from 50 to 100,000 μm^2 .
10. Use the microscope microcentrifuge swinging arm to position the cap of a 1.5-mL microcentrifuge tube containing 40 μL of LCMM lysis buffer ~1 mm above the microscope slide. *Alternatively, 30 μL of TRI reagent may be used instead of LCMM lysis buffer. See Discussion.*
11. Obtain the samples by using the laser pressure catapult (LPC), which catapults the dissected sample into the LCMM lysis buffer contained in the microcentrifuge tube cap. *The sample can be observed in the microcentrifuge tube cap by using the 5X objective long working length lens.*

RNA Extraction from LCMM-Captured Samples

If LCMM lysis buffer was used during the microdissection in Step 10, extract the RNA using the hot phenol method (Steps 12-29). If TRI reagent was used, extract the RNA with the TRI reagent method (Steps 30-42). After RNA extraction, proceed to DNase treatment (Step 43).

RNA Extraction with Hot Phenol

12. Mix 160 μ L of LCMM lysis buffer with 200 μ L of 2% SDS.
13. After LCMM, immediately add the 360 μ L of LCMM lysis buffer/SDS mixture from Step 12 and 1.5 μ L of luciferase control RNA to the microcentrifuge tube containing the sample in the cap. *The control RNA is used to quantify RNA loss during sample preparation.*
14. Cap the tube, mix by inversion, and quickly vortex for 1 sec. Repeat the mixing and vortexing three times.
15. Centrifuge the tube in a microcentrifuge at maximum speed for 10 sec at room temperature, and transfer the sample to a new tube containing 400 μ L of H₂O-equilibrated phenol. Vortex for 5 sec.
16. Incubate for 5 min in a water bath at 65°C. Vortex again every minute during this incubation step. Transfer the tube to ice.
17. Centrifuge the tube in a microcentrifuge at maximum speed for 25 min at 4°C.
18. Centrifuge a 1.5-mL Phase-Lock Gel tube in a microcentrifuge for 30 sec at 12,000-16,000g. *Perform this step immediately before use in Step 19.*
19. Transfer the upper layer from the tube in Step 17 to the pelleted Phase-Lock Gel tube.
20. Add 400 μ L of phenol:chloroform:isoamyl alcohol (25:24:1) and mix by inversion.
21. Centrifuge in a microcentrifuge at maximum speed for 5 min at 4°C. Transfer the aqueous (upper) phase to a 1.5-mL tube. *Discard the organic phase.*
22. (Optional) Add 1.5 μ L of 1:10 diluted PolyAcryl carrier. *Use a carrier substance whenever possible to aid in the precipitation of samples with very little RNA. See Discussion.*
23. Add 30 μ L of 3 M sodium acetate (pH 4.5) and mix. Add 1.1 mL of 100% ethanol and vortex for 5 sec.
24. Incubate for 10 min at room temperature to precipitate the RNA. Store the sample overnight at –80°C.
25. Centrifuge the sample in a microcentrifuge at maximum speed for 10 min at 4°C. Carefully decant the supernatant.

26. Add 1.4 mL of 75% ethanol to the pellet and vortex for 5 sec. Centrifuge the samples in a microcentrifuge at maximum speed for 10 min at 4°C. Carefully decant the supernatant.

27. Repeat Step 26.

28. Air-dry the pellet for 5-10 min.

Alternatively, use a vacuum concentrator to dry the samples for 7 min.

29. Resuspend the RNA in 5 µL of DEPC-treated H₂O and keep on ice.

Samples may be frozen and stored at -80°C.

RNA Extraction with TRI Reagent

30. Aliquot 470 µL of TRI reagent to a 1.5-mL tube. Just before Step 31, add 3 µL of 1:10 diluted PolyAcryl carrier and 1.5 µL of luciferase control RNA.

This method does not work well for LCMM applications without a carrier compound. The control RNA is used to quantify RNA loss during sample preparation.

31. Add 150 µL of the TRI reagent mixture made in Step 30 to the 500-µL tube containing the LCMM sample from Step 11.

32. Cap the tube. Mix by inversion and quickly vortex for 1 sec. Repeat three times.

33. Centrifuge the tube in a microcentrifuge at maximum speed for 10 sec at room temperature. Transfer the sample into the tube containing the remainder of the TRI reagent mixed in Step 30 and vortex for 5 sec.

34. Incubate for 5 min in a water bath at 65°C. Vortex every minute during this incubation step. Transfer the tube to ice.

35. Add 50 µL of BCP and vortex for 15 sec. Incubate for 10 min at room temperature.

36. Centrifuge in a microcentrifuge at 12,000g for 15 min at 4°C. Transfer the top, aqueous phase to a new tube. Keep the interphase and organic phase on ice while finishing the RNA protocol. *The interphase and organic phase contain the DNA, which can be isolated by continuing with Step 48.*

37. Add 250 µL of isopropanol and vortex for 5 sec. Incubate for 10 min at room temperature.

38. Centrifuge in a microcentrifuge at 12,000g for 15 min at 4°C. Carefully decant the supernatant.

39. Add 1.4 mL of 75% ethanol to the RNA pellet and vortex for 5 sec. Centrifuge in a microcentrifuge at maximum speed for 10 min at 4°C, and carefully decant the supernatant.

40. Repeat Step 39.

At this point in the protocol, samples may be frozen and stored at -80°C . In this case, centrifuge the sample in a microcentrifuge at maximum speed for 10 min at 4°C just before Step 41.

41. Air-dry the pellet for 5-10 min.

42. Resuspend the RNA in 5 μL of DEPC-treated H_2O and keep on ice.

Samples may be frozen at this point and stored at -80°C .

DNase Treatment

43. Make a mixture of 3 μL of DEPC-treated H_2O , 1 μL of 10X TURBO DNase Buffer, and 1 μL of TURBO DNase in a 500- μL tube.

44. Add the 5 μL RNA sample from Step 42 and mix gently. Incubate for 20 min in a water bath at 37°C .

45. Add 2 μL of DNase Inactivation Reagent and mix well. Incubate for 2 min at room temperature, mixing two to three times during this incubation period.

46. Centrifuge in a microcentrifuge at maximum speed for 2 min at 4°C .

47. Transfer the RNA to a new tube and keep on ice until it is used for qRT-PCR (see [qRT-PCR of Microbial Biofilms](#) [Pérez-Osorio and Franklin 2008]).

Samples may be frozen at this point and stored at -80°C .

DNA Extraction

In certain experiments, it may be necessary to isolate DNA from LCMM-generated biofilm samples. The method here describes the TRI reagent approach for isolating DNA from the interphase and organic phase of the TRI reagent protocol.

48. Remove any remaining aqueous phase from the DNA sample generated in Step 36.
The DNA is in the underlying interphase and organic phase.

49. Add 3 μL of 1:10 diluted PolyAcryl Carrier and mix well. Add 150 μL of 100% ethanol and mix samples for 30 sec by inversion.

50. Incubate for 2-3 min at room temperature.

51. Centrifuge in a microcentrifuge at 2,000g for 5 min at 4°C . Decant the supernatant.

52. Add 1.4 mL of 75% ethanol and vortex for 5 sec. Incubate for 10 min at room temperature.
53. Centrifuge in a microcentrifuge at maximum speed for 5 min at 4°C. Carefully decant the supernatant.
54. Repeat Steps 52 and 53.
55. Air-dry the sample for 5-10 min.
56. Resuspend the DNA pellet in 23 μ L of 8 mM NaOH.
Samples can be stored overnight at 4°C.
57. Centrifuge in a microcentrifuge at maximum speed for 10 min at room temperature. Transfer the supernatant to a new tube, and discard the cell debris that is left behind.
58. Adjust the pH of the DNA by adding 6.9 μ L of 0.1 M HEPES.

DISCUSSION

Bacteria growing in biofilms are physiologically distinct from cells growing planktonically. Global proteomic and transcriptomic studies have revealed extensive differences in protein abundances and gene expression profiles of cells cultured in biofilms versus their planktonic counterparts (e.g., [Sauer et al. 2002](#); [Waite et al. 2005](#)). The global studies generally analyze entire homogenized biofilm populations, but environmental conditions such as oxygen and nutrient concentrations may vary depending on the distance of the cells within the biofilm from the oxygen or nutrient source. Therefore, the physiological responses of bacteria to the local environmental conditions within biofilms may vary greatly even for cells in close proximity to each other ([Stewart and Franklin 2008](#)).

LCMM allows investigators to separate and isolate individual cells or small groups of cells from surrounding tissue or matrix material ([Emmert-Buck et al. 1996](#); [Bonner et al. 1997](#)). LCMM has now been applied to biofilm studies ([Lenz et al. 2008](#)), in which biofilms are cryoembedded and thin-sectioned. Bacterial cells are then isolated by LCMM from different regions of the biofilm, and RNA is extracted from the cells. An advantage of this approach is that it does not require genetic manipulation of the cells (as is the case for reporter gene studies) and therefore may be used on wild-type cells isolated from their native environment. A drawback of this approach is that it is destructive, and therefore only endpoint analyses are possible.

This article describes two methods for extracting RNA from the microdissected samples. The hot-phenol method ([Chomczynski and Sacchi 1987](#); [Wilderman et al. 2004](#)) is an inexpensive approach that provides

excellent RNA recovery. It can be used when only RNA is needed. The TRI reagent method allows isolation of both RNA and DNA, but it requires a carrier compound if very small amounts of RNA are isolated (e.g., with laser microdissected biofilm samples). It should not be used when the carrier interferes with downstream techniques. An advantage to extracting both DNA and RNA from individual samples is that quantitative PCR may be performed on the DNA, providing an estimate of the number of DNA copies per sample. This in turn may be used as a normalization factor to estimate the number of bacterial cells obtained by LCMM for each sample.

LCMM and quantitative RT-PCR have revealed orders of magnitude differences in gene expression of bacteria at different positions in biofilms ([Lenz et al. 2008](#)). [Lenz et al. \(2008\)](#) discuss details regarding the analytical parameters of this approach, including reproducibility and limits of detection for individual genes. The LCMM/qRT-PCR approach may now be used to better understand why certain subsets of cells within biofilms are more resistant to antibiotics and host defenses, based on their gene expression patterns. It may also be used to localize the bacteria within biofilms with the greatest metabolic response toward certain substrates.

ACKNOWLEDGMENTS

We thank Kerry Williamson, Betsey Pitts, and Paula J. Wilderman for their assistance in developing these protocols. This work was supported by Public Health Service grant AI-065906 (M.J.F.) from the National Institute of Allergy and Infectious Diseases, and the American Society for Microbiology Robert D. Watkins Graduate Research Fellowship (A.P.L.). We acknowledge the Montana State University INBRE Functional Genomics Core Facility supported by NIH grant P20-RR16455.

REFERENCES

- Bonner, R.F., Emmert-Buck, M., Cole, K., Pohida, T., Chuaqui, R., Goldstein, S., and Liotta, L.A. 1997. Laser capture microdissection: Molecular analysis of tissue. *Science* **278**: 1481–1483. [[Free Full Text](#)]
- Chomczynski, P. and Sacchi, N. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**: 156–159. [[Medline](#)]
- Emmert-Buck, M.R., Bonner, R.F., Smith, P.D., Chuaqui, R.F., Zhuang, Z., Goldstein, S.R., Weiss, R.A., and Liotta, L.A. 1996. Laser capture microdissection. *Science* **274**: 998–1001. [[Abstract/Free Full Text](#)]
- Pérez-Osorio, A.C. and Franklin, M.J. 2008. qRT-PCR of microbial biofilms. *Cold Spring Harb. Protoc.* (this issue). doi: 10.1101/pdb.prot5066. [[Abstract/Free Full Text](#)]

Lenz, A.P., Williamson, K.S., Pitts, B., Stewart, P.S., and Franklin, M.J. 2008. Localized gene expression in *Pseudomonas aeruginosa* biofilms. *Appl. Environ. Microbiol.* **74**: 4463–4471. [[Abstract/Free Full Text](#)]

Sauer, K., Camper, A.K., Ehrlich, G.D., Costerton, J.W., and Davies, D.G. 2002. *Pseudomonas aeruginosa* displays multiple phenotypes during development as a biofilm. *J. Bacteriol.* **184**: 1140–1154. [[Abstract/Free Full Text](#)]

Stewart, P.S. and Franklin, M.J. 2008. Physiological heterogeneity in biofilms. *Nat. Rev. Microbiol.* **6**: 199–210. [[Medline](#)]

Waite, R.D., Papakonstantinou, A., Littler, E., and Curtis, M.A. 2005. Transcriptome analysis of *Pseudomonas aeruginosa* growth: Comparison of gene expression in planktonic cultures and developing and mature biofilms. *J. Bacteriol.* **187**: 6571–6576. [[Abstract/Free Full Text](#)]

Wilderman, P.J., Sowa, N.A., FitzGerald, D.J., FitzGerald, P.C., Gottesman, S., Ochsner, U.A., and Vasil, M.L. 2004. Identification of tandem duplicate regulatory small RNAs in *Pseudomonas aeruginosa* involved in iron homeostasis. *Proc. Natl. Acad. Sci.* **101**: 9792–9797. [[Abstract/Free Full Text](#)]

Xu, K.D., Stewart, P.S., Xia, F., Huang, C.T., and McFeters, G.A. 1998. Spatial physiological heterogeneity in *Pseudomonas aeruginosa* biofilm is determined by oxygen availability. *Appl. Environ. Microbiol.* **64**: 4035–4039. [[Abstract/Free Full Text](#)]



Caution

1-Bromo-3-chloropropane (BCP)

1-Bromo-3-chloropropane (BCP) has a narcotic effect and may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses. Do not breathe the vapor.



Caution

Dry ice (Carbon dioxide; CO₂)

CO₂ (Carbon dioxide; Dry ice) in all forms may be fatal by inhalation, ingestion, or skin absorption. In high concentrations, it can paralyze the respiratory center and cause suffocation. Use only in well-ventilated areas. In the form of dry ice, contact with carbon dioxide can also cause frostbite. Do not place large quantities of dry ice in enclosed areas such as cold rooms. Wear appropriate gloves and safety goggles.



Caution

Isopropanol

Isopropanol is flammable and irritating. It may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses. Do not breathe the vapor. Keep away from heat, sparks, and open flame.



Caution

Microtome blades

Microtome blades are extremely sharp! Use care when sectioning. If unfamiliar with the use of a microtome, have someone demonstrate its use.



Caution

NaOH (Sodium hydroxide)

NaOH (Sodium hydroxide) and solutions containing NaOH are highly toxic and caustic and should be handled with great care. Wear appropriate gloves and a face mask. All concentrated bases should be handled in a similar manner.



Caution

OCT

OCT is composed of polyvinyl alcohol, polyethylene glycol, and dimethyl benzyl ammonium chloride. Follow the manufacturer's guidelines for handling OCT.



Caution

Phenol

Phenol is extremely toxic, highly corrosive, and can cause severe burns. It may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves, goggles, and protective clothing. Always use in a chemical fume hood. Rinse any areas of skin that come in contact with phenol with a large volume of water and wash with soap and water; do not use ethanol!



Caution

Phenol:chloroform:isoamyl alcohol

Phenol is extremely toxic, highly corrosive, and can cause severe burns. It may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves, goggles and protective clothing. Always use in a chemical fume hood. Rinse any areas of skin that come in contact with phenol with a large volume of water and wash with soap and water; do not use ethanol!

Chloroform (CHCl_3) is irritating to the skin, eyes, mucous membranes, and respiratory tract. It is a carcinogen and may damage the liver and kidneys. It is also volatile. Avoid breathing the vapors. Wear

appropriate gloves and safety glasses. Always use in a chemical fume hood.

Isoamyl alcohol may be harmful by inhalation, ingestion, or skin absorption and presents a risk of serious damage to the eyes. Wear appropriate gloves and safety goggles. Keep away from heat, sparks, and open flame.



Caution

Sodium dodecyl sulfate (SDS)

Sodium dodecyl sulfate (SDS) is toxic, an irritant, and poses a risk of severe damage to the eyes. It may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety goggles. Do not breathe the dust.



Caution

TRI reagent

TRI reagent combines phenol and guanidine thiocyanate.

Phenol is extremely toxic, highly corrosive, and can cause severe burns. It may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves, goggles, and protective clothing. Always use in a chemical fume hood. Rinse any areas of skin that come in contact with phenol with a large volume of water and wash with soap and water; do not use ethanol!

Guanidine thiocyanate is an irritant and may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses.

Thiocyanate compounds may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and safety glasses, and use in a chemical fume hood.



Recipe

Diethyl pyrocarbonate (DEPC)-treated H₂O

Distilled H₂O

 Diethyl pyrocarbonate (DEPC)

Add 1 mL of fresh DEPC to 1 L of H₂O. Shake well to disperse the DEPC through the H₂O. Incubate at 37°C for at least 12 h and/or autoclave at 15 psi on liquid cycle for 20 min to inactivate the remaining DEPC.



Recipe

LCMM lysis buffer

0.3 M sucrose

0.02 M sodium acetate (pH 4.5)

[Copyright © 2008 by Cold Spring Harbor Laboratory Press.](#) Online ISSN: 1559-6095 [Terms of Service](#)

All rights reserved. Anyone using the procedures outlined in these protocols does so at their own risk. Cold Spring Harbor Laboratory makes no representations or warranties with respect to the material set forth in these protocols and has no liability in connection with their use. All materials used in these protocols, but not limited to those highlighted with the Warning icon, may be considered hazardous and should be used with caution. For a full listing of cautions, [click here](#).

All rights reserved. No part of these pages, either text or images, may be used for any reason other than personal use. Reproduction, modification, storage in a retrieval system or retransmission, in any form or by any means-electronic, mechanical, or otherwise-for reasons other than personal use is strictly prohibited without prior written permission.

This article has been cited by other articles:



Cold Spring Harbor Protocols

[▶ HOME](#)

A. C. Perez-Osorio and M. J. Franklin

qRT-PCR of Microbial Biofilms

CSH Protocols, October 1, 2008; 2008(11): pdb.prot5066 - pdb.prot5066.

[\[Abstract\]](#) [\[Full Text\]](#)