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Transcriptional Profiling of *Candida albicans* in the Host

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Abstract

Gene expression profiling has become an important tool for determining gene functions, performing phenotypic analysis, and quantifying the differential expression of individual transcripts under various conditions. *Candida albicans* gene expression is highly responsive to environmental conditions and rapidly adapts to various niches within the host. Here, we describe a mouse model of gastrointestinal colonization with *C. albicans*, the measurement of colonization in fecal pellets, and the collection of samples for transcriptional profiling. We describe how to extract and purify RNA suitable for analysis via RT-qPCR or microarray.

Keywords

Candida albicans; RNA; Transcriptional profiling; Gene expression; Host

1 Introduction

Transcriptional profiling provides a valuable tool for studying changes in gene expression in response to environmental factors, genetic manipulation, and other experimental conditions. Previous studies have shown that *Candida albicans* gene expression in the gastrointestinal tract differs from that observed during laboratory growth [1, 2]. We describe the extraction of RNA from *C. albicans* in the host using a mouse model of gastrointestinal colonization. Alternative mouse models of gastrointestinal colonization have been used (reviewed in ref. 3).

A variety of techniques have been used to measure *C. albicans* gene expression in the host (reviewed in refs. 4, 5). In vivo expression technology [6] and reverse transcriptase-PCR (RT-PCR) [7, 8] have been used to study the expression of individual *C. albicans* genes during infection. Quantitative (or real-time) reverse transcription PCR (RT-qPCR) is a sensitive and highly reproducible method for measuring individual transcripts. GFP fusion constructs have been used to measure the expression of individual genes in single-*C. albicans* cells [9] in infected tissue. However, neither RT-qPCR nor GFP fusion constructs are well suited to profiling the expression of large numbers of genes. Microarray expression profiling enables the simultaneous and relatively inexpensive measurement of many transcripts. Although early studies using microarrays to measure *C. albicans* gene expression in the host required additional amplification steps in order to increase hybridization signals from the relatively small fungal biomass recovered from infected host tissues [10, 11], a method for enriching fungal cells from host tissues eliminated the need for

this amplification step [12], and the methodology for transcriptional profiling of *C. albicans* has been described in detail [13]. However, microarray profiling is limited to the detection of known genes/splice variants, and it has a limited dynamic range of detection. mRNA-seq is becoming the preferred technology for transcriptional profiling, as it is highly accurate [14, 15] and reproducible [16], has a wide dynamic range [15], and provides data for both known and novel transcripts [17].

Transcriptional profiling of *C. albicans* in the host gastrointestinal tract poses a unique set of challenges. Although the fungal biomass in the gastrointestinal tract is small compared to that obtainable *in vitro*, yield is not a major limitation of the technique (unlike measuring *C. albicans* gene expression from infected host tissues). The primary challenge when measuring *C. albicans* gene expression in the host gastrointestinal tract is the presence of biological and chemical contaminants. Biological “contaminants” include non-*C. albicans* species in the microbiota; contamination by host RNA was found to be minimal [1]. The contribution of bacterial RNAs is minimized first by using an oligo(dT) primer for first-strand cDNA synthesis to select for polyadenylated RNAs. Then a second, technique-specific step can be used to further enrich for *C. albicans*-specific transcripts. Microarrays are particularly well suited for use with this type of impure sample, as *C. albicans*-specific hybridization probes reduce the contribution from other species. The use of *C. albicans*-specific primers serves a similar function during RT-qPCR. While RNA-seq lacks this second enrichment step, sequencing reads from other species can be filtered out during analysis by aligning reads to the *C. albicans* genome.

Chemical impurities from the diet and the digestive process may include lipids, bile acids, and other substances that could interfere with downstream applications. In order to remove chemical contaminants, RNA is extracted using a two-step purification process: guanidinium thiocyanate-phenol-chloroform extraction (using TRIzol Reagent) followed by silica-membrane column purification. This produces RNA of sufficient purity for use in RT-qPCR and microarray experiments. Although RNA-seq experiments have not been performed using RNA prepared according to the method described here, the yield is typically sufficient: a typical yield from the contents of one mouse cecum using this method is between 1 and 6 µg of RNA (see Table 1).

2 Materials

Prepare all solutions using deionized water and analytical grade reagents unless otherwise specified.

2.1 Animal Models

1. Five- to 7-week-old female BALB/c mice (see Note 1): House in pathogen-free conditions in autoclaved enclosed filter-top cages with sterile food and water.

¹Alternative mouse strains have been used, such as 5–7-week-old female C57BL/6 [1] or 19–20 g female Swiss Webster [19] mice. For experiments in an immunocompromised host, 5–7-week-old BALB/c *nu/nu* mice have been used [20]. (Mouse models of gastrointestinal colonization by *C. albicans* are reviewed in ref. 3.)

2. Antibiotic-containing drinking water for mice: 1 g/l tetracycline, 2 g/l streptomycin, 0.1 g/l gentamicin. Weigh antibiotics; mix and make up to appropriate volume. Filter sterilize. Store at room temperature protected from light. Make fresh at least once per week (*see* Notes 2 and ³).
3. Sterile phosphate-buffered saline (PBS).
4. Autoclaved plastic 1 l beakers or other sterile containers of similar size (one per mouse) for collecting fecal pellets.
5. For collecting fecal pellets: forceps and 70 % ethanol for sterilization.
6. Microtube mixer (such as Tomy MT-360) or shaking tissue homogenizer (such as Qiagen TissueLyser) (*see* Note 4).
7. 1000× streptomycin/ampicillin solution (for making YPD-SA plates): Weigh 1 g streptomycin and 0.5 g ampicillin. Make up to 10 ml with water. Filter sterilize. Store 1 ml aliquots at −20 °C.
8. YPD-SA plates (yeast extract-peptone-dextrose agar plus 100 µg/ml streptomycin and 50 µg/ml ampicillin): Add about 700 ml ultrapure water (deionized water purified to 18 MΩ) to a 1-l graduated cylinder or flask. Weigh 10 g yeast extract, 20 g peptone, and 20 g glucose. Mix until no powder can be seen, and then add 20 g agar. Make up to 1 l with water. Autoclave to sterilize. Cool to 50 °C and add 1 ml of 1000× streptomycin/ampicillin solution (from **item 7**, above). Carefully pour into sterile petri dishes.
9. Fresh YPD made with ultrapure molecular biology-grade water: 1 % yeast extract, 2 % peptone, 2 % glucose. Weigh 1 g yeast extract and 2 g peptone. Make up to 90 ml with ultrapure molecular biology-grade water (*see* Note 5) and sterilize. Weigh 2 g of glucose, make up to 10 ml with ultrapure molecular biology-grade water, and filter sterilize. For a 10 ml overnight culture, mix 9 ml of sterile yeast extract/peptone solution and 1 ml of sterile glucose solution.
10. Hemocytometer or other cell counter.
11. Sterile 1 ml disposable syringes and gavage needles (*see* Note 6).
12. RNA stabilization solution such as RNeasy (Qiagen/Life Technologies, Carlsbad, CA; *see* Note 7).

²Antibiotic water is made up fresh weekly; otherwise, antibiotics begin to precipitate.

³Alternative antibiotic treatments have been used (reviewed in ref. 21). For example, for antibiotic-containing drinking water without tetracycline: 1 g/l bacitracin, 2 g/l streptomycin, and 0.1 g/l gentamicin [22].

⁴A mechanical tissue homogenizer will be required for homogenizing tissue (tongue, kidney, liver, or ileum); for measuring colonization from fecal pellets or stomach/ileum/cecum contents, a microtube mixer or other shaking device will make homogenization much easier, but it could be accomplished by vortexing.

⁵We use ultrapure molecular biology-grade water to make YPD for the inoculum because we have had variable growth in medium made from deionized water supplied from our central deionized water source. Make the YPD fresh for each experiment (use within 1–2 weeks).

⁶After use, do not bleach gavage needles, as it destroys stainless steel. When inoculating the mice, have ready a 15 ml tube of water; after gavaging the mice, immediately flush the gavage needle thoroughly with water, and then 70 % ethanol, by repeatedly filling and emptying the syringe. The needles are then ready to be autoclaved for the next use.

⁷RNeasy is a high-salt solution; the salt may interfere with some downstream applications. For techniques that are highly sensitive to salt, an alternative method of RNA preservation may be preferable.

13. Liquid nitrogen or dry ice.

2.2 RNA Extraction

1. Mechanical homogenizer (such as a Mini-Beadbeater from BioSpec Products).
2. Vials for mechanical homogenization (2 or 7 ml polypropylene vials, depending on sample volume; *see* Note 8).
3. 0.5 mm diameter zirconia/silica beads (BioSpec Products, catalog no. 11079105z) for mechanical homogenization: Transfer the beads to a small glass beaker, cover with aluminum foil, and bake at 200 °C for 2 h to eliminate RNase contamination.
4. 250 µm polypropylene mesh filter (Small Parts, Inc., Miami Lakes, FL; *see* Note 9).
5. TRIzol Reagent (Ambion/Life Technologies, Carlsbad, CA).
6. Chloroform and 70 % ethanol.
7. PureLink RNA Mini Kit (Ambion/Life Technologies, Carlsbad, CA) or a similar silica-membrane spin column kit for RNA purification with an on-column DNase I digestion step; *see* Note 10.
8. NanoDrop spectrophotometer.

2.3 Reverse Transcription for qPCR

1. SuperScript III Reverse Transcriptase (Life Technologies, Carlsbad, CA).
2. Oligo(dT)₂₀ (50 µM).
3. dNTP mix: 10 mM each dATP, dGTP, dCTP, and dTTP at neutral pH.
4. RNase inhibitor.

3 Methods

3.1 Animal Models

1. Upon arrival, house mice three to four per cage in autoclaved, enclosed, filter-top cages under pathogen-free conditions with sterile food and water. Let the mice acclimate for 7 days prior to inoculation.
2. Four days prior to inoculation, streak strains from glycerol stocks onto YPD agar plates. Incubate at 25 °C for 3 days or 30 °C for 4 days.
3. Four days prior to inoculation, replace the drinking water in mouse cages with antibiotic-containing water (1 g/l tetracycline, 2 g/l streptomycin, 0.1 g/l

⁸For cecum contents, use 7 ml polypropylene screw-cap vials (available from BioSpec Products, catalog no. 3205). For the contents of two to four ilea/stomachs, use 2 ml screw-cap polypropylene vials with an o-ring seal (available from BioSpec Products or other suppliers).

⁹Cut the polypropylene mesh into 3" squares and trim the corners to make octagons. Sterilize in 70 % ethanol and air-dry. After use, immediately wash thoroughly with deionized water and re-sterilize.

¹⁰If substituting a different column purification kit, substitute the manufacturer's instructions for **steps 24–40** of the RNA extraction. Perform an on-column DNase treatment and elute with 32 µl of RNase-free water.

gentamicin). To ensure adequate antibiotic consumption, also give moist food daily (*see* Note 11).

4. One or two days prior to inoculation, mark mice and verify that mice are not colonized with fungi (**steps 5–11**, below).
5. Prepare and weigh 1.5 ml tubes containing 1 ml sterile PBS (one tube per mouse).
6. In a laminar flow hood, transfer each mouse to a sterile 1 l plastic beaker to collect fecal pellets. Once the mouse has produced at least two fecal pellets, return the mouse to its cage and, using sterile forceps, transfer two fresh fecal pellets to the prepared tube of PBS. Sterilize the forceps in 70 % ethanol between mice.
7. Reweigh the tubes containing the fecal pellets.
8. Homogenize fecal pellets in PBS by shaking for 1 min with a microtube mixer or by vortexing. If pellets are not broken up after 1 min of mechanical homogenization, use a pipette tip to break them up. Shake for 5 more minutes.
9. Vortex for 5 s, let settle for 3 s, and pipette from the top using pipette tips with the tip cut off to avoid clogging.
10. Divide a YPD-SA plate into quadrants; in each, spread 50 μ l of fecal homogenate.
11. Incubate YPD-SA plates at 37 °C overnight (*see* Note 12). Prior to inoculation, there should not be any colonies (*see* Note 13).
12. The morning before you plan to inoculate the mice, pick a colony from the freshly streaked YPD plate (from **step 2**) into 10 ml fresh YPD (*see* Note 5) and incubate on a rotating wheel for 24 h at 37 °C (*see* Note 14).
13. Harvest the 24-h culture by transferring it to a 50 ml conical tube; centrifuge at $3220 \times g$ for 6 min.
14. Discard the supernatant. Wash the pellet with 20 ml sterile PBS. Centrifuge at $3220 \times g$ for 6 min.
15. Repeat **step 14** once.
16. Discard the supernatant. Resuspend the pellet in 3 ml sterile PBS.
17. Dilute 5 μ l of cell suspension in 1 ml PBS (1:200 dilution). Count the cells and prepare 5 ml of cell suspension at a concentration of 5×10^8 cells/ml (in sterile PBS).
18. Inoculate mice via oral gavage: administer 0.1 ml (5×10^7 cells) per mouse.

¹¹Some, but not all, mice will readily drink antibiotic-containing water. BALB/c mice tend not to like antibiotic-containing water, but will readily consume it when it is used to moisten food pellets. Mice will eventually adjust and accept antibiotic-containing water. Fresh moist food can also be given ~30 min prior to sacrifice if stomach contents will be collected.

¹²To be certain that there are no slow-growing fungal colonies, incubate the pre-inoculation plates for an extra day or 2.

¹³Calculate CFU/g fecal pellets using the weights of the 1.5 ml tubes containing PBS before and after adding fecal pellets.

¹⁴The expected density of a 24-h culture is $\sim 3 \times 10^8$ cells/ml.

19. Starting 1 day post-inoculation, measure colonization by collecting fecal pellets and plating homogenates on YPD-SA plates as described above (**steps 5–11** ; *see* Note 15).
20. Prepare tubes prior to sacrificing the mice. For each mouse, prepare tubes as follows for whichever samples you wish to collect (*see* Note 16):

To quantify colonization, prepare and weigh a tube containing 1 ml sterile PBS for each sample to be collected.

To harvest RNA, prepare 15 ml conical tubes containing 5–7 ml RNAlater (one tube per mouse for cecum contents; for ileum or stomach contents, one tube for two to four mice; *see* Note 17).
21. Upon sacrificing the mice, collect all samples in a laminar flow hood using sterile technique.
22. To quantify colonization, collect the desired samples in the prepared tubes (from **step 20**) as follows: first collect the tongue; both kidneys; and/or a piece of the liver. For stomach or cecum contents, collect a small pea-sized drop of contents. For ileum colonization, collect a 1 cm piece of ileum (tissue with contents). Homogenize the tissue and/or GI contents in PBS and plate to determine CFU/g (*see* Note 18).
23. For RNA extraction, collect the contents of desired GI tract organs into prepared tubes of RNAlater. Let sit at room temperature for ~5 min, and then put on dry ice or flash freeze in liquid nitrogen. Store at –80 °C.

3.2 RNA Extraction

1. Thaw samples on ice.
2. Prepare vials for mechanical disruption (*see* Note 8): label the vials and fill $\frac{3}{4}$ full with zirconia/silica beads. Chill the vials at –20 °C while preparing the samples.
3. Weigh an empty 50 ml conical tube for each sample.
4. Fold a sterile polypropylene 250 μ m mesh filter into a cone (*see* Note 19) and push snugly into the top of a pre-weighed 50 ml conical tube. With one hand, hold the mesh filter in the tube to keep it folded.

¹⁵For day 1 post-inoculation, dilute the fecal pellet homogenate 10^{-1} and 10^{-3} . Spread 10 μ l each of the 10^{-1} and 10^{-3} dilutions and 50 μ l of the 10^{-3} dilution on YPD-SA agar. Over the first several days post-inoculation, expect to see $\sim 10^6$ – 10^8 CFU/g. As colonization levels change over time, adjust the volume and dilution of homogenates plated.

¹⁶Colonization can be quantified at many points throughout the GI tract (i.e., stomach, ileum, cecum, colon). We have found that colonization measured in cecum contents correlates well with fecal pellet measurements. Additionally, we typically check for, but do not see, colonization in tongue, kidney, and liver homogenates.

¹⁷For microarray experiments, the cecum contents of three mice were typically pooled and three to four biological replicates were used (i.e., 9–12 mice).

¹⁸Typically, to measure colonization in stomach, ileum, cecum, or colon, plate homogenates at the same dilutions you have been using for fecal pellets. Plate 50 μ l of undiluted homogenate for tongue, kidney, and liver tissue.

¹⁹To fold the polypropylene mesh, fold in half and then fold over a third. Open the large portion as the cone.

5. With the other hand, vortex the thawed sample, pour it into the mesh filter, and let it drip into the 50 ml conical tube (*see* Note 20). Rinse the tube of thawed sample with 1 ml RNAlater to resuspend any remaining material and add to the mesh filter.
6. Once most of the liquid has dripped through the filter, carefully fold down the top of the cone over the outside of the conical tube and firmly screw on the cap over the filter (*see* Note 21).
7. Centrifuge at $3220 \times g$ for 10 min at 4 °C.
8. Remove the mesh filter from the tube and aspirate off the supernatant.
9. Centrifuge at $3220 \times g$ for 10 min at 4 °C. Aspirate off all the remaining liquid (*see* Note 22).
10. Weigh the tube and determine the sample weight.
11. Resuspend the pellet in TRIzol (~1 ml TRIzol per 50–100 mg sample) and add to the pre-chilled homogenization vials (prepared in **step 2**). Rinse the sample tube with additional TRIzol and add to the homogenization vial; there should only be a small amount of air left at the top of the vial (*see* Note 23).
12. Shake the samples with the mechanical homogenizer for 30 s, and then chill samples on ice for 1 min or until cool to the touch (*see* Note 24). Repeat five times (for a total of 3 min of homogenization per sample).
13. Transfer the liquid homogenate to sterile RNase-free 1.5 ml tubes (*see* Notes 25 and 26).
14. Centrifuge at $12,000 \times g$ for 10 min at 4 °C (*see* Note 27).
15. Transfer the cleared supernatant to a new sterile RNase-free 1.5 ml tube (*see* Note 28).
16. Incubate samples at room temperature for 5 min.
17. Add 1/5 volume chloroform (i.e., 0.2 ml chloroform per 1 ml TRIzol).
18. Shake the tubes vigorously by hand for at least 30 s.

²⁰It is often helpful to gently “stir” the sample in the filter with a sterile 1000 µl pipette tip, as solids in the sample can clog the filter and prevent the liquid from passing through.

²¹If the cap is not screwed on securely it may pop off, allowing the filter to unfold and causing loss of sample.

²²RNAlater is a high-salt solution. The salt may interfere with downstream applications, so it is important to remove as much as possible. This is typically sufficient for RT-qPCR.

²³Extra TRIzol is added to nearly fill the vial because the sample will foam during homogenization if the tube contains too much air.

²⁴To keep the samples cold, homogenize the samples in short bursts, resting the samples on ice in between rounds of homogenization. Adjust the length of each burst of homogenization as necessary to keep samples from getting hot; homogenize each sample for a total of 3 min.

²⁵Homogenized cecum contents will typically have to be split into three or four 1.5 ml tubes per sample; homogenized ileum/stomach contents usually fit into one tube per sample.

²⁶Transfer the homogenate using a 1000 µl micropipette; firmly put a sterile 10 µl pipette tip (do not use a filter tip) on over the 1000 µl tip (the beads are too small to easily pass through the opening of the 10 µl tip) and pipette homogenate from the bottom of the vial to avoid transferring beads. After sucking the beads dry, vortex the vial and repeat; vortexing allows additional homogenate to settle to the bottom of the vial.

²⁷In high-fat samples, a layer of fat may collect above the supernatant; carefully remove and discard this layer.

²⁸According to the manufacturer, homogenized sample can be stored at –80 °C for at least a month [18].

19. Incubate the samples at room temperature for 3 min.
20. Centrifuge at $12,000 \times g$ for 15 min at 4 °C.
21. Transfer the upper aqueous phase to a new RNase-free tube (*see* Note 29).
22. Add an equal volume of 70 % ethanol. Mix well by vortexing and/or inverting the tube.
23. Next perform a silica-gel membrane purification with on-column DNase treatment (using a PureLink RNA Mini Kit) according to the manufacturer's instructions (**steps 24–40**, below [18]; *see* Note 10).
24. Prepare the PureLink DNase mixture (80 μ l per sample): in a sterile, RNase-free microcentrifuge tube, mix 10 $\times$ DNase I reaction buffer (8 μ l per sample), resuspended DNase (10 μ l per sample), and RNase-free water (62 μ l per sample).
25. Transfer up to 700 μ l of sample to a PureLink spin cartridge with a collection tube (*see* Note 30).
26. Centrifuge at $12,000 \times g$ for 15 s at room temperature. Discard the flow-through and reinsert the spin cartridge into the same collection tube.
27. Repeat **steps 25** and **26** until the entire sample has been loaded onto the PureLink column.
28. To the spin column, add 350 μ l wash buffer I.
29. Centrifuge at $12,000 \times g$ for 15 s at room temperature. Discard the flow-through and the collection tube. Insert the spin cartridge into a new collection tube.
30. Add 80 μ l of PureLink DNase mixture directly onto the surface of the spin cartridge membrane.
31. Incubate at room temperature for 15 min.
32. To the spin column, add 350 μ l wash buffer I.
33. Centrifuge at $12,000 \times g$ for 15 s at room temperature. Discard the flow-through and the collection tube. Insert the spin cartridge into a new collection tube.
34. To the spin column, add 500 μ l wash buffer II.
35. Centrifuge at $12,000 \times g$ for 15 s at room temperature. Discard the flow-through and reinsert the spin cartridge into the same collection tube.
36. Repeat **steps 34** and **35** once.
37. Centrifuge the spin cartridge at $12,000 \times g$ for 1 min to dry the membrane. Discard the collection tube and insert the spin cartridge into a recovery tube.
38. Add 32 μ l of RNase-free water to the center of the spin cartridge.

²⁹Be careful not to disturb the interphase. It is better to leave 1–2 mm of aqueous phase than to risk contamination with material from the interphase.

³⁰We typically use a single PureLink column for the contents of one cecum even though the amount of RNA typically exceeds the amount recommended by the manufacturer [18].

39. Incubate at room temperature for 1 min.
40. Centrifuge the spin cartridge and recovery tube at $12,000 \times g$ for 1 min at room temperature.
41. Determine the RNA concentration with a NanoDrop spectrophotometer (*see* Note 31). Store at -80°C . (For use in microarray experiments, *see* ref. 13.)

3.3 Reverse Transcription for qPCR

1. Determine how much RNA template to use (*see* Note 32).
2. For each sample, add the following to a nuclease-free 0.2 ml tube (20 μl reaction volume): 1 μl oligo(dT) 20 (50 μM), 1 μl dNTP mix, 10 pg–5 μg RNA (*see* Note 32), and sterile nuclease-free water to 12 μl (*see* Note 33).
3. Incubate at 65°C for 5 min.
4. Incubate on ice for at least 1 min.
5. Make reverse transcription master mix. Per 20 μl reaction: 4 μl of $5\times$ first-strand buffer, 1 μl of DTT (0.1 M), 0.5 μl of RNase inhibitor, and 0.5 μl of SuperScript III RT.
6. Briefly centrifuge the tubes from **step 4** to collect the contents; add 6 μl of reverse transcription master mix to each reaction. Mix gently by pipetting up and down.
7. Incubate at 50°C for 60 min.
8. Inactivate the reaction by incubating at 70°C for 15 min.
9. The cDNA is now ready to be diluted (*see* Note 34) and used as a template for qPCR. Make 20–40 μl aliquots of cDNA diluted 1:10 or 1:20 in nuclease-free water. Store at -20°C . Use 1–2 μl of diluted cDNA per 20 μl qPCR reaction.

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³¹Do not expect absorbance curves with a single, “clean” peak at 260 nm. There will often be a second peak at 230 nm, or a single, broad peak from 230 to 260 nm. We typically obtain concentrations of 50–300 ng/ μl (or a total yield of ~1–6 μg of RNA from the contents of 1 cecum), but it is not uncommon for some samples to have poor yields (10 ng/ μl , or 300 ng total).

³²Typically the amount of RNA template that can be used is limited by the concentration of the sample with the lowest yield. Based on this limitation, we typically use 80–600 ng of RNA in a 20 μl reaction.

³³Add water to the reaction first, then oligo(dT), and RNase inhibitor; add RNA last. Use oligo(dT) instead of random primers because this selects for eukaryotic mRNAs, and eliminates much of the contamination from bacterial RNAs.

³⁴Dilute cDNA 1:10 or 1:20 in nuclease-free water; do not use undiluted cDNA, as it typically contains inhibitors that will interfere with efficient qPCR amplification. If aliquots are prepared in 8-well strip tubes, a multichannel pipette can be used to add cDNA to qPCR reaction tubes.

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Table 1

Typical RNA yield from the contents of one mouse cecum

CFU/g ^a	Sample weight (mg) ^b	RNA concentration (ng/μl)	RNA yield (μg)
10 ⁶ –10 ⁷	100–300	50–300	1–6

^a Measured from fecal pellets or cecum contents^b Weight of centrifuged pellet following filtration through polypropylene mesh (i.e., as measured in **step 10** of Subheading 3.2)