Candida biofilm RNA isolation from venous catheters

1. Cut the end of the catheter into multiple 2- to 3- mm thick doughnuts.
2. Place sliced catheter into eppendorf tube, and add 400 ul AE buffer.
3. Sonicate the tube for 10 minutes in a sonicating water bath, and vigorously vortex for 30 seconds.
4. Add 40 ul 10% SDS.
5. Add equal volume acid phenol (440 ul) warmed in heat block to 65˚C.
6. Incubate at 65˚C for 4-6 minutes, vortexing every 30 seconds.
7. Centrifuge at top speed for 2 minutes at room temperature.
8. Transfer aqueous phase to new 1.5 ml tube.
9. Repeat steps 5 through 8.
10. To aqueous phase, add equal volume phenol:chloroform:isoamyl alcohol, vortex.
11. Centrifuge at top speed for 5 minutes.
12. Transfer aqueous phase to new tube, and add 1/10 volume 3 M NaOAc, pH 5.2, and 2.5 volumes 100% EtOH (-20˚C).
13. Store at -20˚C for 15 minutes to precipitate.
14. Centrifuge at top speed for 5 minutes, carefully remove supernatant.
15. Resuspend pellet in 400 ul DEPC-treated H2O.
16. Add 40 ul 3 M NaOAc, pH 5.2, 1 ml 100% EtOH (-20˚C), and place at -20˚C for 15 minutes.
17. Centrifuge at top speed for 5 minutes, carefully remove supernatant.
18. Wash pellet with 500 ul 80% EtOH, place at -20˚C for 15 minutes.
19. Centrifuge at full speed for 3 minutes, carefully remove as much supernatant as possible.
20. Air dry pellet, 10-15 minutes or as needed.
21. Resuspend in 20 ul H20.
22. Store at -80˚C.

AE Buffer:
50 mM NaOAc, pH 5.3
10 mM EDTA

Acid Phenol: Ambion Cat. No. 9720
Phenol:CHCl3:IAA: Ambion Cat. No. 9730 (do NOT raise pH with provided buffer)