Supplementary Information

The Role of Ammonia Oxidizing Microorganisms in Biofiltration for the Removal of Trace Organic Compounds in Secondary Wastewater Effluent

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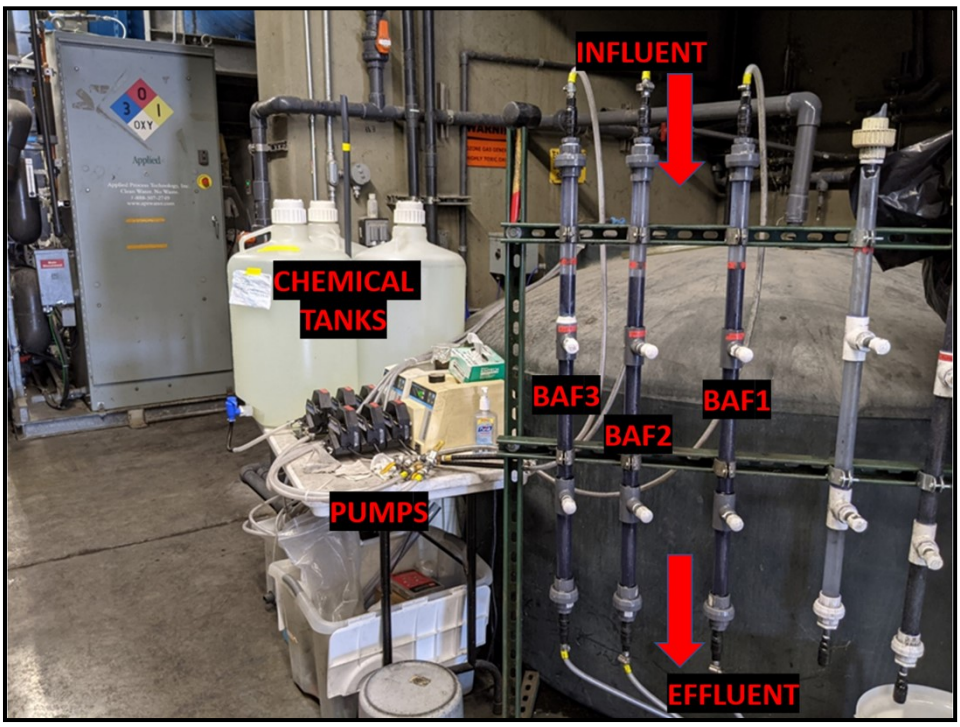

Figure S1. Experimental setup for the biofiltration columns.

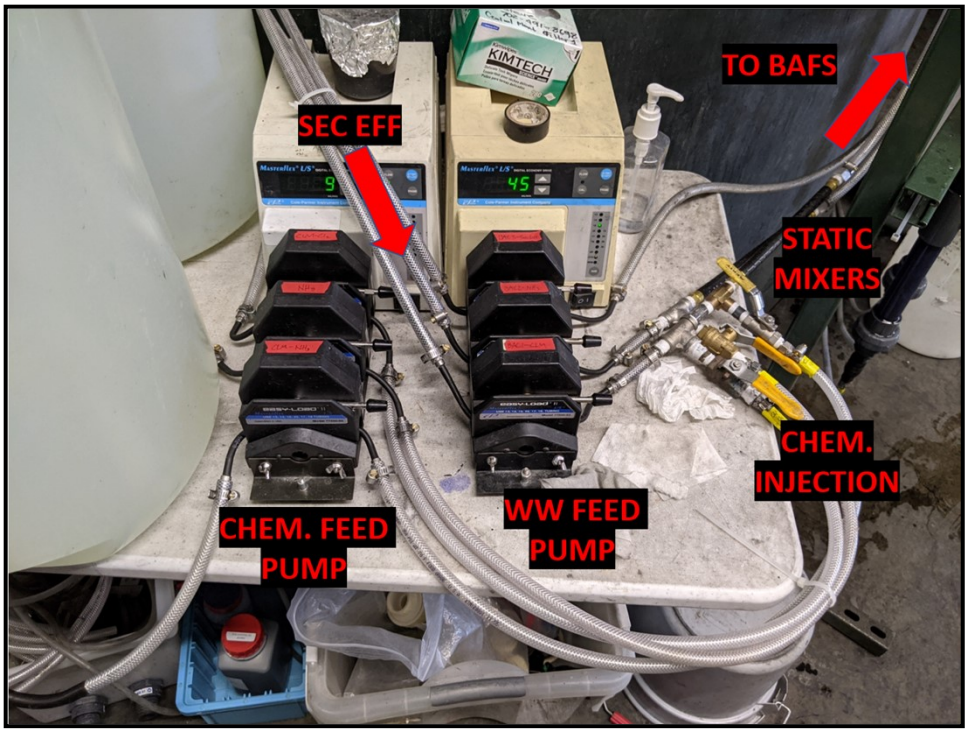

Figure S2. Secondary effluent and chemical feed pumps for the parallel biologically active filter (BAF) columns. During long-term operation, the chemical feeds consisted of sodium hypochlorite and/or ammonium sulfate.

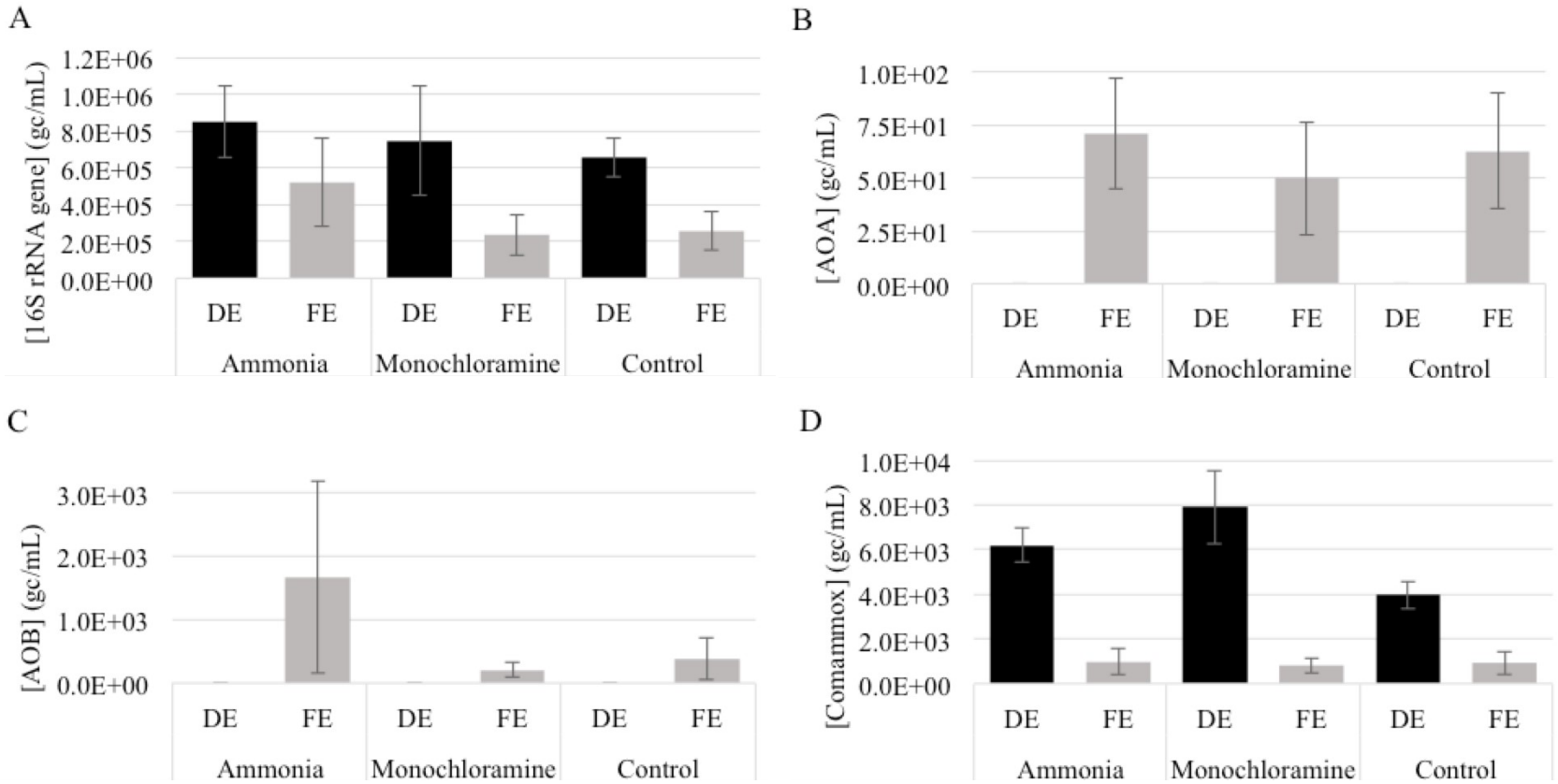

Figure S3. Comparison of the (A) 16S rRNA gene and (B-D) *amoA* gene concentrations: (B) AOA, (C) AOB, and (D) comammox. Data represent aqueous samples (i.e., BAF effluents) processed with direct extraction (DE) or extraction of membrane filters (FE). Data also distinguish BAFs fed with ammonia, chloramine, or ambient secondary effluent (i.e., control).

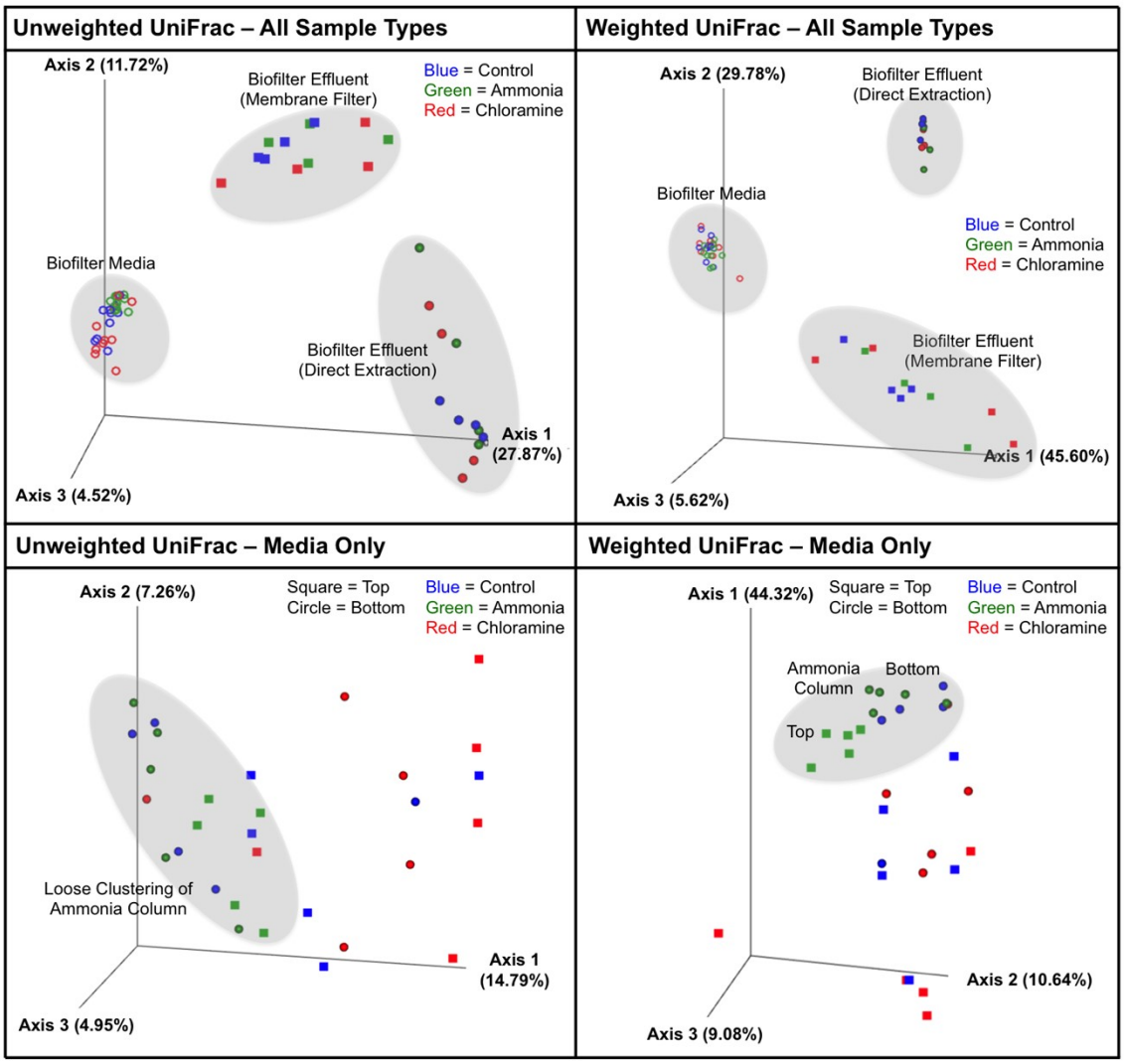

Figure S4. Prinicipal coordinate analyses (PCoAs) showing clustering of samples based on dissimilarities in microbial community structure. Samples are distinguished based on sample type (open circles = BAF media, closed circles = direct extraction of BAF effluent, squares = extraction of membrane-filtered BAF effluent) and treatment (blue = control, green = ammonia-dosed, red = chloramine-dosed).

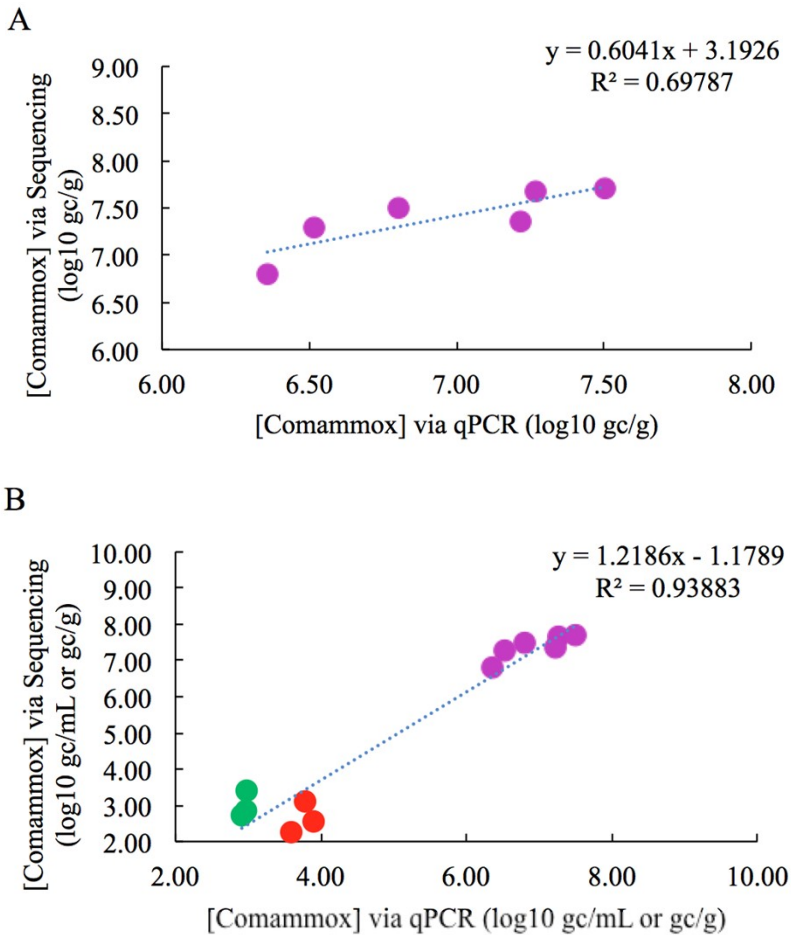

Figure S5. Correlations between comammox concentrations determined by (vertical axis) sequencing vs. (horizontal axis) qPCR. Sequencing-derived concentrations were calculated by multiplying relative comammox abundance by the corresponding 16S rRNA gene concentration. qPCR-derived concentrations were determined directly by quantifying the comammox *amoA* gene. Data are shown for (A) BAF media samples only and (B) a combination of (red) direct extracts of BAF effluents, (green) extraction of BAF effluents after membrane filtration, and (purple) BAF media.

Table S1. Limits of quantification (LoQs) for the qPCR assays.

qPCR assay	LoQ (gc/reaction)	LoQ (Cq value)
16S rRNA gene	31	29.57
AOA *amoA* gene	14	30.74
AOB *amoA* gene	22	30.58
Comammox *amoA* gene	13	30.84

Table S2. AOM taxa identified by 16S rRNA gene sequencing in this study.

AOM Group	Taxon
Recognized AOA	D_0_Archaea;D_1_Thaumarchaeota;D_2_Nitrososphaeria;D_3_Nitrosopumilales;D_4__Nitrosopumilaceae;D_5_Candidatus Nitrosotenuis
	D_0_Archaea;D_1_Thaumarchaeota;D_2_Nitrososphaeria;D_3_Nitrososphaerales;D_4__Nitrososphaeraceae;D_5_Candidatus Nitrocosmicus
Recognized AOB	D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Nitrosomonadaceae;D_5_Nitrosomonas
Other Nitrosomonadaceae	D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Nitrosomnonadaceae;
	D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Nitrosomonadaceae;D_5_966-1
	D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Nitrosomonadaceae;D_5_DSSD61
	D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Nitrosomonadaceae;D_5_GOUTA6
	D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Nitrosomonadaceae;D_5_IS-44
	D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Nitrosomonadaceae;D_5_mle1-7
	D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Nitrosomonadaceae;D_5_Ellin6067
	D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Nitrosomonadaceae;D_5_MND1
	D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Nitrosomonadaceae;D_5_oc32
	D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteriales;D_4_Nitrosomonadaceae;D_5_uncultured
	D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Nitrosococcales;D_4_Nitrosococcaceae;D_5_wb1-P19
Potential Comammox	D_0_Bacteria;D_1_Nitrospirae;D_2_Nitrospira;D_3__Nitrospirales;D_4_Nitrospiraceae;D_5_Nitrospira
Potential NOB	D_0_Bacteria;D_1_Nitrospinae;D_2_P9X2b3D02;D_3_Candidatus Tectomicrobia bacterium RIFCSPLOWO2_12_FULL_69_37
	D_0_Bacteria;D_1_Nitrospinae;D_2_P9X2b3D02;D_3_Ambiguous_taxa; D_4_Ambiguous_taxa; D_5_Ambiguous_taxa
	D_0_Bacteria;D_1_Nitrospirae;D_2_4-29-1;D_3_uncultured bacterium;D_4_uncultured bacterium;D_5_uncultured bacterium
	D_0_Bacteria;D_1_Nitrospirae;D_2_Nitrospira;D_3_Nitrospirales;D_4_Nitrospiraceae;D_5_Leptospirillum
	D_0_Bacteria;D_1_Nitrospirae;D_2_Thermodesulfovibrionia;D_3_uncultured
	D_0_Bacteria;D_1_Nitrospirae;D_2_Thermodesulfovibrionia;D_3_uncultured;D_4_uncultured bacterium;D_5_uncultured bacterium
	D_0_Bacteria;D_1_Nitrospirae;D_2_Thermodesulfovibrionia;D_3_uncultured;D_4_uncultured Nitrospiraceae bacterium;D_5_uncultured Nitrospiraceae
	D_0_Bacteria;D_1_Nitrospirae;D_2_Thermodesulfovibrionia;D_3_uncultured;D_4_uncultured Sphingobacteriales bacterium;D_5_uncultured Sphingobacteriales
	D_0_Bacteria;D_1_Nitrospirae;D_2_Thermodesulfovibrionia;D_3_uncultured;D_4_Ambiguous_taxa;D_5_Ambiguous_taxa

Table S3. Relative abundances of the three AOM groups based on 16S rRNA gene sequencing. Taxon grouping into AOA, AOB, or comammox was based on the classification shown in Table S1. Data are shown for secondary effluent, BAF effluent with direct extraction (DE) or membrane filter extraction (FE), and BAF media. Data also distinguish BAFs fed with ammonia, chloramine, or ambient secondary effluent (i.e., control).

Sample Type	Treatment	Sampling/ processing	AOA	AOB	Comammox
Secondary	N/A	HFUF[1]	ND	0.080±0.055%	0.126±0.083%
BAF Effluent	Ammonia	DE[2]	ND	0.196±0.216%	0.193±0.193%
BAF Effluent	Chloramine	DE[2]	ND	0.046±0.049%	0.054±0.014%
BAF Effluent	Control	DE[2]	ND	0.013%[5]	0.031±0.025%
BAF Effluent	Ammonia	FE[3]	0.008±0.007%	0.142±0.172%	0.484±0.606%
BAF Effluent	Chloramine	FE[3]	0.004±0.008%	0.037±0.019%	0.324±0.377%
BAF Effluent	Control	FE[3]	0.054±0.001%	0.060±0.049%	0.277±0.134%
Media	Ammonia	Top	0.047±0.038%	0.622±0.142%	2.933±0.707%
Media	Ammonia	Bottom	0.030±0.012%	0.157±0.033%	0.826±0.232%
Media	Chloramine	Top	0.056±0.048%	0.210±0.229%	5.351±1.810%
Media	Chloramine	Bottom	0.042±0.022%	0.094±0.038%	1.743±0.845%
Media	Control	Top	0.032±0.023%	0.097±0.045%	4.081±1.975%
Media	Control	Bottom	0.049±0.032%	0.107±0.070%	1.138±1.088%

[1]HFUF = hollow fiber ultrafiltration; [2]DE = direct extraction; [3]FE = membrane filter extraction; [4]ND = non-detect; [5]identified in only one sample

Text S1. DNA extraction protocol.

1. Add 4 g of media to 50 mL tube
2. Add 3 mL of lysis buffer
4. Mix by gently inverting the tube by hand for 5 min
5. Add 200 µL lysozyme (50 mg/mL) and 100 µL proteinase K (10 mg/mL)
5. Incubate at 37°C for 30 min in an incubator
6. Add 1 mL sodium dodecyl sulfate (SDS) (20%)
7. Incubate at 55°C for 1 hour while gently inverting by hand every 30 min
8. Centrifuge at 3500×g for 10 min
9. Collect the supernatant
10. Re-extract the pellet twice as follows:
    a) Add 2 mL of lysis buffer
    b) Mix gently by inverting the tube
    c) Incubate at 55°C for 10 min
    d) Centrifuge at 3500×g for 10 min
    e) Collect the supernatant
    f) Repeat steps (a-e) one more time
11. Combine the 3 supernatants
12. Add equal volume of chloroform isoamyl alcohol and parafilm the tube
13. Mix gently by swirling
14. Centrifuge at 3500×g for 10 min
15. Transfer the aqueous phase to a new tube
16. Add 0.6×volume of isopropanol
17. Split sample into 2 mL aliquots
18. Incubate at 4°C overnight
19. Centrifuge at 13,000 rpm for 20 min
20. Carefully discard the supernatant
21. Wash the pellet with 500 µL of cold ethanol (70%)
22. Resuspend the pellet in 100 µL of Tris-EDTA (TE) buffer

Buffers

Lysis buffer, pH 8

100 mM Tris-HCl
100 mM sodium EDTA
1.5 M NaCl
1% CTAB

TE buffer, pH 8

10 mM Tris-HCl
1 mM sodium EDTA