

Supplementary Materials:

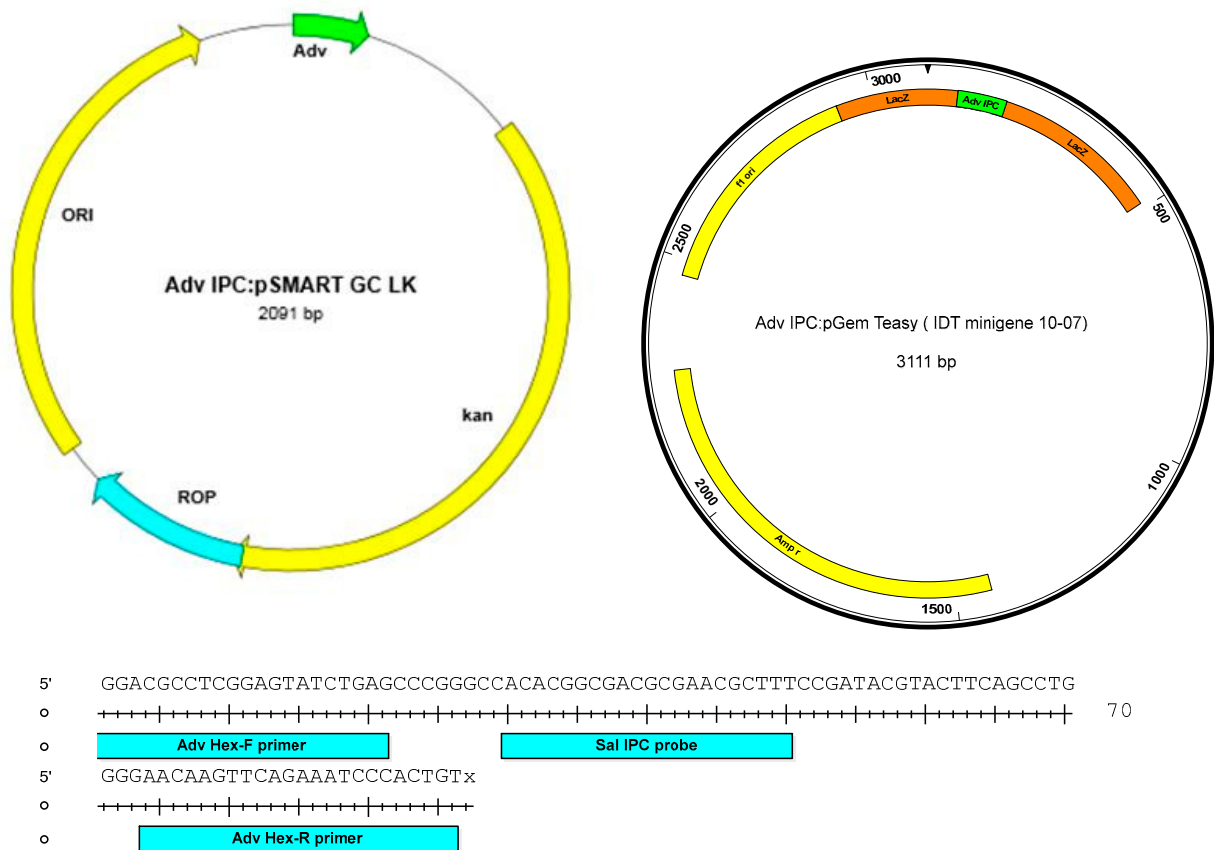


Figure S1. Graphical representation of the Adv IPC plasmids.

A synthetic DNA sequence, AdvIPC, was inserted into two different vectors: pSMART (left) and pGEM-Teasy plasmid (Right). The inserted sequence is shown below (bottom). The synthetic sequence consisted of the portion of the adenovirus hexon gene between nucleotides 17651 and 17746 of Adenovirus 41 Tak (NCBI accession number DQ315364) with nucleotides to 17679 to 17700 replaced with a probe sequence not found in nature [41].

Table S1. QPCR Parameters using the Roche LC480 Thermocycler

Target	Cycling Parameters	Number of Cycles	Temperature (°C)/Time (mm:ss)
blaSHV/TEM	Initial denaturation	1	95/01:00
	Denaturation of DNA		95/00:10
	Annealing and extension	50	60/00:45
sul1	Initial denaturation	1	
	Denaturation of DNA		95/00:10
	Annealing of primers/probe	50	50/00:15
	Extension		72/00:15
	Melt	1	60-95°C
Bacterial 16S rDNA	Initial denaturation	1	95/00:30
	Denaturation of DNA		95/00:10
	Annealing of primers/probe	45	50/01:00
	Extension		72/00:20
AdvIPC	Initial denaturation	1	95/02:00
	Denaturation of DNA		95/00:15
	Annealing and extension	50	65/00:30
Salmon SPC	Initial denaturation	1	95/10:00
	Denaturation of DNA	50	95/00:30
	Annealing and extension		60/00:60

Table S2. QPCR Parameters using the RotorGene Thermocycler

Target	Cycling Parameters	Number of Cycles	Temperature (°C)/Time (mm:ss)
Bacterial 16S rDNA	Initial denaturation	1	95/00:30
	Denaturation of DNA		95/00:10
	Annealing	45	50/01:00
	Extension		72/00:20
AdvIPC	Initial denaturation	1	95/01:00
	Denaturation of DNA		95/00:10
	Annealing	45	55/00:30
	Extension		68/00:20
Salmon SPC	Initial denaturation	1	95/10:00
	Denaturation of DNA		95/00:30
	Annealing and extension	45	60/00:60

The limit of detection (LOD) was determined for each qPCR according to rational provide by Bustin et. al. (2009) and Kralik et. al. (2017). At least ten replicate qPCRs were performed with ten-fold dilutions of plasmid standards. The lowest concentration that was detected in at least 90% of the assays was identified as the LOD. In addition, the efficiency and mean square error (LightCycler480) or r^2 (RotorGene) were compiled for each qPCR. Limit of quantification (LOQ) was also determined according to Kralik et. al. (2017) as the lowest detectable concentration with a coefficient of variation below 25%. The coefficient of variation for the *sul1*, *bla_{SHV/TEM}*, and *16S* qPCRs at the LOD concentrations were all below 25% indicating that there was no difference between the LOD and LOQ values.

The *bla_{SHV/TEM}* hydrolysis probe qPCR was designed to detect a wide variety of SHV and TEM β -lactamase variants to increase the likelihood of detecting the resistance genes in the treatment matrices. Alignments of both TEM and SHV-class β -lactamase genes were performed using the DNASTAR software (Megalign application) and sequences downloaded from the NCBI sequence database. Eighteen SHV and 51 TEM-type β -lactamase genes were aligned and scanned manually for probable qPCR primers and probes. The target region identified as being most favorable for the detection of both SHV and TEM genes was between nucleotide 370 and 695 yielding a PCR product of 325 nucleotides (based on β -lactamase TEM-1 sequence accession number EF035622). The primers were examined *in silico* for specificity using the NCBI primer BLAST tool (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and were shown to match only β -lactamase genes. Furthermore, DNA sequencing of fifteen amplicons from different wastewater matrices showed that all readable sequences matched β -lactamase genes in the NCBI Genbank sequence database.

The *bla_{SHV/TEM}* qPCR was linear over eight orders of magnitude (Figure S2) and showed a limit of detection (LOD) of 70 copies/ μ l (positive in $\geq 95\%$ of qPCRs) with an average efficiency of $95\% \pm 17\%$ and error of 0.12 ± 0.18 (n=68).

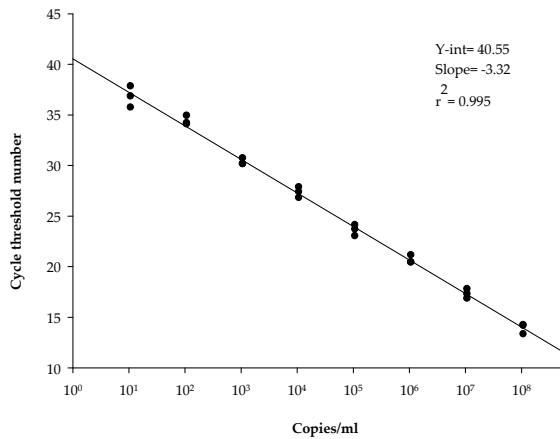


Figure S2. TEM-SHV β -lactamase qPCR characterization. Serial ten-fold dilutions of a plasmid containing a *bla*_{SHV/TEM} gene were analyzed in triplicate with the *bla*_{SHV/TEM} qPCR using the LightCycler480. The plot shows qPCR results from a single representative experiment. Linear regression statistics and the r^2 value are depicted in the box located in the upper right corner.

The *sul1* qPCR showed a LOD of 6 copies/ μ l (positive in $\geq 95\%$ of qPCRs) with an average efficiency of $86\% \pm 6\%$ (n=18) and error (calculated as mean square error not r^2) of 0.095.

Assessment of the total bacteria was done by a qPCR targeting a portion of the 16S ribosomal DNA gene that is well conserved across a broad spectrum of bacteria, as described by Harms et. al. (2003). Samples were pretreated with a heat-labile dsDNase prior to addition of the template to reduce the amount of bacterial DNA in the commercial master mix preparation. The detection limit was determined to be 57 copies/ μ l (positive in $\geq 95\%$ of qPCRs) with an average efficiency of $99\% \pm 5\%$ (n= 17) using the Qiagen RotorGene thermocycler. The linearity was assessed using the r^2 metric resulting in an average of $0.99 (\pm 0.01)$.

The salmon SPC qPCR was performed as described previously [44]. The AdvIPC qPCR targeted an artificial DNA insert not found in nature. It demonstrated a detection limit of 10 copies/ μ l with an average efficiency of $97\% \pm 6\%$ and an r^2 of 0.998 ± 0.002 (n=22).

Table S3. The quantity of *bla_{SHV/TEM}*, *sul1* and bacterial 16S genes in full-scale WRP treatment processes: solids fraction.

WRP matrix ^a	<i>bla_{SHV/TEM}</i> (copies/L) ^b	positive samples	<i>sul1</i> (copies/L) ^b	positive samples	16S rDNA (copies/L) ^b	positive samples
Raw	1.41x10 ⁷ ±3.31x10 ⁶	3/3	7.61x10 ⁷ ±4.43x10 ⁷	3/3	6.58x10 ⁹ ±5.14x10 ⁹	3/3
AS	1.95x10 ⁷ ±1.06x10 ⁷	9/9	1.82x10 ¹⁰ ±2.36x10 ¹⁰	9/9	1.21x10 ¹² ±7.22x10 ¹¹	9/9
SE	1.09x10 ⁵ ±9.24x10 ⁴	3/3	3.03x10 ⁷ ±1.85x10 ⁷	3/3	5.77x10 ⁸ ±1.74x10 ⁸	3/3
FE	<5.30x10 ³	0/6	7.89x10 ³ ±9.18x10 ³	3/6	4.57x10 ⁵ ±6.30x10 ⁵	6/6
Filter backwash	1.55x10 ⁶ ±1.91x10 ⁶	5/5	ND	-	ND	-

^a Samples were collected from a full-scale tertiary WRP. Final effluent refers to tertiary-treated water that was chlorinated and de-chlorinated. Backwash was collected from the filtration tanks during the backwash cycle after approximately 24-hours of continuous use. SE and FE samples were concentrated by HFF. AS: activated sludge; SE: secondary effluent; FE: final effluent.

^b Results are in qPCR copies per L of original matrix ± standard deviation. Final effluents were obtained as grab samples prior to entering the distribution piping. ND= Not done. Averages were calculated using the qPCR concentrations of positive samples and the limit of detection for all samples that were negative. "Less than" values denote all samples were negative and the concentration given represents the LOD for each assay.

Table S4. The quantity of *bla_{SHV/TEM}*, *sul1* and bacterial 16S genes in full-scale WRP treatment processes: dissolved fraction.

WRP matrix ^a	<i>bla_{SHV/TEM}</i> (copies/L) ^b	positive samples	<i>sul1</i> (copies/L) ^b	positive samples	16S rDNA (copies/L) ^b	positive samples
Raw	6.31x10 ⁶ ±2.32x10 ⁶	3/3	1.04x10 ⁹ ±1.70x10 ⁹	3/3	6.22x10 ⁹ ±3.03x10 ⁹	3/3
AS	9.29x10 ⁵ ±4.46x10 ⁵	3/9	3.57x10 ⁷ ±2.57x10 ⁷	9/9	1.46x10 ⁹ ±1.43x10 ⁹	9/9
SE	9.17x10 ³ ±4.55x10 ²	3/3	1.88x10 ⁶ ±1.39x10 ⁶	3/3	3.60x10 ⁸ ±2.43x10 ⁸	3/3
FE	<5.30x10 ³	0/6	1.68x10 ⁴ ±1.85x10 ⁴	5/6	6.32x10 ⁵ ±8.38x10 ⁵	6/6
Filter backwash	3.29x10 ⁵ ±2.16x10 ⁵ ^d	2/5	ND	-	ND	-

^a Samples were collected from a full-scale WRP. Final effluent refers to tertiary-treated water that was chlorinated and de-chlorinated. Backwash was collected from the filtration tanks during the backwash cycle after approximately 24-hours of continuous use. SE and FE samples were concentrated by HFF. AS: activated sludge; SE: secondary effluent; FE: final effluent.

^b Results are in qPCR copies per L of original matrix ± standard deviation. Final effluents were obtained as grab samples prior to entering the distribution piping. ND= Not done. Averages were calculated using the qPCR concentrations of positive samples and the limit of detection for all samples that were negative. "Less than" values denote all samples were negative and the concentration given represents the LOD for each assay.

Table S5. The ratio of solids and dissolved fractions for different wastewater matrices.

Solids (SA) to dissolved (DF) ratios						
	16S	St. Dev.	sul1	St. Dev.	blas _{HV/TEM}	St. Dev.
<i>Raw (SA/DF)</i>	1.94E+00		2.67E+00		3.96E+00	
	1.08E+00		1.48E+00		2.42E+00	
	1.65E-01		9.82E-03		1.28E+00	
<i>Average=</i>	1.06E+00	8.86E-01	1.38E+00	1.33E+00	2.55E+00	1.34E+00
<i>AS (SA/DF) (collected with HFF FE samples)</i>	1.01E+04		1.32E+03		1.09E+01	
	2.75E+03		7.61E+02		1.53E+01	
	3.84E+03		1.47E+03		8.85E+00	
	1.43E+04		3.77E+03		1.12E+01	
	1.75E+02		3.82E+02		2.31E+01	
	6.57E+02		6.19E+02		4.17E+00	
<i>Average=</i>	5.31E+03	5.68E+03	1.39E+03	1.24E+03	1.23E+01	6.44E+00
<i>AS (SA/DF) (collected with HFF SE samples)</i>	6.42E+02		9.38E+01		1.80E+02	
	6.21E+02		2.01E+02		1.57E+02	
	3.03E+02		7.33E+01		3.73E+01	
<i>Average=</i>	5.22E+02	1.90E+02	1.23E+02	6.84E+01	1.25E+02	7.67E+01
<i>HFF SE (SA/DF)</i>	2.31E+00		9.98E+00		7.42E+00	
	2.08E+00		2.09E+01		5.12E+00	
	1.18E+00		3.72E+01		2.26E+01	
<i>Average=</i>	1.86E+00	6.00E-01	2.27E+01	1.37E+01	1.17E+01	9.51E+00
<i>HFF FE (SA/DF)</i>	9.91E-01		5.25E-01		1.00E+00	
	7.29E-01		4.98E-01		1.00E+00	
	1.41E+00		8.37E-01		1.00E+00	
	4.69E-01		2.64E-01		1.00E+00	
	7.54E-02		1.73E+00		1.00E+00	
	5.06E-01		1.00E+00		1.00E+00	
<i>Average=</i>	6.96E-01	4.62E-01	8.09E-01	5.20E-01	1.00E+00	0.00E+00
<i>Combined AS data</i>						
<i>Average=</i>	3.71E+03	5.09E+03	9.65E+02	1.17E+03	4.98E+01	6.38E+01

Table S6. ANOVA statistical analysis of ARGs and 16S qPCR concentration between different water types.

Sample ^a	<i>bla</i> _{SHV/TEM} vs <i>sul1</i>		Number of	
	^b	<i>bla</i> _{SHV/TEM} vs 16S ^b	<i>sul1</i> vs 16S ^b	samples
Raw to AS (SF)	1.00E-03	1.00E-03	1.88E-01	3
Raw to AS (DF)	>0.05	>0.05	>0.05	3
AS to SE HFF (SF)	>0.05	3.00E-03	1.00E-03	3
AS to SE HFF (DF)	>0.05	>0.05	>0.05	3
SE HFF to FE HFF (SF)	2.00E-03 ^c	2.00E-03 ^c	>0.05	6
SE HFF to FE HFF (DF)	2.00E-03 ^c	2.00E-03 ^c	>0.05	6
AS to FE HFF (SF)	2.00E-03 ^c	2.00E-03 ^c	>0.05	6
AS to FE HFF (DF)	3.00E-03 ^c	3.00E-03 ^c	>0.05	6
Raw to FE HFF (SF)	>0.05 ^c	1.00E-03 ^c	>0.05	3
Raw to FE HFF (DF)	8.00E-03 ^c	>0.05 ^c	>0.05	3

^a The qPCR data (copy/L) was log transformed and subtracted between two matrices to determine the log increase or decrease between two stages. The log difference data were compared via one way ANOVA (SigmaPlot) between the three gene targets. AS: activated sludge; SE: secondary effluent; FE final effluent; HFF: hollow fiber filtration concentrated; SF: solids associated fraction; DF: dissolved fraction. Where normality or equal variance tests failed the Kruskal-Wallis One Way Analysis of Variance on Ranks was used for statistical comparisons.

^b ANOVA *p*-values listed. Statistically significant differences are indicated by *p*-values less than 0.05.

^c Note that the *bla*_{SHV/TEM} concentrations were below the LOD for all final effluent samples and the assay's LOD was used in these calculations however, the statistical relevance should be interpreted cautiously.

Table S7. Statistical comparison of the *bla* or *sul1* to 16S rDNA ratio between different water types.

<i>Water matrices</i> ^a	Solids-associated		Dissolved	
	<i>bla</i> :16S t-test ^a	<i>Sul</i> :16S t-test ^a	<i>bla</i> :16S t-test ^a	<i>Sul</i> :16S t-test ^a
<i>Raw vs AS</i>	0.015	0.244	0.035	0.807
<i>AS vs SE</i>	0.074	0.359	0.036	0.145
<i>SE vs FE</i>	0.018 ^b	0.005	0.044 ^b	0.016
<i>Raw vs SE</i>	0.004	0.358	0.035	0.807
<i>Raw vs FE</i>	0.671 ^b	0.006	0.499 ^b	0.077
<i>AS vs FE</i>	0.036 ^b	0.002	0.389 ^b	0.015

^a The qPCR concentrations (copies/L) for each gene target were log transformed and divided by the log transformed 16S concentrations. Each ratio was compared between different wastewater matrices via t-test (Microsoft Excel). Statistically significant differences are indicated by p-values less than 0.05. SE and FE samples were HFF concentrated. AS: activated sludge; SE: secondary effluent; FE final effluent; HFF: hollow fiber filtration concentrated; SF: solids associated fraction; DF: dissolved fraction.

^b The red font denotes samples where the ARG was below LOD for all samples analyzed. The assay's LOD was used in these situations.

Table S8. Reduction of exogenous plasmid DNA in tertiary filtration and chlorination processes.

Sample ^a	Dissolved	Dissolved	Solids	Solids
	fraction ^b	fraction + chlorine ^b	fraction ^b	fraction + chlorine ^b
<i>Pre-filtration</i>	1.23×10 ⁸ ±	2.06×10 ⁶ ±	8.79×10 ⁶ ±	1.11×10 ⁵ ±
	1.17×10 ⁸	5.29×10 ⁶	1.39×10 ⁷	2.27×10 ⁵
<i>20 min. filtrate</i>	1.07×10 ⁷ ±	2.84×10 ² ±	1.33×10 ⁵ ±	<3.00×10 ²
	7.47×10 ⁶	9.10×10 ¹ ^c	9.28×10 ⁴	
<i>90 min. filtrate</i>	1.39×10 ⁷ ±	<3.16×10 ²	1.60×10 ⁶ ±	<3.00×10 ² ^c
	1.04×10 ⁷		1.56×10 ⁶	

^a Samples collected prior to filtration but after plasmid addition and at different times during the filtration. Filtrate refers to the water collected immediately after filtration. The last sample was taken after the last volume had entered the filter column.

^b Data for the dissolved and solids-associated fractions represent the averages and standard deviations from eight independent filtration/disinfection experiment (seven for the solids fraction). Samples that were below the LOD were assigned the LOD. Units are copies per ml of original matrix.

^c One of the eight experiments showed a positive qPCR signal but below the quantification limit of the assay and therefore the LOD value was assigned.

Table S9. Comparison of plasmid concentrations between the solids-associated and dissolved fractions during filtration and disinfection.

Sample ^a	Average log difference		Rank sum test p-
	(n=7)^b	t-test p-value ^c	value ^c
<i>Pre-filtration</i>	1.39	0.028	0.021
<i>Pre-filtration + chlorine</i>	0.506	0.342	0.867
<i>Post-filtration</i>	1.17	0.007	0.014
<i>Post-filtration + chlorine</i>	-	Below LOD	Below LOD

^a Upon addition of the plasmid, samples were collected before and after filtration. Post-filtration refers to the 90 minute time point after filtration had begun.

^b Each individual sample was log transformed and the difference calculated between both fractions. The average of the seven log differences is given.

^c Two-tailed t-test was performed assuming unequal variance between the solids-associated and dissolved fractions of each type of sample using MS Excel (n=7). Rank sum performed on SigmaPlot version 11 (n=7). "Below LOD" signifies that seven of the data points were below LOD therefore accurate statistical determinations cannot be made.

Table S10. Turbidity measurements on full-scale WRP waters prior to HFF concentration.

Sample date	Water type	Turbidity (NTU)
8/1/16	Secondary effluent	1.58
8/8/16	Secondary effluent	0.98
8/29/16	Secondary effluent	1.57
9/7/16	Tertiary, disinfected effluent	0.37
9/19/16	Tertiary, disinfected effluent	0.55
9/28/16	Tertiary, disinfected effluent	0.62
10/11/16	Tertiary, disinfected effluent	0.53
10/18/16	Tertiary, disinfected effluent	0.69
10/24/16	Tertiary, disinfected effluent	0.72

Table S11. Individual qPCR data from all full-scale WRP samples.

Raw qPCR data in copies/L (not log transformed)							
Units are qPCR copies/L of matrix	qPCR concentrations: Solids-associated fraction				qPCR concentrations: Dissolved fraction		
	16S	sul1	bla		16S	sul1	bla
Raw (SA)	8.48E+09	8.12E+07	1.47E+07	Raw (DF)	4.38E+09	3.04E+07	3.71E+06
	1.05E+10	1.18E+08	1.70E+07		9.73E+09	7.96E+07	7.05E+06
	7.54E+08	2.94E+07	1.05E+07		4.57E+09	3.00E+09	8.17E+06
Average=	6.58E+09	7.61E+07	1.41E+07	Average=	6.22E+09	1.04E+09	6.31E+06
AS (SA)	3.03E+12	7.98E+10	1.28E+07	AS (DF)	2.98E+08	6.05E+07	1.18E+06
	9.56E+11	1.38E+10	1.81E+07		3.48E+08	1.81E+07	1.18E+06
	6.00E+11	9.94E+09	1.04E+07		1.56E+08	6.78E+06	1.18E+06
	1.40E+12	1.15E+10	1.33E+07		9.77E+07	3.05E+06	1.18E+06
unconcentrated samples collected along with HFF FE	7.48E+11	1.90E+10	2.73E+07	unconcentrated samples collected along with HFF FE	4.28E+09	4.97E+07	1.18E+06
	9.80E+11	7.44E+09	4.92E+06		1.49E+09	1.20E+07	1.18E+06
Average=	1.28E+12	2.36E+10	1.45E+07	Average=	1.11E+09	2.50E+07	1.18E+06
AS (SA)	1.14E+12	6.00E+09	3.23E+07	AS (DF)	1.78E+09	6.40E+07	1.79E+05
unconcentrated samples collected along with HFF SE	1.17E+12	1.30E+10	1.94E+07	unconcentrated samples collected along with HFF SE	1.88E+09	6.48E+07	1.23E+05
	8.60E+11	3.09E+09	3.66E+07		2.84E+09	4.22E+07	9.80E+05
Average 3 samples above=	1.06E+12	7.37E+09	2.94E+07	Average 3 samples above=	2.17E+09	5.70E+07	4.28E+05
Average of all AS samples=	1.21E+12	1.82E+10	1.95E+07	Average of all AS samples=	1.46E+09	3.57E+07	9.29E+05
HFF SE (SA)	5.69E+08	2.97E+07	6.42E+04	HFF SE (DF)	2.46E+08	2.98E+06	8.66E+03
	4.07E+08	4.91E+07	4.78E+04		1.95E+08	2.35E+06	9.34E+03
	7.54E+08	1.20E+07	2.15E+05		6.40E+08	3.23E+05	9.52E+03
Average=	5.77E+08	3.03E+07	1.09E+05	Average=	3.60E+08	1.88E+06	9.17E+03
HFF FE (SA)	5.96E+05	2.14E+04	5.30E+03	HFF FE (DF)	6.01E+05	4.08E+04	5.30E+03
	1.68E+06	1.72E+04	5.30E+03		2.30E+06	3.45E+04	5.30E+03
	1.43E+05	8.40E+02	5.30E+03		1.02E+05	1.00E+03	5.30E+03
	1.90E+05	6.18E+03	5.30E+03		4.06E+05	2.34E+04	5.30E+03
	1.02E+04	8.40E+02	5.30E+03		1.35E+05	4.86E+02	5.30E+03
	1.25E+05	8.40E+02	5.30E+03		2.47E+05	8.40E+02	5.30E+03
Average=	4.57E+05	7.89E+03	5.30E+03	Average=	6.32E+05	1.68E+04	5.30E+03

Red font indicates samples resulted in a negative qPCR and were assigned the assays LOD.

Data set includes the AS data from the HFF FE and HFF SE samples. SE grab and FE grab data were omitted because of the high percentage of negative samples. HFF samples showed a higher rate of qPCR signals above LOD. The qPCR data for the AS(DF) samples collected with the HFF SE samples had a standard curve that detected one-log lower than the LOD resulting in positive concentrations that were below the LOD but are reported here because they were within the quantifiable range and above background.

Table S12. Solids and dissolved plasmid qPCR data from pilot-scale media filtration experiments.

Units for all results are copies per ml matrix. Eluted in 30 ul EB													
Experiment Date	Chlorine Solution made		Diluted date	2nd Eff+DNA SN		2nd Eff+DNA SN+C		2nd Eff+DNA Pellet		2nd Eff+DNA P+C			
	7/26/2012	7/13/2012	7/26/2012	4.96E+07		7.39E+05		1.07E+06		1.26E+05			
	8/6/2012	7/13/2012	8/6/2012	3.66E+05		4.55E+02		--		--			
	9/5/2012	9/4/2012	9/5/2012	7.52E+07		3.16E+02		1.86E+06		7.14E+02			
	9/10/2012	9/7/2012	9/7/2012	1.17E+08		1.51E+07		3.60E+06		6.15E+05			
Post-Shock Treatment 10/9/2012		10/9/2012	10/9/2012	3.51E+07		1.21E+02		7.77E+06		2.26E+04			
Post-Shock Treatment 10/16/12		10/16/2012	10/16/2012	2.92E+08		1.40E+04		1.85E+06		9.63E+03			
Post-Shock Treatment 10/22/12		10/22/2012	10/22/2012	3.12E+08		2.77E+05		3.99E+07		2.74E+03			
Post-Shock Treatment 10/29/12		10/29/2012	10/29/2012	1.01E+08		2.07E+04		5.46E+06		3.00E+02			
			average	1.23E+08		2.02E+06		8.79E+06		1.11E+05			
			St. Dev.	1.17E+08		5.29E+06		1.39E+07		2.27E+05			
Note that the qPCR for the 8-6-12 pellet fractions did not meet the efficiency requirement and thus the data was not used.													
All data checked and valid as of 8-4-17. RR													
Legend. 2nd Eff= secondary effluent; +DNA= with AdvIPC plasmid added, P=pellet fraction; SN= supernatant fraction; C or Chl= chlorinated; copies= qPCR copies				Note that the limit of detection for this set of qPCR runs was 50 copies/PCR tube. 5ul template added per PCR tube. DNA extracted from 1ml matrix (0.95ml for SN) was eluted in 30ul									
Chlorine treated vs no chlorine		Copies/ml matrix		Copies/ml matrix		Conc in pellet vs SN		Copies/ml matrix		2nd Eff+DNA Pellet		2nd Eff+DNA P+C	
Log difference (copy/ml)		2nd Eff+DNA Pellet		2nd Eff+DNA P+C		% of total				Log transformed		Log transformed	
0.93		1.07E+06		1.26E+05		2.10		7/26/2012		6.03		5.10	
-- --				--				8/6/2012					
3.42		1.86E+06		7.14E+02		2.42		9/5/2012		6.27		2.85	
0.77		3.60E+06		6.15E+05		2.98		9/10/2012		6.56		5.79	
2.54		7.77E+06		2.26E+04		18.14		Post-Shock Treatment		6.89		4.35	
2.28		1.85E+06		9.63E+03		0.63		Post-Shock Treatment		6.27		3.98	
4.16		3.99E+07		2.74E+03		11.35		Post-Shock Treatment		7.60		3.44	
4.26		5.46E+06		3.00E+02		5.11		Post-Shock Treatment		6.74		2.48	
2.62		8.79E+06		1.11E+05		6.11		Average difference		2.62		Pellet + Chlorine	
1.42		1.39E+07		2.27E+05		6.36		St. Dev difference		1.42			

Chlorine treated											
Units for all results are copies per ml matrix. Eluted in 30 ul EB											
Experiment Date	Chlorine Solution made		Diluted date	20-min Pellet	20-min P+C	90-min Pellet	90-min P+C	20-min SN	20-min SN+C	90-min SN	90-min SN+C
	7/26/2012	7/13/2012	7/26/2012	7.23E+04	3.00E+02	1.91E+05	5.76E+00	2.35E+07	5.84E+01	3.02E+07	3.16E+02
	8/6/2012	7/13/2012	8/6/2012	--	-- --	--	--	4.26E+04	3.16E+02	7.71E+04	3.16E+02
	9/5/2012	9/4/2012	9/5/2012	1.82E+05	3.00E+02	4.68E+05	3.00E+02	1.34E+07	3.16E+02	9.57E+06	3.16E+02
	9/10/2012	9/7/2012	9/7/2012	8.61E+04	3.00E+02	1.47E+05	3.00E+02	1.26E+07	3.16E+02	6.44E+06	3.16E+02
Post-Shock Treatment 10/9/2012		10/9/2012	10/9/2012	1.43E+05	3.00E+02	4.44E+06	3.00E+02	2.74E+06	3.16E+02	4.48E+06	3.16E+02
Post-Shock Treatment 10/16/12		10/16/2012	10/16/2012	8.70E+04	3.00E+02	1.30E+06	3.00E+02	1.58E+07	3.16E+02	2.39E+07	3.16E+02
Post-Shock Treatment 10/22/12		10/22/2012	10/22/2012	4.32E+04	3.00E+02	2.49E+06	3.00E+02	1.07E+07	3.16E+02	1.82E+07	3.16E+02
Post-Shock Treatment 10/29/12		10/29/2012	10/29/2012	3.15E+05	3.00E+02	2.18E+06	3.00E+02	7.07E+06	3.16E+02	1.84E+07	3.16E+02
			Average cpy/ml	1.33E+05	3.00E+02	1.60E+06	3.00E+02	1.07E+07	2.84E+02	1.39E+07	3.16E+02
			St. Dev. cpy/ml	9.28E+04	0.00E+00	1.56E+06	1.11E+02	7.47E+06	9.10E+01	1.04E+07	6.08E-14
Red font= below LOD.											
Less than values were given the value of the detection limit for the assay which is 50 copies/PCR											
Note that the 90 min P+C gave qPCR signal but beyond the detection limit of the assay. The raw data is shown but the detection limit of the assay was used for calculation purposes.											
Log difference (copy/ml matrix)											
	Sample date			2nd Eff+DNA Pellet	20-min Pellet	20-min P+C	90-min Pellet	90-min P+C	2nd Eff+DNA		
1.07E+06	2.38			7/26/2012	6.03	4.86	2.48	5.28	0.76	5.10	
--	#VALUE!			8/6/2012							
1.86E+06	3.16			9/5/2012	6.27	5.26	2.48	5.67	2.48	2.85	
3.60E+06	2.22			9/10/2012	6.56	4.94	2.48	5.17	2.48	5.79	
7.77E+06	2.78			Post-Shock Treatment 10/9/2012	6.89	5.15	2.48	6.65	2.48	4.35	
1.85E+06	2.46			Post-Shock Treatment 10/16/12	6.27	4.94	2.48	6.11	2.48	3.98	
3.99E+07	1.97			Post-Shock Treatment 10/22/12	7.60	4.64	2.48	6.40	2.48	3.44	
5.46E+06	3.70			Post-Shock Treatment 10/29/12	6.74	5.50	2.48	6.34	2.48	2.48	
				Average difference		1.58	4.14	0.68	4.39	2.62	
				St. Dev difference		0.66	0.52	0.47	0.60	1.42	
Log removal= log (2nd eff+DNA)-log(Sample + chlorine)											
Log difference (copy/ml matrix)											
2nd Eff+DNA Supernatant (cpy/ml matrix)	Sample date			2nd Eff+DNA Super	20-min SN	20-min SN+C	90-min SN	90-min SN+C	2nd Eff+DNA SN	2nd Eff+DNA SN+C	
4.96E+07	7/26/2012			7.70	7.37	1.77	7.48	2.50	7.70	5.87	
3.66E+05	8/6/2012			5.56	4.63	2.50	4.89	2.50	5.56	2.66	
7.52E+07	9/5/2012			7.88	7.13	2.50	6.98	2.50	7.88	2.50	
1.17E+08	9/10/2012			8.07	7.10	2.50	6.81	2.50	8.07	7.18	
3.51E+07	Post-Shock Treatment 10/9/2012			7.54	6.44	2.50	6.65	2.50	7.54	2.08	
2.92E+08	Post-Shock Treatment 10/16/12			8.47	7.20	2.50	7.38	2.50	8.47	4.15	
3.12E+08	Post-Shock Treatment 10/22/12			8.49	7.03	2.50	7.26	2.50	8.49	5.44	
1.01E+08	Post-Shock Treatment 10/29/12			8.01	6.85	2.50	7.26	2.50	8.01	4.32	
				Average difference		1.00	5.31	0.88	5.21	3.44	
				St. Dev difference		0.35	0.96	0.34	0.93	1.61	

Units for all results are copies per ml matrix. Eluted in 30 ul EB						
Experiment Date	Chlorine Solution made	Diluted date	Post-Column BLANK SN	Post-Column BLANK SN+C	Post-Column BLANK Pellet	Post-Column BLANK P+C
7/26/2012	7/13/2012	7/26/2012	--	0.00E+00	0.00E+00	0.00E+00
8/6/2012	7/13/2012	8/6/2012	0.00E+00	0.00E+00	--	--
9/5/2012	9/4/2012	9/5/2012	0.00E+00	0.00E+00	0.00E+00	0.00E+00
9/10/2012	9/7/2012	9/7/2012	0.00E+00	0.00E+00	3.16E+03	0.00E+00
Post-Shock Treatment 10/9/2012	10/9/2012	10/9/2012	0.00E+00	0.00E+00	0.00E+00	0.00E+00
Post-Shock Treatment 10/16/12	10/16/2012	10/16/2012	0.00E+00	0.00E+00	0.00E+00	0.00E+00
Post-Shock Treatment 10/22/12	10/22/2012	10/22/2012	0.00E+00	0.00E+00	0.00E+00	0.00E+00
Post-Shock Treatment 10/29/12	10/29/2012	10/29/2012	0.00E+00	0.00E+00	0.00E+00	0.00E+00
Post column blank= filtrate sample taken before the plasmid was added, negative control. BLANK= sample of the secondary effluent collected before the addition of the plasmid, negative control. Post shock treatment= after the filter matrix was treated with a high dose of chlorine to decrease bio-fouling						
Experiment Date	Chlorine Solution made	Diluted date	2nd Eff BLANK SN	2nd Eff BLANK SN+C	2nd Eff BLANK Pellet	2nd Eff BLANK P+C
7/26/2012	7/13/2012	7/26/2012	<50	<50	<50	<50
8/6/2012	7/13/2012	8/6/2012	<50	<50	--	--
9/5/2012	9/4/2012	9/5/2012	<50	<50	<50	<50
9/10/2012	9/7/2012	9/7/2012	<50	<50	<50	<50
Post-Shock Treatment 10/9/2012	10/9/2012	10/9/2012	<50	<50	<50	<50
Post-Shock Treatment 10/16/12	10/16/2012	10/16/2012	<50	<50	<50	<50
Post-Shock Treatment 10/22/12	10/22/2012	10/22/2012	<50	<50	<50	<50
Post-Shock Treatment 10/29/12	10/29/2012	10/29/2012	<50	<50	<50	<50