



Title	Free-Chlorine Disinfection as a Selection Pressure on Norovirus
Author(s)	Rachmadi, Andri Taruna; Kitajima, Masaaki; Watanabe, Kozo; Yaegashi, Sakiko; Serrana, Joeselle; Nakamura, Arata; Nakagomi, Toyoko; Nakagomi, Osamu; Katayama, Kazuhiko; Okabe, Satoshi; Sano, Daisuke
Citation	Applied and Environmental Microbiology, 84(13), UNSP e00244-18 <a href="https://doi.org/10.1128/AEM.00244-18">https://doi.org/10.1128/AEM.00244-18</a>
Issue Date	2018-07
Doc URL	<a href="http://hdl.handle.net/2115/71221">http://hdl.handle.net/2115/71221</a>
Rights(URL)	<a href="https://creativecommons.org/licenses/by/4.0/">https://creativecommons.org/licenses/by/4.0/</a>
Type	article
Additional Information	There are other files related to this item in HUSCAP. Check the above URL.
File Information	Supplemental material.pdf



[Instructions for use](#)

Table S1. Growth rate and doubling time for each clone isolated from the chlorine-treated population (A1, A2, A3) and the control population (X1, X2, X3)

Clone	Growth Rate ( $\mu$ )/h	Doubling Time (H)
A1	$0.76 \pm 0.02$	$0.91 \pm 0.02$
A2	$0.77 \pm 0.02$	$0.90 \pm 0.02$
A3	$0.80 \pm 0.01$	$0.87 \pm 0.01$
X1	$0.83 \pm 0.01$	$0.86 \pm 0.05$
X2	$0.82 \pm 0.02$	$0.84 \pm 0.03$
X3	$0.81 \pm 0.01$	$0.85 \pm 0.01$

Table S2. Relative replicative fitness between clones isolated from the chlorine-treated population (A1, A3, A2) and the control population (X1, X2, X3)

Selection coefficient (s) value			
Clone	X1	X2	X3
A1	0.07	0.07	0.06
A2	0.06	0.07	0.05
A3	0.03	0.04	0.02

Table S3. Temperature profile of PCR for each seven regions from MNV ORF2 and ORF3.

Region	Temperature (°C)	Time	Cycle number
1	94	2 min	1
	94	30 s	20
	66	45 s	
	72	45 s	
	72	7 min	1
	4	∞	1
2	94	2 min	1
	94	30 s	20
	68	45 s	
	72	45 s	
	72	7 min	1
	4	∞	1
3	94	2 min	1
	94	30 s	20
	67	45 s	
	72	45 s	
	72	7 min	1
	4	∞	1
4	94	2 min	1
	94	30 s	20
	66	45 s	
	72	45 s	
	72	7 min	1
	4	∞	1
5	94	2 min	1
	94	30 s	20
	70	45 s	
	72	45 s	
	72	7 min	1
	4	∞	1
6	94	2 min	1
	94	30 s	20
	64	45 s	
	72	45 s	
	72	7 min	1
	4	∞	1
7	94	2 min	1
	94	30 s	20
	64	45 s	
	72	45 s	
	72	7 min	1
	4	∞	1

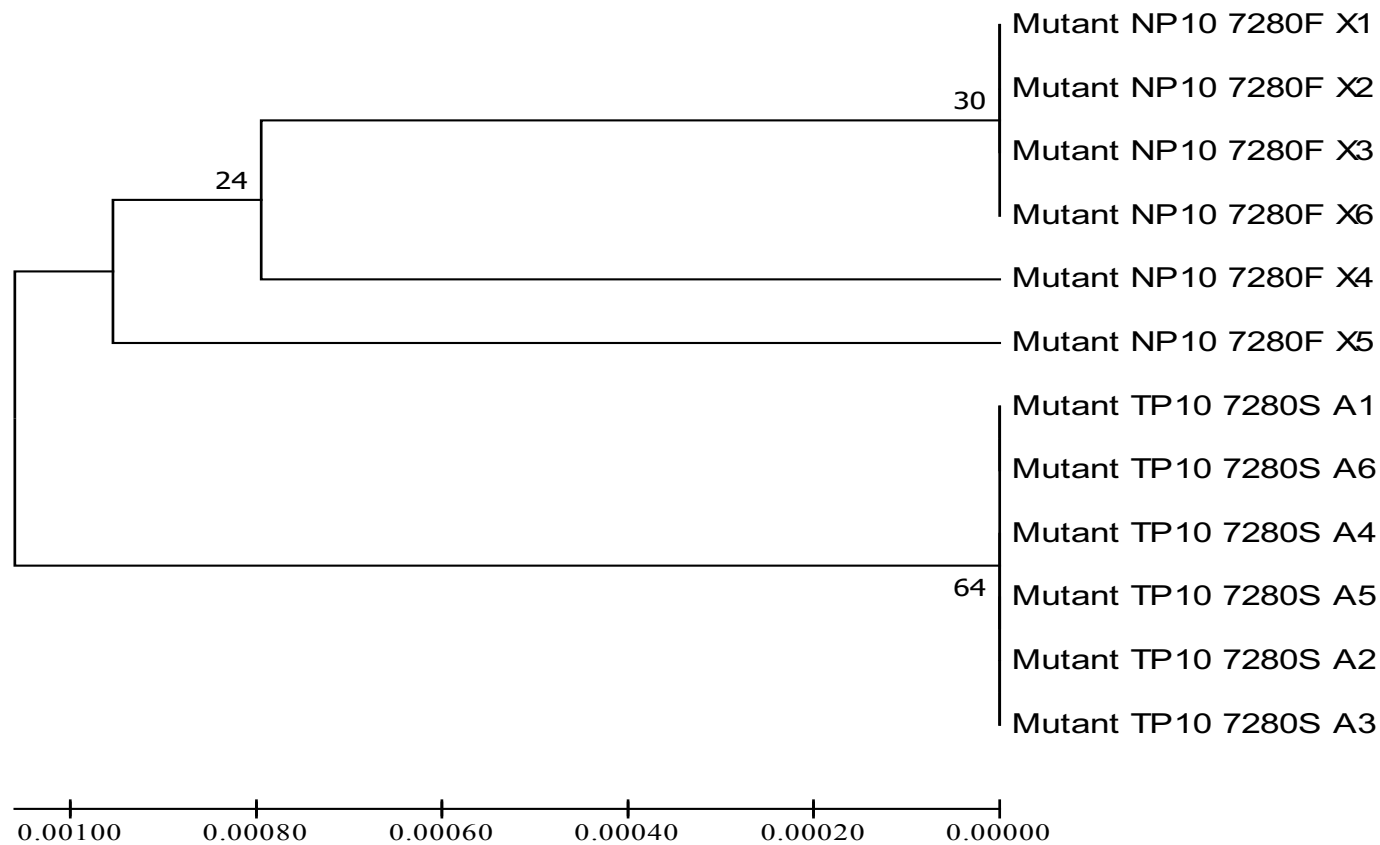


Fig. S1. A phylogenetic tree of the chlorine-treated clones (A1, A2, A3, A4, A5, A6) and the control clones (X1, X2, X3, X4, X5, X6) derived from a murine norovirus S7 lineage. Forward and reverse SANGER sequences for each strain were merged using ExPasy website. Merged sequences were aligned by MUSCLE and a phylogenetic tree was generated using bootstrap method with 5000 replications in MEGA 7 software. Evolutionary distances were computed using Tamura-Nei model, and evolutionary history was inferred using UPGMA method.

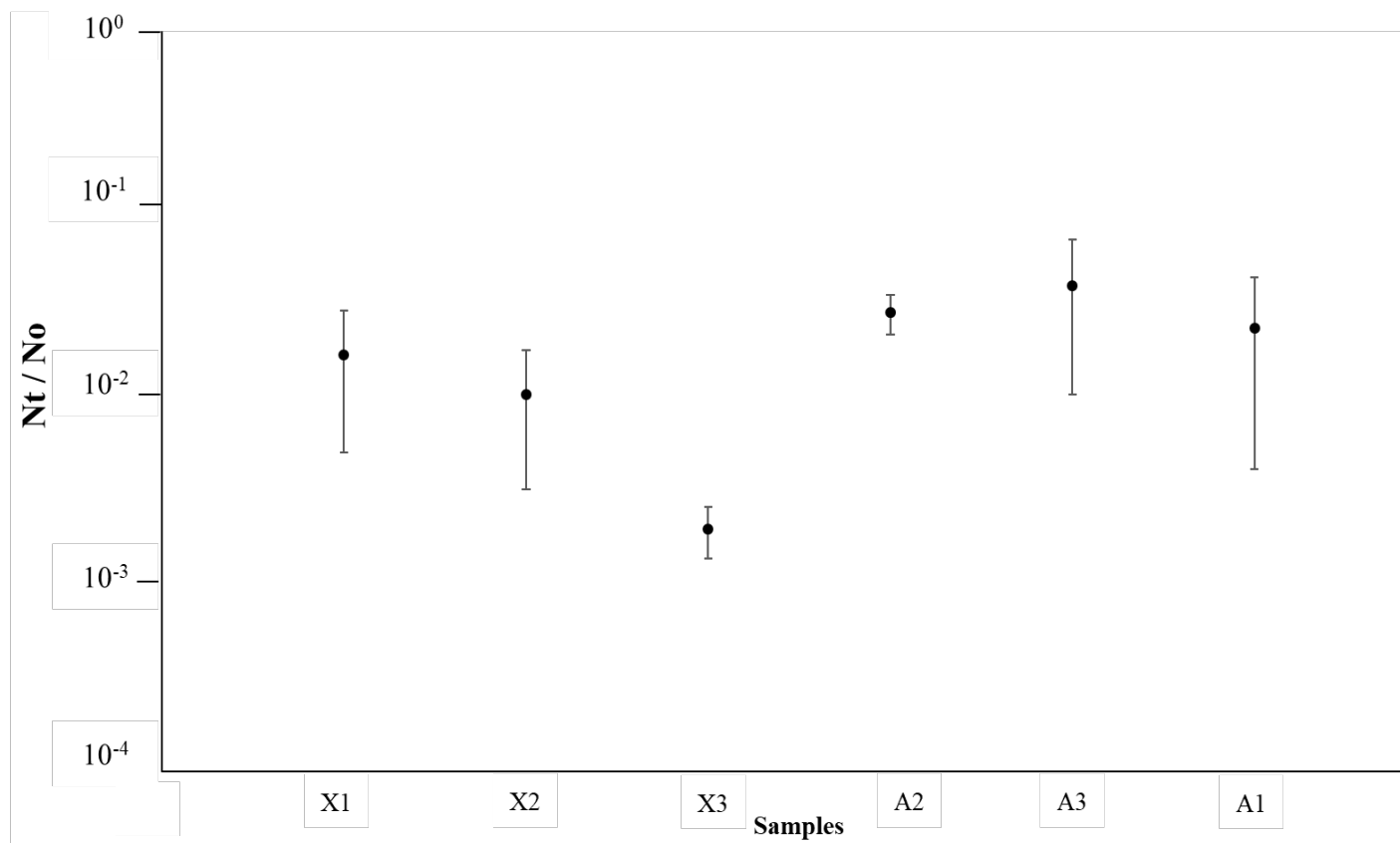


Fig. S2. The ratio of MNV concentration in the supernatant after incubation at 4 °C for 90 minutes ( $N_t$ ) to the initial MNV concentration ( $N_0$ ).