## 1 SUPPLEMENTARY TABLES

<sup>3</sup> *coli* O157:H7 suppression in the August 2008 experiment  $(n=4)^{a}$ 

	Frequency of 31 candidate $TRF^{b}$								
Dpi <sup>c</sup>	Bedding source <sup>d</sup>	Fluorescence NH > HT (A) <sup>e</sup>	Negative Regression (B) <sup>f</sup>	Fluorescence and Regression $(A + B)^{g}$	$\mathrm{TRF}^h$				
1	Fresh	10	8	4	99, 466, 480, 488				
1	In-use <sup><i>i</i></sup>	6	2	0					
1	Washed <sup><i>i</i></sup>	6	0	0					
1	Recycled <sup><i>i</i></sup>	0	17	0					
15	Fresh	5	18	4	75, <b>130</b> , <b>139</b> , <b>156</b>				
15	In-use <sup><i>i</i></sup>	11	23	10	66, <b>130</b> , 136, <b>139</b> ,				
					142, 153, <b>156</b> , 490,				
					492, 495				
15	Washed <sup><i>i</i></sup>	6	0	0					
15	Recycled	3	10	0					

<sup>a</sup> Farm-derived livestock bedding samples each split into a non-heated (NH) and a heated (HT) sample were used for 456789 a TRF analysis: TRFs <300 nt were at an accuracy of  $\pm 1$  nt; TRFs >300 nt were binned at  $\pm 2$  nt. Fragments that occurred in more than one context are in bold.

<sup>b</sup> Thirty-one candidate TRFs with higher fluorescence in NH than the corresponding HT of a particular bedding source were identified by visual inspection of interval plots.

<sup>c</sup> After cooling, samples were inoculated with a suspension of *E. coli* O157:H7, and incubated at 21 °C in the dark in 10 the laboratory. One and 15 dpi, colony forming units (CFU) of E. coli O157:H7 were determined, and DNA was 11 extracted.

12 <sup>d</sup> Livestock bedding from a sand recycling system at four independent strata: (i) Fresh, front of the cow box; (ii) In-

13 use, back of the cow box; (iii) Washed, used material from the pile following the sand manure separation; and (iv)

14 Recycled, rested ( $\geq 2$  days) in a pile of washed material.

15 <sup>e</sup> The frequency of NH with stronger fluorescence than HT equivalents was determined by Mood's Median test at P 16 = 0.05; parameter (A).

17 <sup>f</sup> The frequency of the thirty-one candidate fragments that had a negative regression with CFUs of *E. coli*; parameter 18 **(B)**.

19 <sup>g</sup> The frequency of TRFs that fulfilled both requirement (A) and (B).

20 <sup>h</sup> Identification of TRFs that fulfilled both requirements (A) and (B).

21 <sup>*i*</sup> CFU of *E. coli* O157:H7 were only numerically higher in HT than NT bedding material.

<sup>2</sup> TABLE S1. Terminal restriction fragments (TRF) of heat-sensitive bacteria associated with E.

Dpi <sup>c</sup>	Bedding source <sup>d</sup>	Fluorescence $NH > HT (A)^{e}$	Negative Regression (B) <sup>f</sup>	Fluorescence and Regression $(A + B)^{g}$	$\mathrm{TRF}^h$
1	Fresh	18	2	0	
1	In-use <sup>i</sup>	17	16	7	<b>96, 145, 151, 299, 488,</b> 545, <b>548</b>
1	Washed <sup>i</sup>	13	12	4	<b>96, 99, 299</b> , 556
1	Recycled	11	5	1	118
7	Fresh	31	16	11	<b>87</b> , <b>89</b> , <b>94</b> , <b>112</b> , <b>115</b> , <b>142</b> , 149, <b>151</b> , <b>299</b> , 496, 541
7	In-use <sup>i</sup>	28	44	26	<b>87</b> , <b>89</b> , 92, <b>94</b> , <b>96</b> , <b>99</b> , <b>112</b> , <b>115</b> , <b>130</b> , <b>136</b> , 139, <b>142</b> , <b>145</b> , 147, 149, <b>297</b> , <b>299</b> , 456, 466, 469, <b>483</b> , 486, <b>488</b> , 496, 541, 550
7	Washed	35	0	0	
7	Recycled	32	24	19	<b>94</b> , <b>99</b> , <b>118</b> , 122, <b>130</b> , <b>136</b> , 156, 202, 272, 275, 278, 280, 282, <b>297</b> , <b>299</b> , <b>483</b> , 546, <b>548</b> , 551

TABLE S2. Terminal restriction fragments (TRF) of heat-sensitive bacteria associated with *E*. *coli* O157:H7 suppression in the November 2008 experiment,  $n=8^a$ 

<sup>*a*</sup> Farm-derived livestock bedding samples each split into non-heated (NH) and heated (HT), and used for TRF analysis; TRFs <300 nt were at an accuracy of  $\pm 1$  nt; TRFs >300 nt were binned at  $\pm 2$  nt. Fragments that occurred in more than one context are in bold.

<sup>b</sup> Sixty-seven candidate TRFs with higher fluorescence in NH than the corresponding HT of a particular source were determined by visual inspection of interval plots.

<sup>c</sup> After cooling, samples were inoculated with a suspension of *E. coli* O157:H7, and incubated in the dark at 21 °C in the laboratory. One and seven dpi, colony forming units (CFU) of *E. coli* O157:H7 were determined and DNA extracted.

<sup>*d*</sup> Livestock bedding from a sand recycling system at eight independent strata of each of the following: (i) Fresh, front of the cow box; (ii) In-use, back of the cow box; (iii) Washed, used material from the pile following the sand manure separation; and (iv) Recycled, rested ( $\geq 2$  days) in a pile of washed material.

<sup>*e*</sup> The frequency of NH with stronger fluorescence than HT equivalents was determined by Mood's Median test at P = 0.05; parameter (A).

<sup>*f*</sup> The frequency of the sixty-seven candidate fragments that had a negative regression with CFUs of *E. coli* O157:H7; parameter (B).

<sup>g</sup> The frequency of TRFs that fulfilled both requirement (A) and (B).

<sup>h</sup> Identification of TRFs that fulfilled both requirements (A) and (B).

Dpi <sup>c</sup>	Bedding	Fluorescence	Negative $\mathbf{P}_{\text{regariser}}(\mathbf{P})^{f}$	Fluorescence and $\mathbf{P}_{accession}$	$\mathrm{TRF}^h$
	source	$N\Pi > \Pi I (A)$	Regression (B)	Regression $(A + B)^{\circ}$	
1	Fresh	33	19	11	<b>89</b> , 139, <b>142</b> , <b>145</b> ,
					<b>149</b> , <b>156</b> , <b>184</b> , 465,
					486, 533, <b>537</b>
1	In-use	27	38	17	66, <b>85</b> , <b>89</b> , <b>96</b> , <b>142</b> ,
					149 152, 156, 184,
					299, 308, 492, 495,
					<b>537</b> , 548, <b>550</b> , 554
1	Washed	21	24	5	<b>87, 89, 142, 492</b> , 508
1	Recycled	21	28	17	<b>89</b> , <b>96</b> , 99, <b>147</b> , <b>156</b> ,
					<b>299</b> , 304, <b>308</b> , 488,
					<b>492</b> , <b>495</b> , 500, 503,
					505, <b>547</b> , <b>550</b> , 555
10	Fresh	15	17	2	163, <b>550</b>
10	In-use	30	35	15	<i>85</i> , <b>87</b> , 136, <b>142</b> , <b>145</b> ,
					<b>147</b> , <b>152</b> , 163, 202,
					222, 304, 488, <b>492</b> ,
					<b>495</b> , 499
10	Washed	24	16	3	92, 94, <b>547</b>
10	Recycled	17	15	1	122

TABLE S3. Terminal restriction fragments (TRF) of heat-sensitive bacteria associated with *E. coli* O157:H7 suppression in the March 2009 experiment,  $n=8^{a}$ 

<sup>*a*</sup> Farm-derived livestock bedding samples each split into non-heated (NT) and heated (HT), and used for TRF analysis; TRFs <300 nt were at an accuracy of  $\pm 1$  nt; TRFs >300 nt were binned at  $\pm 2$  nt. Fragments that occurred in more than one context are in bold.

<sup>b</sup> Seventy-eight candidate TRFs with higher fluorescence in NH than the corresponding HT of a particular source were determined by visual inspection of interval plots.

<sup>c</sup> After cooling, samples were inoculated with a suspension of *E. coli* O157:H7, and incubated at 21 °C in the dark in the laboratory. One and ten dpi, colony forming units (CFU) of *E. coli* O157:H7 were determined and DNA extracted.

<sup>*d*</sup> Livestock bedding from a sand recycling system at eight independent strata of each of the following: (i) Fresh, front of the cow box; (ii) In-use, back of the cow box; (iii) Washed, used material from the pile following the sand manure separation; and (iv) Recycled, rested ( $\geq 2$  days) in a pile of washed material.

<sup>*e*</sup> The frequency of NH with stronger fluorescence than HT equivalents was determined by Mood's Median test at P = 0.05; parameter (A).

<sup>f</sup> The frequency of the seventy-eight candidate fragments that had a negative regression with CFUs of *E. coli* O157:H7 were noted; parameter (B).

<sup>g</sup> The frequency of TRFs that fulfilled both requirement (A) and (B).

<sup>h</sup> Identification of TRFs that fulfilled both requirements (A) and (B).

- 1 TABLE S4. Terminal restriction fragments (TRF) of bacteria found to be more abundant
- 2 in non-heated (NH) recycled or washed bedding samples on 1 dpi or 7 dpi, respectively,
- 3 associated with *E. coli* O157:H7 suppression in the 10 November Experiment,  $n=8^{a}$

Recyc Dpi <sup>d</sup>	cled <sup>c</sup> versus	Frequence Fluorescence Suppressive > other (A) <sup>e</sup>	ey of 29 candidat Negative regression (B) <sup>f</sup>	te TRF <sup>b</sup> Fluorescence and Regression $(A + B)^g$	$\mathrm{TRF}^h$
1	Fresh <sup>c</sup>	13	9	6	118, 297, 299,
1	In-use <sup>c</sup>	9	9	5	302, 306, 508 83, 118, 299, 302, 306
1	Washed <sup>c</sup>	7	9	4	83, 118, 302, 508
Washed <sup>c</sup> versus		Frequenc	TRFs <sup>h</sup>		
7	Fresh <sup>c</sup>	12	n/a'	n/a	<b>118, 202</b> , 269, 272, 275, 276, <b>278, 280, 282</b> , 297, <b>543</b> , 548
7	In-use <sup>c</sup>	10	n/a	n/a	92, 118, 202, 272, 275, 276, 278, 280, 282, 543
7	Recycled <sup>cj</sup>	10	n/a	n/a	200, 202, 543 92, 118, 202, 272, 275, 276, 278, 280, 282, 543

4 <sup>a</sup> Farm-derived livestock bedding samples each split into a non-heated (NH) and a heated (HT) sample were 5 used for TRF analysis; TRFs <300 nt were at an accuracy of  $\pm 1$  nt; TRFs >300 nt were binned at  $\pm 2$  nt. 6 TRFs that occurred in more than one context are in **bold**; values of NH samples were compared. 7 <sup>b</sup> Twenty-nine (or 28) candidate TRFs with higher fluorescence in the most suppressive NH source 8 compared to the other NH sources at 1 dpi or 7 dpi were determined by visual inspection of interval plots. 9 <sup>c</sup> Livestock bedding from a sand recycling system at eight independent strata of each of the following: (i) 10 Fresh, front of the cow box; (ii) In-use, back of the cow box; (iii) Washed, used material from the pile 11 following the sand manure separation; and (iv) Recycled, rested ( $\geq 2$  days) in a pile of washed material. 12 <sup>d</sup> After cooling, samples were inoculated with a suspension of *E. coli* O157:H7, and incubated in the dark at 13 21 °C in the laboratory. One and seven dpi, colony forming units (CFU) of E. coli O157:H7 were 14 determined and DNA extracted. 15 <sup>e</sup> The frequency of the more suppressive source with stronger fluorescence than the less suppressive 16 sources was determined by Mood's Median test at P = 0.05; parameter (A). 17 <sup>f</sup> The frequency of the 29 candidate fragments that had a negative regression with CFUs of *E. coli*; 18 parameter (B). 19 <sup>g</sup> The frequency of TRFs that fulfilled both requirement (A) and (B). 20 <sup>h</sup> Identification of TRFs that fulfilled both requirements (A) and (B); only (A) for samples from 7 dpi. 21 <sup>i</sup> No CFUs were detected in the Washed bedding material. No test for regression could be conducted. The 22 higher frequency of TRFs is considered an overestimate of those involved in E. coli O157:H7 23 suppressiveness. 24 <sup>1</sup> CFU of *E. coli* O157:H7 were only numerically higher in the Recycled than the Washed bedding material. 25

- 1 TABLE S5. Terminal restriction fragments (TRF) of bacteria found to be more abundant
- 2 in non-heated (NH) recycled bedding samples and concomitant with E. coli O157:H7

				-	_	-
3	suppression	in a	23	March	Experiment,	$n=8^a$

	Frequency of 14 candidate $TRF^{b}$						
Dpi <sup>c</sup>	Recycled <sup>d</sup>	Fluorescence	Negative	Fluorescence and	$\mathrm{TRF}^h$		
-	versus	Suppressive > other	regression in	Regression (A +			
		$(A)^e$	Recycled $(B)^{f}$	$\mathbf{B})^{g}$			
1	Fresh <sup><i>id</i></sup>	8	10	7	99, <b>308</b> , <b>500</b> , <b>505</b> ,		
					<b>508</b> , 555, <b>623</b>		
1	In-use <sup>d</sup>	5	10	4	500, 505, 508, 623		
1	Washed <sup>d</sup>	4	10	3	304, <b>308</b> , <b>505</b>		
	Washed <sup>d</sup>	Frequen	cy of 11 candidate	TRFs			
	versus	-					
10	Fresh <sup>d</sup>	3	3	0			
10	In-use <sup>dj</sup>	2	3	0			
		Frequen	cy of 11 candidate	TRFs			
	Recycled <sup>d</sup>	-	-				
	versus						
10	Fresh <sup>d</sup>	3	0	0			
10	In-use <sup>dj</sup>	2	0	0			

<sup>a</sup> Farm-derived livestock bedding samples each split into a non-heated (NH) and a heated (HT) sample, and

5 used for TRF analysis; TRFs <300 nt were at an accuracy of  $\pm 1$  nt; TRFs >300 nt were binned at  $\pm 2$  nt.

6 TRFs that occurred in more than one context are high-lighted in bold; values of NH samples were compared.

<sup>b</sup> Fourteen (11, 11) candidate TRFs with higher fluorescence in more suppressive than the less suppressive
were determined by visual inspection of interval plots.

10 <sup>c</sup> After cooling, samples were inoculated with a suspension of *E. coli* O157:H7, and incubated at 21 °C in

the dark in the laboratory. One and ten dpi, colony forming units (CFU) of *E. coli* O157:H7 were
determined and DNA extracted.

13 <sup>d</sup> Livestock bedding from a sand recycling system at eight independent strata of each of the following: (i)

14 Fresh, front of the cow box; (ii) In-use, back of the cow box; (iii) Washed, used material from the pile

following the sand manure separation; and (iv) Recycled, rested ( $\geq 2$  days) in a pile of washed material.

<sup>e</sup> The frequency of the more suppressive source with stronger fluorescence than the less suppressive

17 sources was determined by Mood's Median test at P = 0.05 and recorded; parameter (A).

<sup>f</sup> The frequency of the candidate TRFs that had a negative regression with CFUs of *E. coli* O157:H7;
parameter (B).

20 <sup>g</sup> The frequency of TRFs that fulfilled both requirement (A) and (B).

<sup>*h*</sup> Identification of TRFs that fulfilled both requirements (A) and (B).

22 <sup>*i*</sup> The CFU of *E. coli* O157:H7 were not significantly different from the counts in Recycled samples.

<sup>23</sup> <sup>*j*</sup> The CFU of *E. coli* O157:H7 were not significantly different from the counts in Washed, Recycled, or

Fresh samples.