

Measurement of DNA degradation in *E coli* by DuoMax using comparative qPCR analysis of three amplicons

TEST PRODUCT: DUOMAX GENERAL PURPOSE CLEANER

TESTED FOR: DUOTECH LTD

PROJECT CODE: BT-DUO-17A-02

SAMPLE RECEIVED: 16 December 2011

TEST PERFORMED: 31 July to 13 August 2012

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Purpose

To assess the DNA degradation resulting from treatment of *E. coli* bacterial cells with DuoMax General Purpose Cleaner from Duotech Ltd. This was performed as a modification of an EN 13697 surface test where analysis was performed for cell viability and this compared to the degradation of *E. coli* DNA.

Methodology

The procedure was carried out according to EN 13697 against *E. coli* ATCC 10536 with modifications. Saline was used to replace water in the “water control”. The viability of controls was assessed for EN 13697, and NT, NC and water (SALINE) controls were also performed for DNA extraction and qPCR analysis, to ensure that residual product did not affect the performance of qPCR. An additional Ultraviolet (UV 254nm wavelength) light treatment was performed for 400 seconds.

The aim was to utilize the property that damaged DNA inhibits PCR, using quantitation of long (~1,500 nt), medium (~500 nt) and short (~100 nt) amplicons from bacterial 16S DNA is used to measure the degree of DNA damage in a cell. For all qPCR for the small, medium and large amplicons, the relationship between bacterial cell DNA equivalents/ml and Ct is determined. This data can then be used to calibrate the effects of treatment to give an apparent loss of bacterial DNA, and therefore assess the damage to DNA caused by treatment, using three amplicon lengths. The details of the amplification are shown below and are taken from: Knut et al. Int J Environ Res Public Health 2010 7:3376 – 3381.

Amplicons			Thermocycling		
Name	Primer sequence	Position *	Denaturation	Annealing	Synthesis
Short F	GAAGAAGCACCGGCTAAC	529	95 °C – 30s	50 °C – 30s	72 °C – 30s
Short R	GCT TTACGCCAGTCATTC	611			
Medium F	TCCTACGGGAGGCAGCAGT	375	95 °C – 30s	63 °C – 30s	72 °C – 45s
Medium R	GGACTACCAGGGTATCTAATCCTGTT	841			
Long F	AAGAGTTTGATCATGGCTCA	42	95 °C – 30s	55°C – 30s	72 °C – 90s
Long R	CGGTTACCTTGTACGACTT	1546			

Control viability data according to the EN 13697 procedure

Test organisms	Bactericidal test suspension			Validation test				Water control (SALINE)			Test procedure at concentrations % V/V of the <u>working concentration</u>											
				NT		NC		Nc			1.00			50.00			100.00					
<i>Escherichia coli</i>	10 ⁻⁶ :	>330	; >330	10 ⁻³ :	>330 ; >330	10 ⁻³ :	>330 ; >330	10 ⁻³ :	17	; 31	10 ⁰ :	ND	; ND	10 ⁰ :	ND	; ND	10 ⁰ :	ND	; ND			
	10 ⁻⁷ :	>330	; >330	10 ⁻⁴ :	6 ; 21	10 ⁻⁴ :	10 ; 11	10 ⁻⁴ :	12	; 4	10 ⁻¹ :	ND	; ND	10 ⁻¹ :	ND	; ND	10 ⁻¹ :	ND	; ND			
ATCC 10536	10 ⁻⁸ :	37	33	10 ⁻⁵ :	2 ; 4	10 ⁻⁵ :	0 ; 2	10 ⁻⁵ :	1	; 3	10 ⁻² :	ND	; ND	10 ⁻² :	ND	; ND	10 ⁻² :	ND	; ND			
								10 ⁻⁶ :	0	; 0	Nts:	18		Nts:	0		Nts:	0				
								Nts:	>100		Nd:	ND		Nd:	ND		Nd:	ND				
	N:	8.24		NT:	6.13	NC:	6.02	Nc:	5.38		ME:	ND		ME:	ND		ME:	ND				

Definitions

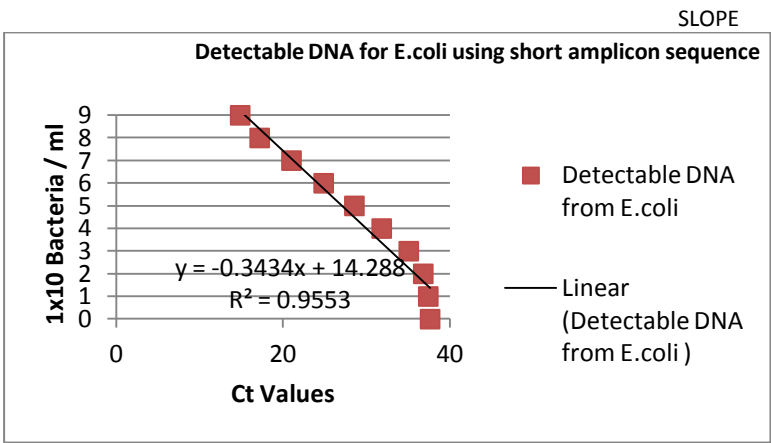
Cfu = Colony forming units; N = Log₁₀ number of cfu per 0.05ml of test suspension; Nc = Log₁₀ number of cfu recovered from the test surface of the water control (SALINE); Nd = Log₁₀ number of cfu recovered from the test surface of the disinfectant test; Nts = number of cfu remaining on test surface; NC = Log₁₀ number of cfu recovered from the neutralisation control; NT = Log₁₀ number of cfu recovered from the neutralisation test; ME = Microbiocidal Effect (Log₁₀ reduction)

The test procedure was carried out on treated samples according to EN 13697 then as detailed below, following DNA extraction.

Calibration of bacterial cell count/ml against Ct value. Short amplicon sequence

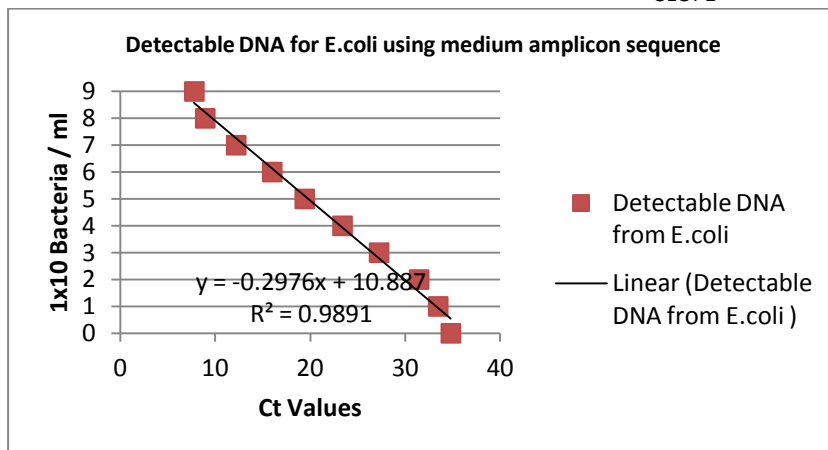
Reporter	Start	End
SYBR	6	15
Bacterial cells/ml	Ct	AVERAGE Ct
10^9	14.8161	14.875
10^8	17.2194	17.1519
10^7	20.884	21.1097
10^6	24.6562	25.0503
10^5	28.9449	28.1209
10^4	32.056	31.5601
10^3	34.2227	35.8762
10^2	37.2659	36.3105
10^1	37.8972	36.8807
10^0	36.4711	38.7408

-
3.06735
112%



Calibration of bacterial cell count/ml against Ct value. Medium amplicon sequence

Reporter	Start	End	
SYBR,	6	15	
Bacterial cells/ml	Ct	AVERAGE Ct	
10 ⁹	6.8959	8.6911	7.7935
10 ⁸	9.0762	8.8034	8.9398
10 ⁷	12.2403	12.1497	12.195
10 ⁶	16.1247	15.8485	15.9866
10 ⁵	19.3842	19.4352	19.4097
10 ⁴	23.2365	23.5287	23.3826
10 ³	27.0752	27.3817	27.22845
10 ²	31.0232	31.8605	31.44185
10 ¹	33.3334	33.565	33.4492
10 ⁰	34.663	34.9019	34.78245
			0
			-3.459864167
			SLOPE
			95%



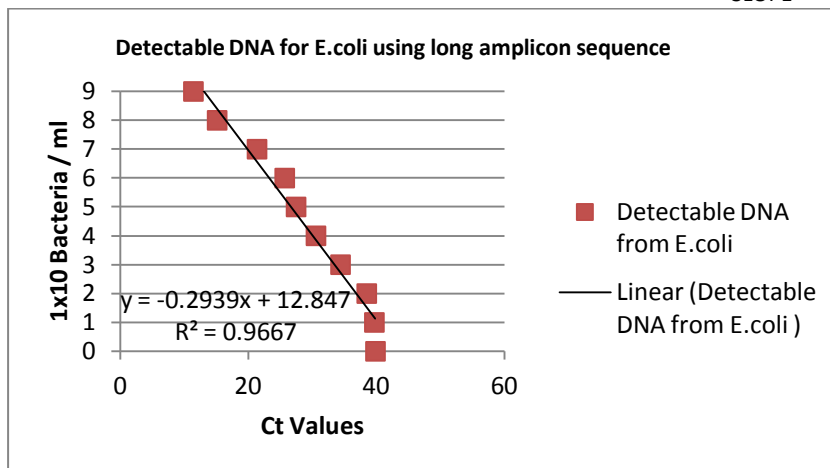
Calibration of bacterial cell count/ml against Ct value. Long amplicon sequence

Reporter	Start	End	Threshold
SYBR,	6	15	0.365455
Bacterial cells/ml	Ct	AVERAGE Ct	
10 ⁹	11.4368	11.409	11.4229
10 ⁸	15.0031	15.2522	15.12765
10 ⁷	20.4376	22.2774	21.3575
10 ⁶	25.905	25.4632	25.6841
10 ⁵	27.8569	27.0505	27.4537
10 ⁴	31.1548	29.9718	30.5633
10 ³	34.787	34.0136	34.4003
10 ²	38	38.9874	38.4937
10 ¹	39.7937	39.5796	39.68665
10 ⁰	39.8946	39.822	39.8583

-3.5686325

SLOPE

91%



DNA detected following treatment according to an EN 13697 procedure. Short amplicon sequence

	Ct Value	AVERAGE Ct	DNA Detected using standard curve equation (10^x bacterial cell DNA/ml)	
100%	27.6193	27.89045	4.710419	
100%	28.1616			
50%	28.8389	28.9246	4.355292	
50%	29.0103			
1.00%	27.1203	26.8039	5.083541	
1.00%	26.4875			
NT	21.3683	21.85295	6.783697	
NT	22.3376			
NC	26.299	26.684	5.124714	
NC	27.069			
SALINE	22.1607	22.2319	6.653566	Untreated control
SALINE	22.3031			
UV _{254nm}				
DAMAGED 10 ⁶	20.4764	20.70055	7.179431	
UV _{254nm}				
DAMAGED 10 ⁶	20.9247			
NPC	38.5648	38.5104	1.063529	No primer control
NPC	38.456			
NTC	37.1086	37.1248	1.539344	No template control
NTC	37.141			

Treatment according to the EN 13697 procedure

Saline is the 'Water' (untreated) control. UV damage was induced by treatment for 400 seconds.

DNA detected following treatment according to an EN 13697 procedure. Medium amplicon sequence

	Ct Value	AVERAGE Ct	DNA Detected using standard curve equation (10^x bacterial cell DNA/ml)	
100%	25.1272	25.42105	3.321696	
100%	25.7149			
50%	26.5164	26.33095	3.050909	
50%	26.1455			
1%	24.5359	24.6457	3.55244	
1%	24.7555			
NT	19.643	19.7508	5.009162	
NT	19.8586			
NC	22.7911	22.96305	4.053196	
NC	23.135			
UV _{254nm} DAMAGED 10 ⁶	18.83	19.145	5.189448	
UV _{254nm} DAMAGED 10 ⁶	19.46			
NPC	35.2739	35.564	0.303154	No primer control
NPC	35.8541			
NTC	34.7738	34.4154	0.644977	No template control
NTC	34.057			
SALINE	18.9544	18.7057	5.320184	Untreated control
SALINE	18.457			

Treatment according to the EN 13697 procedure

Saline is the 'Water' (untreated) control. UV damage was induced by treatment for 400 seconds.

DNA detected following treatment according to an EN 13697 procedure. Long amplicon sequence

	Ct Value	AVERAGE Ct	DNA Detected using standard curve equation (10 ^x bacterial cell DNA/ml)	
100%	29.0332	29.15775	4.277537275	
100%	29.2823			
50%	29.4052	29.6107	4.14441527	
50%	29.8162			
1%	28.4353	28.23035	4.550100135	
1%	28.0254			
NT	25.4979	25.54495	5.339339195	
NT	25.592			
NC	27.0595	27.4878	4.76833558	
NC	27.9161			
SALINE	26.7553	26.30175	5.116915675	Untreated control
SALINE	25.8482			
UV _{254nm}				
DAMAGED 10 ⁶	25.4887	25.29115	5.413931015	
UV _{254nm}				
DAMAGED 10 ⁶	25.0936			
NPC	39.8254	39.7645	1.16021345	No primer control
NPC	39.7036			
NTC	39.1534	39.34495	1.283519195	No template control
NTC	39.5365			

Treatment according to the EN 13697 procedure

Saline is the 'Water' (untreated) control. UV damage was induced by treatment for 400 seconds.

Conclusion

For all qPCR for the small, medium and large amplicons, there was a linear range relationship between bacterial cell DNA/ml and Ct (R^2 = short, 0.955; medium, 0.989; long 0.967, MicroSoft Excel linear regression). This data could therefore be used to calibrate the effects of treatment to give an apparent loss of bacterial DNA, and therefore assess the damage to DNA caused by treatment. To compare the saline control in each case at 100% V/V for the product (5 minutes) and sterilising UV 254nm (6 minutes, 40 seconds):

Amplicon	Log₁₀ bacterial cell DNA/ml equivalents detectable by qPCR
Short	
SALINE	6.65
100% V/V	4.71
UV 254nm	7.17
Medium	
SALINE	5.32
100% V/V	3.32
UV 254nm	5.19
Long	
SALINE	5.12
100% V/V	4.28
UV 254nm	5.41

DNA damage induced by DuoMax treatment reduces amplifiable 16S sequences by a factor of approximately 2.0 log₁₀ for the short and medium amplicons. This is in the region of a 1.0 log₁₀ reduction for the long amplicon, possibly as a result of lower amplification efficiency in general for this qPCR. In all cases, however, DuoMax is more efficient than sterilising UV light in inducing DNA damage in a similar contact time. UV light 254nm specifically damages DNA by formation of thymidine dimers.

DuoMax does not completely destroy bacterial DNA according to this analysis, because PCR-amplifiable bacterial 16S DNA sequences can still be detected after treatment. However, it does cause damage to DNA, as shown by a reduction in bacterial cell DNA equivalents/ml detectable by qPCR. The least sensitive qPCR assay using amplification of a 100 nt amplicon was inhibited by the effects of DuoMax on bacterial cell DNA. Inhibition of qPCR was not related to inhibition of the qPCR reaction as judged by the comparison of the NT, NC controls with the treated samples. Therefore, the effect of DuoMax, when compared to UV light treatment is likely cause sufficient DNA lesions to inactivate DNA.

Signed



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15 August 2012

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