Decontamination of the KingFisher Duo Prime processing chamber using UV light

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Goal

The new Thermo Scientific™ KingFisher™ Duo Prime magnetic particle processor contains an ultraviolet C (UVC) light inside the instrument to eliminate most bacterial and viral contaminants caused by sample handling, for example. The UVC irradiation induces damage to the DNA of the organisms, thereby inhibiting their growth. The light is designed to reach over the entire work surface of the instrument to quickly eliminate contaminants. This note represents how easily bacterial growth is inhibited by the UVC irradiation inside KingFisher Duo Prime. The deactivation of genomic DNA subjected to UVC irradiation prior to a PCR amplification is also shown.

Introduction

Short wavelength ultraviolet light between 200–280 nm, classified as UVC light, is generally accepted to be germicidal and inactivates bacterial growth effectively. The optimal germicidal wavelengths are in the range of 255–265 nm. The KingFisher Duo Prime instrument contains a mercury lamp with peak emission around 255 nm. Micro-organisms are destroyed due to the UVC radiation causing DNA lesions, which block transcription and replication with lethal consequences. An example of the UVC exposure effect on DNA is shown in figure 1. The efficiency of nucleic acid inactivation depends on the organism. The specific genetic composition and other molecules may protect the organism from UVC-induced damage. Bacteria and viruses are readily inactivated, whereas fungal cells and spores from both bacteria and fungi typically require higher UV dosages (Kowalski 2009).

In this application note, the lethal effect of UVC irradiation on bacterial growth is shown, as well as the deactivation of human genomic DNA (gDNA). A typical laboratory bacteria, *E. Coli*, was used to verify the inhibition efficiency of the UV lamp inside the KingFisher Duo Prime. The gDNA deactivation efficiency of KingFisher Duo Prime UV lamp was compared to a commonly used PCR UV Cabinet.

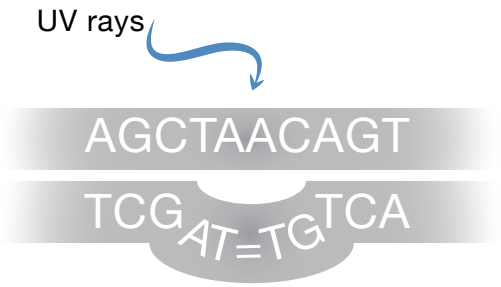

Figure 1. An example of DNA lesion caused by UVC radiation. Thymine bases cross-link, preventing DNA replication. The cross-links may also occur between adjacent strands of nucleic acids or protein molecules.

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Materials and Methods

Power of the UVC lamp

Inside the KingFisher Duo Prime instrument, there is an 8.0 W UV bulb. The output power of the lamp in the UVC spectra is 2.0 W. In comparison to other similar-type automated DNA purification instruments, the UVC power of the KingFisher Duo Prime instrument is significantly higher (Table 1).

	UV bulb electrical power	UVC radiation power
KingFisher Duo Prime	8.0 W	2.0 W
Maxwell® 16 MDx*	4.5 W	0.8 W

Table 1. Technical specifications of the UV lamp integrated in the DNA purification instrument

Microbial growth inhibition

An *E. coli* BL-21 bacterial strain was grown in Tryptone Soya Broth for four hours to exponential phase. The grown bacteria was diluted 10^{-5} and 100 µl was plated onto Tryptone Soya Agar petri dishes. Five bacteria containing agar dishes were placed to cover the working surface of three KingFisher Duo Prime instruments (Figure 2).

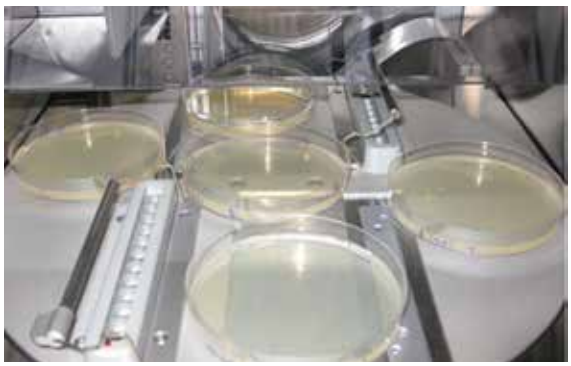

Figure 2. The orientation of the bacteria-containing agar dishes on the KingFisher Duo Prime round table

* Information based on manufacturer specifications in Maxwell® 16 MDx Instrument User Manual, accessed 4/15 on promega.com.

A control dish containing the same amount of bacteria was placed into equal environmental conditions outside the instrument. The bacteria were subjected to 30 minutes of UVC exposure inside the instrument. The control dish and the UVC-exposed dishes were incubated overnight at 37°C. The next day the colonies were counted with OpenCFU software (Geissmann 2013). (Figure 3)

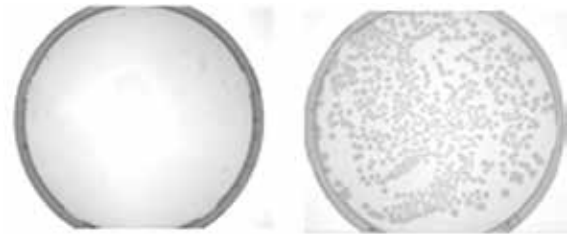

Figure 3. The UV-irradiated agar dish, left, and the control dish, right

Genomic DNA deactivation

Human genomic DNA was subjected to UV light to determine the amount of inhibition UV would cause to the PCR amplification of the template. The UVC/T-M-AR PCR UV Cabinet (from Grant Instruments, Cambridge, UK) was used as a reference for the effect of UVC irradiation.

The 24-well Thermo Scientific™ Piko™ PCR Plate with the dried human gDNA was placed inside three KingFisher Duo Prime instruments and the UV lamp was set for 16 hours. Similar settings were done with the PCR UV Cabinet. Positive controls on the PCR plates were covered during the UV irradiation to protect the samples from UV rays. Two samples and one positive control with 10,000 copies of human gDNA, all in three replicates, were used for each instrument.

As standards, the 1:10 serial dilution of the gDNA was prepared and NTC was added into each PCR plate, all in three replicates (Table 2). Cathepsin K gene amplification was determined using Thermo Scientific™ Maxima™ SYBR qPCR reagents with the Thermo Scientific™ PikoReal™ 24 real-time PCR system.

Standards					UV-irradiated samples		Pos. control
NTC	10	100	1,000	10,000	10,000	10,000	10,000
NTC	10	100	1,000	10,000	10,000	10,000	10,000
NTC	10	100	1,000	10,000	10,000	10,000	10,000

Table 2. The orientation of samples on the 24-well Piko PCR Plate

Results

Microbial growth inhibition test

The colony count on the control dish outside the instrument had roughly 700 colonies. The number of colonies on the UVC exposed dishes is shown in table 3.

KF Duo Prime	Dish replicate no.				
	1	2	3	4	5
A	1	0	1	0	0
B	0	0	0	0	0
C	0	0	0	0	2

Table 3. The number of bacterial colonies on the agar dishes after 30 minutes of UVC exposure in three parallel KingFisher Duo Prime instruments (A, B and C)

The average number of bacterial colonies on the test plate was 0.27: (1+1+2)/15 = 0.27. In comparison, the number of colonies on the control plate was 700. Therefore the reduction in colony count due to the UVC exposure was 99.96%: 100%-(0.27/700x100) = 99.96%.

Genomic DNA inactivation

The effect of UVC irradiation to gDNA was determined by calculating the difference of the qPCR amplification of the UVC-treated sample to the amplification of the positive control. PikoReal software was used to analyze the amplification. The example of the amplification from one qPCR run is shown in figure 4. The average Cq values of the UV irradiated samples and positive controls from three KingFisher Duo Prime instruments and the PCR UV Cabinet are listed in table 4. Average values are from three replicates.

The average Cq value difference (ΔCq) between UV irradiated samples and the gDNA control sample from three instruments was 11.4. The effect of the UVC irradiation can be calculated as follows: $2^{(-\Delta Cq)}$ = 0.0004 = 0.04%. The 99.96% reduction in the amplifiable genomic target with this assay was achieved. In comparison, the amplification reduction was 99.98% in the UV PCR Cabinet.

Instruments	Sample	Cq average	ΔCq	Average ΔCq
KF Duo Prime A	gDNA UV_1	33.17	11.13	10.7
	gDNA UV_2	32.34	10.30	
	gDNA +ctrl	22.04		
KF Duo Prime B	gDNA UV_1	34.19	11.99	12.2
	gDNA UV_2	34.70	12.49	
	gDNA +ctrl	22.20		
KF Duo Prime C	gDNA UV_1	32.97	10.85	11.2
	gDNA UV_2	33.75	11.63	
	gDNA +ctrl	22.12		
UV Cabinet	gDNA UV_1	33.68	11.67	12.4
	gDNA UV_2	35.14	13.14	
	gDNA +ctrl	22.00		

Table 4. The Cq values of the UVC irradiated gDNA samples and the positive controls treated in three KingFisher Duo Prime instruments and the PCR UV Cabinet

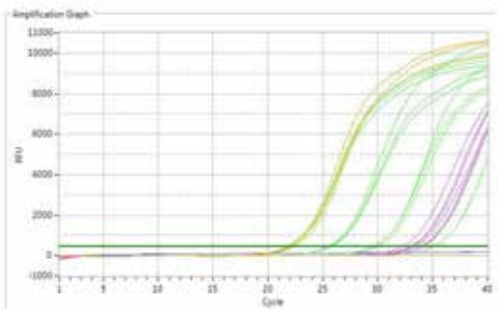

Figure 4. The amplification graph from PikoReal Software (samples from KF Duo Prime C). Colors of the curves: purple – UVC treated samples, orange – positive controls, green – 1:10 serial dilution standards, and blue – NTC

Conclusions

KingFisher Duo Prime with UVC light is highly efficient for decontaminating the instrument from bacteria and genomic DNA. The UVC irradiance level is on par with that of a typical PCR UV Cabinet and works well in practice.

References

Geissmann Q. OpenCFU, a New Free and Open-Source Software to Count Cell Colonies and Other Circular Objects. PLoS ONE 8(2)

Kowalski W. Ultraviolet Germicidal Irradiation Handbook: UVGI for Air and Surface Disinfection. Springer, English, 2009.

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