UV Decontamination of MDA Reagents for Single Cell Genomics

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UV Decontamination of MDA Reagents for Single Cell Genomics

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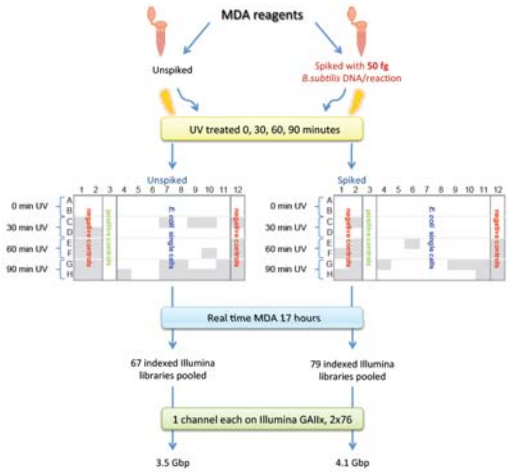
Abstract

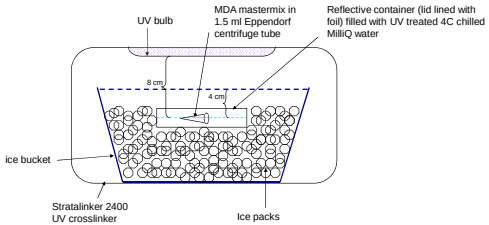
Single cell genomics, the amplification and sequencing of genomes from single cells, can provide a glimpse into the genetic make-up and thus life style of the vast majority of uncultured microbial cells, making it an immensely powerful and increasingly popular tool. This is accomplished by use of multiple displacement amplification (MDA), which can generate billions of copies of a single bacterial genome producing microgram-range DNA required for shotgun sequencing. Here, we address a key challenge inherent to this approach and propose a solution for the improved recovery of single cell genomes. While DNA-free reagents for the amplification of a single cell genome are a prerequisite for successful single cell sequencing and analysis, DNA contamination has been detected in various reagents, which poses a considerable challenge. Our study demonstrates the effect of UV irradiation in efficient elimination of exogenous contaminant DNA found in MDA reagents, while maintaining Phi29 activity. Consequently, we also find that increased UV exposure to Phi29 does not adversely affect genome coverage of MDA amplified single cells. While additional challenges in single cell genomics remain to be resolved, the proposed methodology is relatively quick and simple and we believe that its application will be of high value for future single cell sequencing projects.

Methods

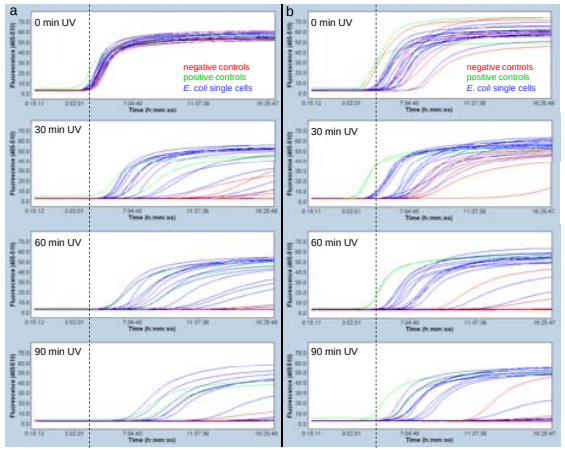


Unspiked and spiked (50 fg of *B. subtilis* DNA per reaction as intentional contamination) MDA reagents were UV treated for 0, 30, 60 and 90 min, then used to amplify sorted single *E. coli* cells and controls. Wells that did not generate an MDA DNA product are marked in grey. Indexed Illumina libraries were constructed from each MDA product, followed by low-level shotgun sequencing at ~10x coverage.

Schematic cross section of the UV irradiation setup.



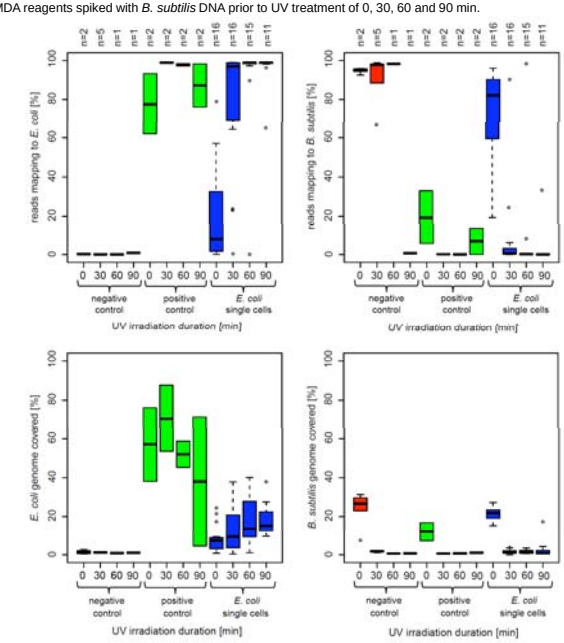
Real time MDA



Real-time MDA of single *E. coli* cells using (a) unspiked MDA reagents and (b) spiked MDA reagents, UV irradiated for 0, 30, 60 and 90 min. Fluorescence was measured real-time for 17 hours to quantify the amount of DNA produced during MDA. Without any UV treatment of the MDA mastermix, no template controls generated substantial amounts of MDA product. With increasing UV irradiation times, DNA amplification in negative controls was suppressed, suggesting that contaminating DNA was successfully removed with a minimal effect on the overall amplification kinetics.

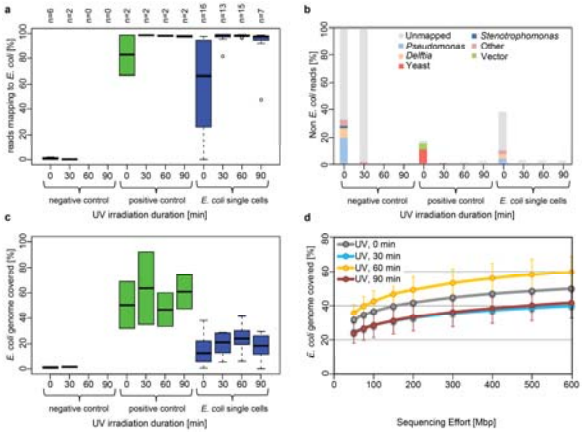
Shotgun sequence analysis

MDA reagents spiked with *B. subtilis* DNA prior to UV treatment of 0, 30, 60 and 90 min.



Shotgun sequence analysis for single *E. coli* cells amplified with *B. subtilis* DNA spiked into MDA reagents prior to UV irradiation for 0, 30, 60 and 90 min. With 60 min UV treatment, the contaminant (*B. subtilis* DNA) has largely been eliminated as suggested by the majority of the reads (median=98.9%) mapping to the *E. coli* genome, while the median percentage of reads mapping to the *Bacillus* genome drop from 82.2% (no UV irradiation) to 0.5% (30 min UV irradiation) to 0.2 % (60 min UV irradiation).

MDA reagents UV treated for 0, 30, 60 and 90 min.



Shotgun sequence analysis of single *E. coli* cells amplified with MDA reagents that were UV irradiated for 0, 30, 60 and 90 min (a-c). In untreated samples, a large number of sequences mapping to *Pseudomonas*, *Delftia* and *Stenotrophomonas* genomes were found in no template controls (negative controls), as well as a substantial number unmappable reads, which may represent self priming of random hexamers. With 60 min UV irradiation, the contamination in the negative controls was successfully eliminated, leaving no DNA for library generation. Positive controls (10-100 *E. coli* cells) and individual *E. coli* cells were free of contamination after 30 min of UV treatment, with ~98% (median) of reads mapping to the *E. coli* genome and covering approximately 64% and 21% respectively, which is to be expected at the given sequence effort. (d) Genome coverage rarefaction analysis for 12 *E. coli* single cells sequenced at ~120x depth suggests that UV irradiation did not negatively impact on the genome recovery.

Conclusions

Generating genomic sequence data for > 100 *E. coli* single cells, we comprehensively demonstrate the utility of UV irradiation to provide a simple and highly efficient decontamination method of MDA reagents used for whole genome amplification of single cells enabling contamination-free single cell genomics. Our data shows that UV radiation eliminates contaminating DNA while maintaining Phi29 enzyme activity without introducing additional genome coverage bias and artifacts.

Acknowledgements

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