# Decontamination of MDA reagents for single cell genomics

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## INTRODUCTION

Single cell genomics, the sequencing of genomes from single cells, provides 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. 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. In this study we aimed to test the effect of UV radiation in reducing or eliminating the availability of the contaminated DNA for MDA. Exposure of the MDA buffer and enzyme to UV radiation prior to the amplification can reduce the amount of contaminating DNA in the end products, and this effect increases with an increasing amount of UV radiation. Although the enzymatic activity of phi-29 is also affected by the UV treatment, the remaining activity seems to have no problem in amplifying the target DNA to a similar level of DNA quantity. The methodology is quick, simple, and highly efficient and exhibits a low impact on the MDA reaction efficiency

## Methods

**Part 1:** Screen commercial kits for contamination:

20ul MDA reactions with no target DNA were constructed for the REPLI-g® Ultrafast, RepliPHI™, GenomiPhi HY Kits and a homemade kit based upon NEB's phi29 enzyme (1-4). Reactions ran for 20 hours at 30C. MDA products were screened for contamination via PCR for 16S and 18S genes. All of the commercial kits had 16S hits, but none had 18S. Sequencing of the 16S found the contaminates to be: *Delftia* in the UltraFast, *E.coli* in the Repliphi, and a *Cytophage* in the Genomiphi. Larger data sets with the NEB phi29 has shown that it has a minimal amount of *E.coli* contamination.

**Figure 1a**: MDA products from manufacture's recommended kit concentrations with no target DNA. 1ul of product on a 1% agarose gel, EtBr staining, 120V, 45 minutes.

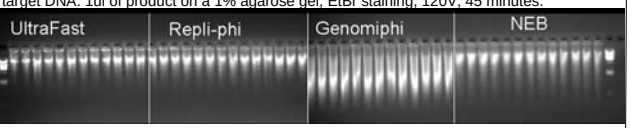
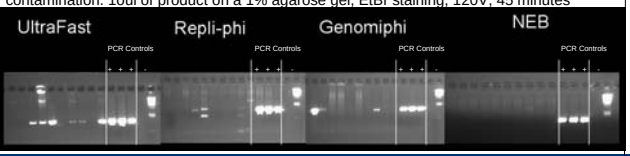
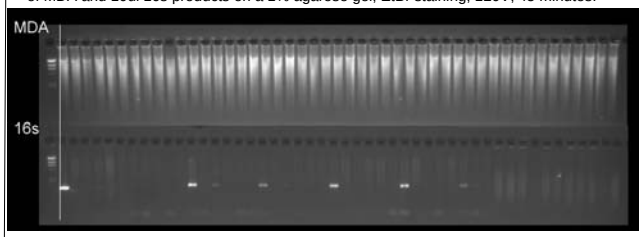



**Figure 1b**: 16S PCR products run on ~50ng of MDA DNA. Commercial kits show bacterial contamination. 1ou of product on a 1% agarose gel, EtBr staining, 120V, 45 minutes




Since the MDA kit based upon the NEB phi29 polymerase had the least amount of contamination in the previous experiment it became the focus of the rest of the testing. To understand the level of contamination from the MDA kit itself, 30 20ul reactions were run without UV exposure and probed with 16S PCR. 30% of the reactions showed bands for 16S.

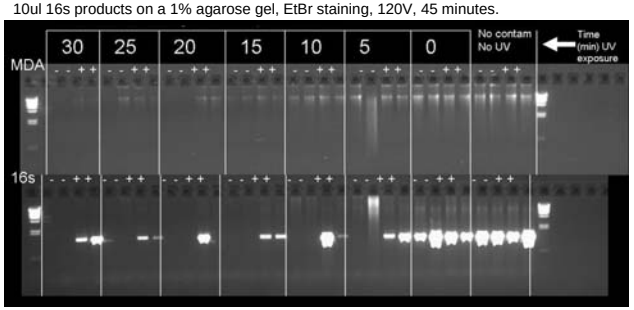
**Figure 1c**: 20ul MDA reactions with no target DNA using the NEB phi29 polymerase kit. 1ul of MDA and 10ul 16s products on a 1% agarose gel, EtBr staining, 120V, 45 minutes.




**Part 2:** Use UV to eliminate contaminating DNA

MDA kits created with the NEB phi29 polymerase were contaminated with 50fg of *E.coli* DNA per reaction and exposed to 254 nm UV light in a Stratalinker 2400 (~4000uwatts/cm$^2$) for up to 30 minutes. All UV exposure is performed on ice to keep the temperature of the enzyme beneath 40C. 20ul MDA reactions were then run with and without 50fg of *E.coli* DNA for 20 hours. 1ul of the MDA product was run on a gel and demonstrated the kit was still active even after 30 minutes of UV exposure.16S PCR run on the MDA products showed the contaminating DNA was able to be eliminated within the first 5 minutes of UV exposure.

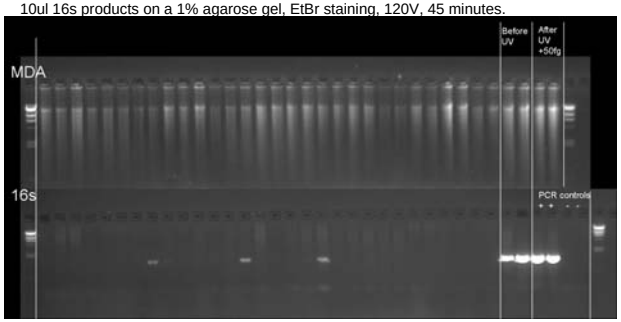
**Figure 2a**: UV exposure of reactions contaminated with 50fg *E.coli* DNA. 1ul of MDA and 10ul 16s products on a 1% agarose gel, EtBr staining, 120V, 45 minutes.




20 minutes of UV exposure was decided upon as the optimal condition, because according to the gel series above it was far past the point that 50fg of contaminating DNA could be eliminated, but not so much exposure that the MDA reaction could not amplify 50fg of target DNA.

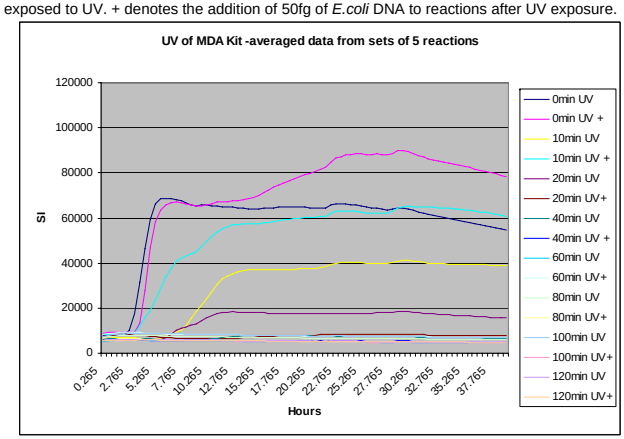
30 20ul MDA reactions contaminated with 50fg of *E.coli* DNA were exposed to UV for 20 minutes and then run for 20 hours at 30C. The MDA products were probed with 16S PCR. 10% of the reactions showed bands for 16S.

**Figure 2b**: UV exposure of reactions contaminated with 50fg *E.coli* DNA. 1ul of MDA and 10ul 16s products on a 1% agarose gel, EtBr staining, 120V, 45 minutes.




Kits created from the NEB phi29 polymerase, contaminated with 50fg *B.subtilis* per reaction and exposed to UV for up to 120 minutes had 20ul Real Time MDA reactions run using SYTO13 at 1uM on a BioRad iCycler IQ for 40 hours at 30C. A dramatic drop off in the ability of the kit to amplify DNA was seen just passed 20 minutes of UV exposure and most reactions were able to reach their product plateau by 13 hours.

**Figure 2c**: rtMDA data of NEB phi29 kits contaminated with 50fg of DNA that were then exposed to UV. + denotes the addition of 50fg of *E.coli* DNA to reactions after UV exposure.




## References

1. "REPLI-g® UltraFast Mini Handbook." Qiagen. October 2006.
2. "RepliPHI™ Phi29 DNA Polymerase." Epicentre. July 2006.
3. "illustra GenomiPhi HY DNA Amplification Kit." GE HealthCare. 2007.
4. "Phi 29 DNA Polymerase" New England Biolabs Inc.29 MAY 2009. <http://www.neb.com/nebecomm/products/productM0269.asp>

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