Title
Overcoming some of the challenges to single cell genomics

Permalink
https://escholarship.org/uc/item/3d18t54n

Authors
Lee, Janey
Tighe, Damon
Wang, Mei
et al.

Publication Date
2010-03-24
Overcoming some of the challenges to single cell genomics

Janey Lee, Damon Tighe, Mei Wang, Stephanie Malfatti, Erika Lindquist, Feng Chen, Jan-Fang Cheng and Tanja Woyke

US Department of Energy Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, California 94598 USA

Abstract

Eliminating MDA reagent contamination using UV

Real-time MDA was used to monitor the effect of UV treatment on the kinetics of the MDA reaction, in addition to cross linking of contamination. 50 μl MDA reactions were created from the Epicentre RepliPhi kit according to the manufacturer’s recommended concentrations, and were contaminated with 25 fg of λ. subtilis DNA per reaction. These were UV treated in a Stratalinker 2400 (254 nm, ~4000 pJ/cm2) for up to 60 minutes at different time points. All UV exposures were performed in 96-well plate format in chilled, sterile MQ water in order to keep the temperature of the enzyme below 30°C. 5 fg of E.coli DNA was added to each reaction as template and were run in real time using SYTO13 at 0.5 μM on a Roche LightCycler 480 for 17 hours at 30°C. Preliminary data (not pictured) suggested an optimal UV treatment in the 20-30 minute range. UV exposures of longer than 30 minutes showed a significantly reduced ability of the kit to amplify product.

To show a comparison between a 25 and 30 minute UV exposure, 6X replicates of 50 μl MDA reactions were contaminated and UV treated at 0, 25 and 30 minute exposures. Template (5 fg E.coli DNA) and ‘no template’ negative controls were run in parallel to gauge the efficacy of eliminating contamination within the kit, for a total of 48 reactions.

Normalized 454 Titanium Std library construction

The 30 minute UV treatment is shown to be the most effective at eliminating contamination within the MDA reagents, as compared to the 25 minute treatment, which remained clean in only 4 out of 6 reactions (it is also possible this amplification could be a non-template product, i.e. hexamer buildup. Further 16S analysis is required to determine if this is true contamination). The rMDA also demonstrated that the 30 minute UV treated reactions still had enough activity to amplify 5 fg of DNA, comparable to a single copy of a bacterial genome.

Conclusions

• The inherent contamination found within commercial MDA kits can be successfully eliminated with a 30 minute exposure of reagents to UV light.

• MDA produces bias in amplification of target genomes, leaving some regions of the genome highly overrepresented and others without sufficient coverage. Normalization of 454 Std Titanium libraries reduces this large bias and produces a more even depth of coverage across the genome, increasing sequencing efficiency and ease of genome assembly.

The normalization steps, shown in green, were performed within the regular 454 Titanium library construction protocol. Because the process is also relatively quick and requires no specialized equipment, creation of normalized libraries is amenable for high throughput library construction, potentially in 96-well plate format.

There was no detectable contamination within the kit. The normalization steps, shown in green, were performed within the regular 454 Titanium library construction protocol. Because the process is also relatively quick and requires no specialized equipment, creation of normalized libraries is amenable for high throughput library construction, potentially in 96-well plate format.

No template control

50 μl MDA reactions run in real time on a Roche LightCycler 480 with SYTO13 for 17 hours at 30°C.

MDA products from manufacturer’s recommended kit concentrations with no template DNA. 1 μl of product on a 1% agarose gel, EBB staining. 120V, 45 minutes.

MDA products were screened for contamination via 16S/18S PCR. All the commercial kits were positive for 16S but, had none for 18S. Sequencing of the 16S contaminants identified them as Delftia in the Ultrafast, RepliPhi™, Genomiphi HY Kits and a homemade kit based upon NEB’s ph29 enzyme. Reactions were incubated for 20 hours at 30°C.

MDA products were screened for contamination via 16S/18S PCR. All the commercial kits were positive for 16S but, had none for 18S. Sequencing of the 16S contaminants identified them as Delftia in the Ultrafast, RepliPhi™, and a Cytophage in the Genomiphi. Larger data sets with the NEB ph29 has shown it has a minimal amount of E.coli contamination

Normal 454 Titanium Std library construction

The inherent contamination found within commercial MDA kits can be successfully eliminated with a 30 minute exposure of reagents to UV light.

MDA bias as evaluated by sequence depth distribution. Normalization results in significant improvements in the uniformity of coverage. The un-normalized library was sequenced to an average depth of ~45X with 454 Titanium, but the representation of specific regions of the genome ranged from 0 to ~35X, with a mean coverage of 47X. After normalization, the difference in depth of coverage between the two libraries was reduced approximately 3.5 fold. Highly represented regions of the genome were drastically reduced, while under-represented regions saw an increase in depth of coverage.

Conclusions

The inherent contamination found within commercial MDA kits can be successfully eliminated with a 30 minute exposure of reagents to UV light.

MDA produced bias in amplification of target genomes, leaving some regions of the genome highly overrepresented and others without sufficient coverage. Normalization of 454 Std Titanium libraries reduces this large bias and produces a more even depth of coverage across the genome, increasing sequencing efficiency and ease of genome assembly.

The inherent contamination found within commercial MDA kits can be successfully eliminated with a 30 minute exposure of reagents to UV light.

MDA produced bias in amplification of target genomes, leaving some regions of the genome highly overrepresented and others without sufficient coverage. Normalization of 454 Std Titanium libraries reduces this large bias and produces a more even depth of coverage across the genome, increasing sequencing efficiency and ease of genome assembly.

To screen commercial MDA kits for contamination, 20 μl MDA reactions were created from the REPLI-g® Ultrafast, RepliPHI™, GenomiPHI HY Kits and a homemade kit based upon NEB’s ph29 enzyme. Reactions were incubated for 20 hours at 30°C.

MDA products were screened for contamination via 16S/18S PCR. All the commercial kits were positive for 16S but, had none for 18S. Sequencing of the 16S contaminants identified them as Delftia in the Ultrafast, RepliPhi™, and a Cytophage in the Genomiphi. Larger data sets with the NEB ph29 has shown it has a minimal amount of E.coli contamination

Despite the lowest level of contamination being found within the NEB kit, the RepliPhi kit was chosen as the primary kit with which to continue our studies because of cost analysis. To suit our needs for a current project, further optimization was applied to 50 μl reactions instead of 20 μl.

To screen commercial MDA kits for contamination, 20 μl MDA reactions were created from the REPLI-g® Ultrafast, RepliPHI™, GenomiPHI HY Kits and a homemade kit based upon NEB’s ph29 enzyme. Reactions were incubated for 20 hours at 30°C.

MDA products were screened for contamination via 16S/18S PCR. All the commercial kits were positive for 16S but, had none for 18S. Sequencing of the 16S contaminants identified them as Delftia in the Ultrafast, RepliPhi™, and a Cytophage in the Genomiphi. Larger data sets with the NEB ph29 has shown it has a minimal amount of E.coli contamination

16S PCR products run on ~50ng of MDA DNA. Commercial kits show bacterial contamination. 1 μl of product on a 1% agarose gel, EBB staining. 120V, 45 minutes.

Despite the lowest level of contamination being found within the NEB kit, the RepliPhi kit was chosen as the primary kit with which to continue our studies because of cost analysis. To suit our needs for a current project, further optimization was applied to 50 μl reactions instead of 20 μl.