1. Introduction

- The analysis of genomes at the single cell level offers unprecedented biological insights in diverse fields such as cancer research, immunology & microbiology. To enable single cell genomics, a technology for amplification of genomic DNA is required that provides utmost sensitivity, accuracy & robustness.
- We have previously developed a method of multiple strand displacement amplification (MDA) by Phi29 DNA polymerase.
- This single subunit, proofreading DNA polymerase has excellent processivity and possesses strand displacement properties that enable the high-fidelity amplification of input DNA using random hexamer primers.
- Phosphorothioate modification of the primers prevents degradation by the DNA polymerase and dramatically stimulates reaction kinetics. This system is commercially available as the Illustra™ Single Cell GenomiPhi™ DNA Amplification Kit.
- The project presented here builds on the merits of conventional MDA to develop methods to recover and amplify DNA from single cells of bacterial and mammalian origin. Quality of the output DNA in terms of genome coverage, uniformity of amplification, and error rate is critical to obtain useful single cell data in various applications. Microarray analysis and NGS are used to validate our novel single cell MDA protocols and formulations.

2. Methods

- A number of improved GenomiPhi formulations, named GRC1, GRC2 and CD, were developed for single cell WGA. These formulations were then optimized to develop the Single Cell GenomiPhi product.
- New manufacturing processes, including UV & enzymatic reagent clean-up, help to ensure that all kit components are free from any detectable DNA contamination & enable sensitivity of amplification down to 1 fg of gDNA. An optional, proprietary, enzymatic clean-up step ensures that any potential DNA contaminants introduced during setup are removed before each individual reaction.
- Amplified DNA quality was assessed by performing downstream sequencing (Illumina™ HiSeq™) and SNP analysis (Affymetrix™).

3. Results

A) New Formulation is Sensitive Enough to Amplify from

- Figure 3: Bacterial Genomic DNA was amplified with formulation GRC2. Amplification kinetics were monitored on a Tecan plate reader in real-time by the addition of SYBR™ Green I.

B) New Formulations Consistently Amplify from

- Single Microbes with Suppressed Background Amplification

- Figure 4: a. Single cell solutions made in TEN (100 mM NaCl + 50 mM glycerol + 0.01% Tween-20) with the addition of Phi29-F4-PF-fluor for visualization. b. Single cell post- amplification yields. c. Real-time amplification kinetics using PCR formulation E on E. coli sub-saturated single cells and live GenomiPhi current GenomicPhi V2 formulation + enzymatic cleaning reagent on E. coli single cells.

C) Modified Cell Lysis Method Improves Genome Coverage of DNA Amplified from Single Microbial Cells

- Figure 5: a. Whole Genome Sequencing (Illumina™ HiSeq™) of DNA amplified from single E. coli and B. subtilis cells with GRC formulation E. RSD = Relative Standard Deviation = Mean Coverage ± Standard Deviation/Mean coverage x 100.

D) New Formulations Reduce Amplification Bias in DNA Amplified from Single Microbial Cells

- Figure 6: Whole Genome Sequencing (Illumina™ HiSeq™) of DNA amplified from single E. coli and B. subtilis cells with GRC formulation E. RSD = Relative Standard Deviation = Mean Coverage ± Standard Deviation/Mean coverage x 100.

E) Single Cell GenomiPhi Enables Robust Mammalian Cell Lysis and Amplification Kinetics

- Figure 7: Three replicates of single HCC2218 human B-lymphoblast cells were amplified with Single Cell GenomiPhi formulation. Amplification kinetics were monitored in real-time by the addition of SYBR™ Green I (cycle = 5 min).

F) Single Cell GenomiPhi Shows Improved Coverage and Accuracy from Mammalian Cells in SNP Analysis

- Figure 8: gDNA amplified from 5 cell Jurkat samples using different WGA technologies was run on an Affymetrix™ Genome Wide Human 6.0 SNP Array and compared to unamplified control gDNA. SNP copy number graph showing WGA normalized to the unamplified bulk control gDNA samples. Graphs represent overview of 5 separate samples. Colored dots ± mean ± standard deviation of 3 replicates. Representative graph of chromosome 2 as the GenomiPhi shows amplification bias of different WGA technologies. Each spot intensity was compared to that same spot on the other chromosome, standard deviation of each spot data for control is shown in (c). "See footnote below.

4. Summary

- Single Cell GenomiPhi provides a robust and representative method for whole genome amplification from single cells.
- New optimized formulation increases the amplification sensitivity down to 1 fg amount of DNA.
- New manufacturing process has been developed that includes UV decontamination and enzymatic clean-up to eliminate any contaminating DNA from whole genome amplification reagents.
- Single cell whole genome amplification, SNP arrays and the NGS results suggest that the Single Cell GenomiPhi kit provides improved sensitivity and coverage with reduced amplification bias.

Figure 3: Bacterial Genomic DNA was amplified with formulation GRC2. Amplification kinetics were monitored on a Tecan plate reader in real-time by the addition of SYBR™ Green I.

Figure 4: a. Single cell solutions made in TEN (100 mM NaCl + 50 mM glycerol + 0.01% Tween-20) with the addition of Phi29-F4-PF-fluor for visualization. b. Single cell post-ampilification yields. c. Real-time amplification kinetics using PCR formulation E on E. coli sub-saturated single cells and live GenomiPhi current GenomicPhi V2 formulation + enzymatic cleaning reagent on E. coli single cells.

Figure 5: a. Whole Genome Sequencing (Illumina™ HiSeq™) of DNA amplified from single E. coli and B. subtilis cells with GRC formulation E. RSD = Relative Standard Deviation = Mean Coverage ± Standard Deviation/Mean coverage x 100.

Figure 6: Whole Genome Sequencing (Illumina™ HiSeq™) of DNA amplified from single E. coli and B. subtilis cells with GRC formulation E. RSD = Relative Standard Deviation = Mean Coverage ± Standard Deviation/Mean coverage x 100.

Figure 7: Three replicates of single HCC2218 human B-lymphoblast cells were amplified with Single Cell GenomiPhi formulation. Amplification kinetics were monitored in real-time by the addition of SYBR™ Green I (cycle = 5 min).

Figure 8: gDNA amplified from 5 cell Jurkat samples using different WGA technologies was run on an Affymetrix™ Genome Wide Human 6.0 SNP Array and compared to unamplified control gDNA. SNP copy number graph showing WGA normalized to the unamplified bulk control gDNA samples. Graphs represent overview of 5 separate samples. Colored dots ± mean ± standard deviation of 3 replicates. Representative graph of chromosome 2 as the GenomiPhi shows amplification bias of different WGA technologies. Each spot intensity was compared to that same spot on the other chromosome, standard deviation of each spot data for control is shown in (c). "See footnote below.

Figure 9: gDNA amplified from 5 Jurkat cell samples using different WGA technologies was run on an Affymetrix™ Genome Wide Human 6.0 SNP Array and compared to unamplified control gDNA. a. Copy number graph showing WGA normalized to the unamplified bulk control gDNA samples. Graphs represent overview of 5 separate samples. Colored dots ± mean ± standard deviation of 3 replicates. Representative graph of chromosome 2 as the GenomiPhi shows amplification bias of different WGA technologies. Each spot intensity was compared to that same spot on the other chromosome, standard deviation of each spot data for control is shown in (c). "See footnote below.

Figure 10: Five HCC2218 human B-lymphoblast cells were (w/o) and the gDNA was amplified using Single Cell GenomiPhi and a competitor WGA kit. SureSelect™ Custom Panel Target Enrichment System Human All Exon Kit, Agilent™ was used to capture the exomes of amplified test and unamplified control samples. All samples then underwent sequencing on Illumina™ HiSeq™ 2500 platform. DNA sequences were aligned to hg19 using BWA-GLE. Figure shows the percent of data-matching the whole genome and percent exome coverage at 10x read-depth (n=2). "See footnote below.

Figure 11: Five HCC2218 human B-lymphoblast cells were directly dispersed into 96-well gDNA plate containing lysis buffer using BioTek Dickinson FACSAria IV. gDNA was amplified using Single Cell GenomiPhi and a competitor WGA kit. SureSelect™ Custom Panel Target Enrichment System Human All Exon Kit, Agilent™ was used to capture the exomes of amplified test and unamplified control samples. All samples then underwent sequencing on Illumina™ HiSeq™ 2500 platform. DNA sequences were aligned to hg19 using BWA-GLE. Figure shows the percent of data-matching the whole genome and percent exome coverage at 10x read-depth (n=2). "See footnote below.

Figure 12: Single HCC2218 human B-lymphoblast cells were directly dispersed into 96-well gDNA plate containing lysis buffer using BioTek Dickinson FACSAria IV. gDNA was amplified using Single Cell GenomiPhi and a competitor WGA kit. SureSelect™ Custom Panel Target Enrichment System Human All Exon Kit, Agilent™ was used to capture the exomes of amplified test and unamplified control samples. All samples then underwent sequencing on Illumina™ HiSeq™ 2500 platform. DNA sequences were aligned to hg19 using BWA-GLE. Figure shows the percent of data-matching the whole genome and percent exome coverage at 10x read-depth (n=2). "See footnote below.