Use of *Bst* DNA polymerase for whole genome amplification

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ESPP2 Environmental Stress Pathway Project

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DOE GENOMICS:GTL ACCELERATING DISCOVERY FOR ENERGY AND ENVIRONMENT

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ABSTRACT

Many environmental sites (e.g., groundwater, seawater, contaminated sites) contain low microbial biomass, which can make it difficult to effectively analyze these samples using genomic techniques requiring large amounts of DNA. Functional gene microarrays, for example, require microgram quantities of DNA. Whole community genome amplification (WCGA) amplifies the entire community in a sample using random priming. As such, the use of WCGA would be beneficial for samples containing low concentrations of DNA or that would be difficult to resample. Two DNA polymerases are known to catalyze this reaction: phi29 and *Bst*. Phi29 has been previously shown to be effective for amplifying microbial community DNA with a low amplification bias. This study evaluated the use of *Bst* for WCGA. Initial experiments compared the amplification of *Desulfovibrio vulgaris* using phi29 and *Bst*. An aliquot of DNA (10 ng) was amplified using random heptamers containing two additional 5-nitroindole residues on the 5' end and a phosphorothioate linkage on the 3' end. Amplified DNA (1 µg) and the same amount of unamplified DNA were labeled with Cy5-dUTP and Cy3-dUTP, respectively. The unamplified DNA was pooled and then co-hybridized with the amplified DNA to a *D. vulgaris* microarray at 45 °C for 10 h. All hybridizations were done in triplicate. The signal-to-noise ratio (SNR) was calculated for the unamplified sample and all genes with SNR <3 in any of the triplicates was removed and the remaining genes were used for analysis. The average amplified to unamplified signal intensity was 1.02 (±0.005) and 1.05 (±0.005) for *Bst* and phi29, respectively. The representational bias (DjTotal) values were similar for both the *Bst* and phi29 amplifications (0.181 and 0.157, respectively). The percentage of genes whose ratios were > 1.5, 2, 3, and 4 were 12.56, 2.79, 0.15, and 0.03%, respectively, for *Bst* and 12.22, 3.08, 0.23, and 0.03%, respectively, for phi29. These results suggest that *Bst* would be a viable substitute for phi29. Further experiments are underway to compare the amplification of other pure culture DNA and examine microbial community DNA amplification.

BACKGROUND

Whole genome amplification (WGA) – amplifies the entire genome/community. Based on two principles: 1) Ability of the polymerase to cause strand displacement and 2) random priming initiation sites

Two enzymes catalyze this reaction:

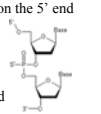
1. **Phi29 DNA polymerase** [Templiphi/Gemniphi (GE Healthcare); REPLI-g (QIAGEN)]
   - ✓ Proof-reading activity
   - ✓ Lower error rate, more efficient amplification
   - ✓ Most often used for WGA
2. ***Bst* DNA polymerase** from *Bacillus stearothermophilus*
   - ✓ Contains the 5'– 3' polymerase activity, but not the 5'– 3' exonuclease activity
   - ✓ Moves through secondary DNA structures

METHODS

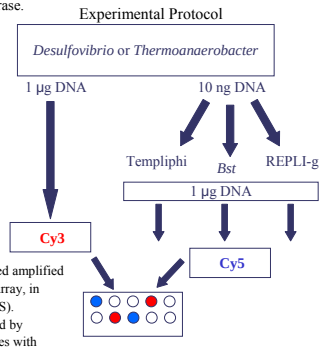
DNA (10 ng) from *Desulfovibrio vulgarus* or *Thermoanaerobacter ethanolicus* was amplified using phi29 DNA polymerase [Templiphi (GE Healthcare) or REPLIg (Qiagen)] following the manufacturer's recommended protocol or *Bst* DNA polymerase.

For *Bst* DNA ploymerase, modified random primers were used:

- Random heptamers
- Two additional **5-nitroindole residues** on the 5' end
  - Universal base
  - Stabilizes primer binding
  - Less background amplification
- **Phosphorothioate linkage** on the 3' end
  - Modified phosphate group
  - Resistant to exo- and endonucleases
  - Resists degradation
  - Enhanced stability



Experimental Protocol

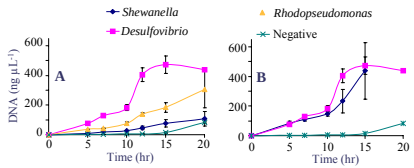


DNA (amplified and unamplified) was labeled with Cy-5 or Cy-3. Labeled amplified and unamplified DNA (1 µg) was co-hybridized to the appropriate microarray, in triplicate, at 45 °C for 10 h using a TECAN hyridization station (Tecan US). Arrays were scanned and signal intensities for each probe were determined by Imagene software (Biodiscovery Inc.). Poor spots were removed and genes with signal-to-noise ratios of ≥3 in all triplicates from unamplified hybridizations were used for analysis.

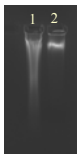
RESULTS

Amplification by phi29 DNA polymerase was performed with Templiphi (GE Healthcare) or REPLIg (Qiagen) kits following the manufacturer's instructions. Amplification by *Bst* DNA polymerase followed protocols from Lage et al., 2003 with modifications in the amplification time.

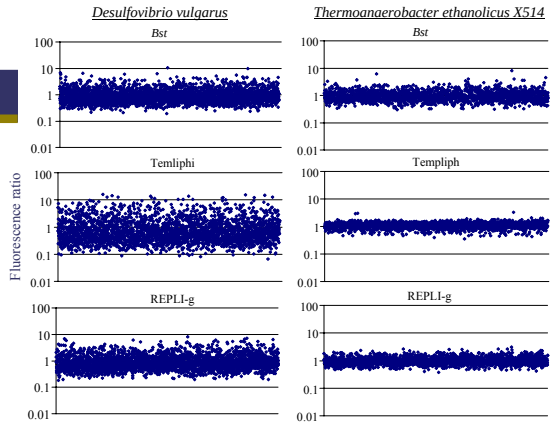
Determine the optimum amplification time for *Bst*



Initial amplification reactions showed a low amount of amplification with *Shewanella* DNA (A). A gel of the unamplified DNA showed degraded DNA (lane 1). Extraction of fresh *Shewanella* DNA (lane 2) produced a band of high molecular weight DNA. Amplification with the fresh DNA produced much higher quantities of DNA (B). Based on results of the amplification timecourse, 10 h amplificaiton was chosen for remaining studies.

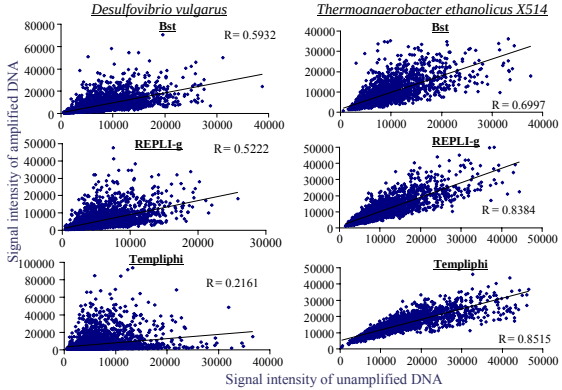


Fresh DNA is critical for optimum amplification



Evaluation of amplification bias with pure culture DNA using three whole genome amplification methods. The signal intensity ratio of amplified to unamplified DNA was calculated for each effective gene (all probes for a particular gene had SNR>3).

Amplification of *Desulfovibrio* showed more bias than *Thermoanaerobacter* with all amplification methods (as evidenced by data points clustering further from 1). With *Desulfovibrio* DNA, *Bst* appeared to have less bias than Templiphi, and a similar level of bias to REPLI-g. With *Thermoanaerobacter* DNA, Templiphi appeared to provide the least amount of bias.



Correlations between signal intensities of amplified and unamplified pure culture DNA. Higher correlations were observed between amplified and unamplified *Thermoanaerobacter* DNA than with *Desulfovibrio* DNA. The highest correlation was observed with Templiphi and REPLI-g phi29 for *Thermoanaerobacter*. For *Desulfovibrio*, *Bst* showed the highest correlations.

Three indices were used to further evaluate amplification bias and compare amplification methods

1. Amount of representational bias (D_j^{Total})
2. Percentage of genes whose ratios of amplified to unamplified DNA are significantly different from 1
3. Percentage of genes whose ratios are > 1.5, 2, 3, and 4

$$D_j^{total} = \sqrt{\sum_{i=1}^{N_j} (LR_{i,j})^2 / N_j}$$

$R_{i,j}$ = ratio of signal intensity of amplified to unamplified DNA
L = log
N = number of genes detected in the unamplified DNA
$D_j^{Total} = 0$ if no bias is present
No upper limit to D_j^{Total}

	Desulfovibrio			X514		
	Bst	**Repli-g**	**Templiphi**	**Bst**	**Repli-g**	**Templiphi**
Total no. of genes	3063	3067	2818	1941	2049	1941
representational bias	**0.2418**	0.2668	0.4147	0.1795	0.1370	**0.1128**
$SDG_{0.01}$	0.6208	0.0316	0.2602	0.5286	0.4397	0.3658
$F_{1.5}$	0.0519	0.0785	0.3407	0.0134	0.0005	0.0021
$F_{2.0}$	0.0114	0.0196	0.1794	0.0021	0.0000	0.0000
$F_{3.0}$	0.0007	0.0010	0.0238	0.0000	0.0000	0.0000
$F_{4.0}$	0.0000	0.0000	0.0018	0.0000	0.0000	0.0000

SUMMARY

- Amplified DNA from *Desulfovibrio* showed higher amplification bias than *Thermoanaerobacter* with all amplification methods tested
- *Bst* showed the least amount of amplification bias with *Desulfovibrio* DNA
- Templiphi showed the least amount of amplification bias with *Thermoanaerobacter* DNA
- Results suggest that variation in amplification bias between different organisms may be less with Bst than with Templiphi

FUTURE STUDIES

Additional studies with different microorganisms and mixtures of microorganisms are required to further evaluate the amplification bias of phi29 and *Bst* DNA polymerases:

Shewanella, *Rhodopseudomonas*, and mixtures with *Desulfovibrio* and *Thermoanaerobacter*

Evaluation of amplification bias of community DNA is also needed.

ACKNOWLEDGEMENT

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