

NEXT GENERATION DNA POLYMERASES FOR SEQUENCING AND AMPLIFICATION

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ABSTRACT

Next generation sequencing platforms utilize one or more polymerases for nucleic acid amplification, library construction, labeling and/or base discrimination. We have combined enzyme discovery and protein engineering to develop a suite of 30 distinct viral and bacterial DNA polymerases (DNAP) and derivatives with enhanced characteristics including thermostability, affinity for template, efficiency in incorporating both standard and modified nucleotides, extension of bead-immobilized primer/templates, reaction kinetics, processivity and strand displacement. Among these, ThermoPhi DNAP has thermostability and strand displacement that allows efficient Whole Genome Amplification of very low amounts of DNA. Accura DNAP is a high fidelity PCR enzyme with superior performance on GC-rich, long and single molecule templates. PyroScript reverse transcriptase allows single enzyme RT PCR and RT LAMP amplification of RNA. We also engineered classical enzymes such as T4, Klenow and Phi29, including an engineered Taq DNAP derivative with improved performance in sequencing GC-rich and secondary structures as well as direct Sanger sequencing of single colonies or liquid cultures. These new enzyme tools are available for evaluation of critical steps in different sequencing platforms including sample preparation for RNA or DNA templates or single molecules, flexible labeling chemistries, improved base discrimination, improved sequencing of difficult templates and enhanced signal generation.

| Lucigen Thermo Polymerases | | | | | | |
|----------------------------|---------|--------------|-----|------------|---------------------|-----------------------|
| Polymerases | 3' Exo- | Temp Optimum | PCR | Processive | Strand Displacement | Reverse Transcriptase |
| Taq | N | 72 | Y | L | N | N |
| Taq98 | N | 72 | Y | L | N | N |
| | | | | | | |
| Accura HiFi | Y | 72 | Y | M | Y | Y |
| PyroScript RT | N | 72 | Y | M | Y | Y |
| ThermoPhi | Y | 65 | N | M | Y | ? |
| | | | | | | |
| Bst | N | 65 | N | M | Y | N |
| Bst Y | N | 65 | N | M | Y | Y |
| Bst W | N | 65 | N | M | Y | Y |
| Bst DS/SS | N | 65 | N | M | Y | N |

Taq98® DNA Polymerase

An engineered form of Taq DNA was constructed and evaluated for sequencing (Fig. 1-3) and amplification (Fig. 4,5). Exceptional thermostability and tolerance of template impurities distinguishes this enzyme, resulting in superior performance

IMPROVED SANGER SEQUENCING

Modified Taq DNAP (Taq98) synthesizes through a difficult hairpin that has confounded other polymerases (Fig. 1).

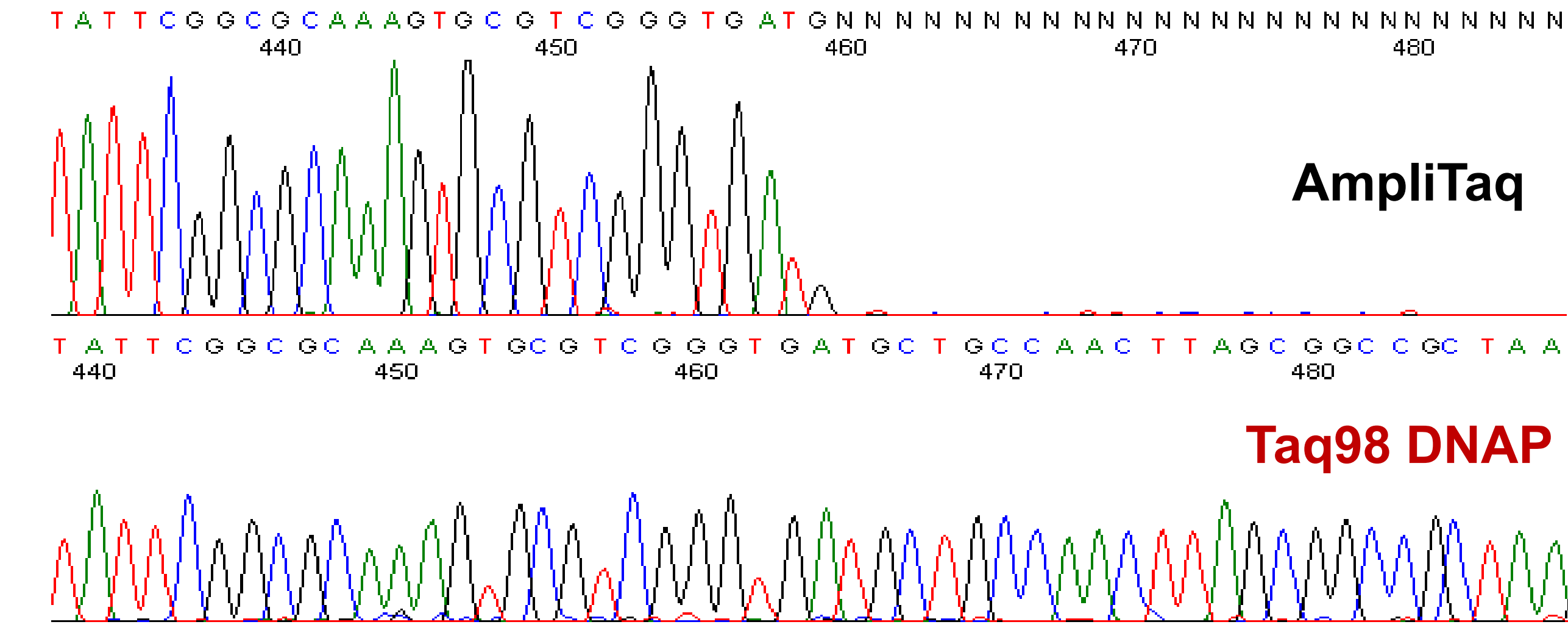


Figure 1. Taq98 DNAP permits sequencing of hairpin DNA templates. ABRF tough template #3 was sequenced with Taq98 DNAP while ABRF study participants were unable to read through the 24bp hairpin using existing DNAPs.

Taq98 DNAP was able to sequence low amounts of template from a single colony or crude lysates, a task for which AmpliTaq DNAP is inefficient (Fig. 2,3).

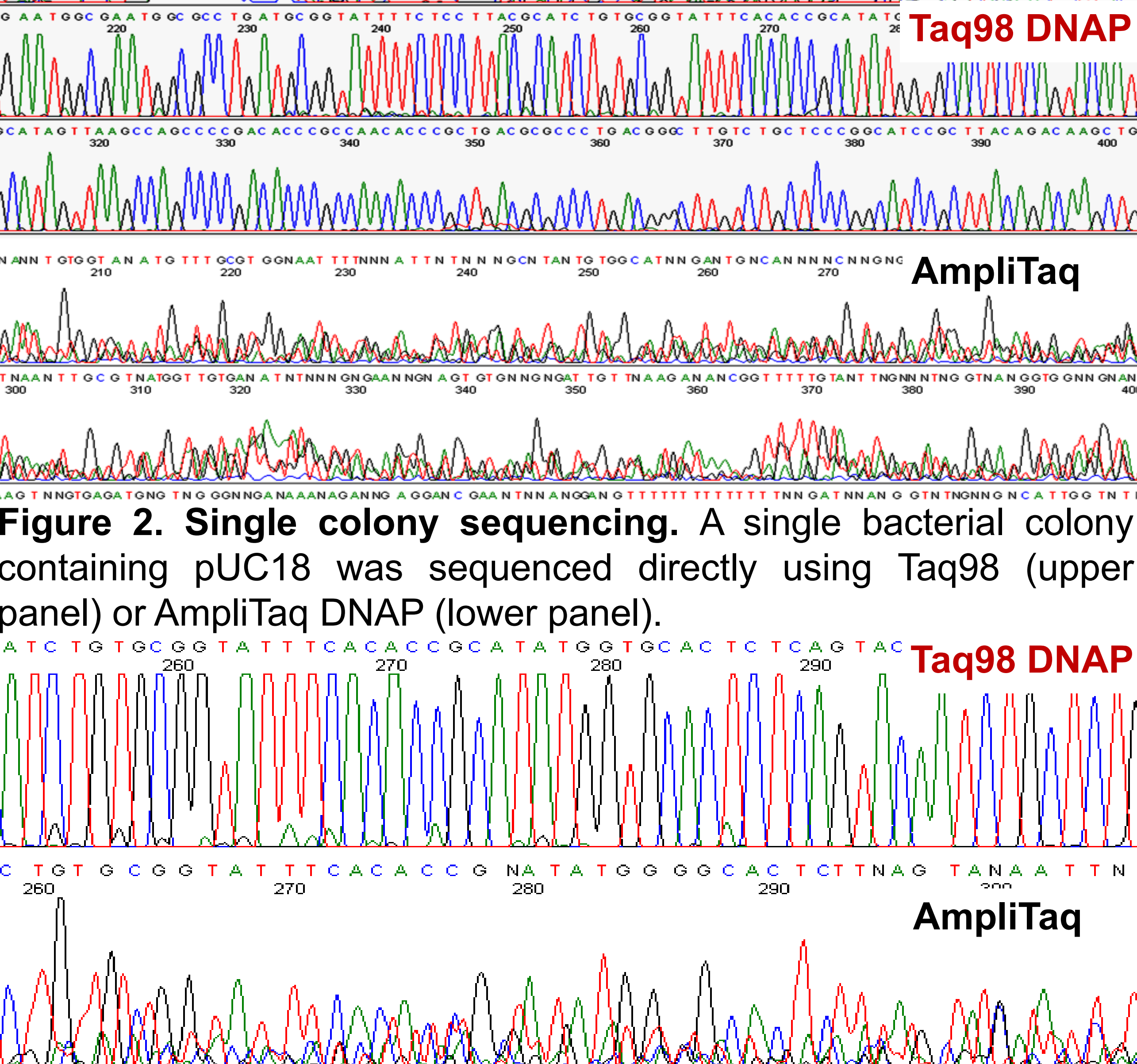
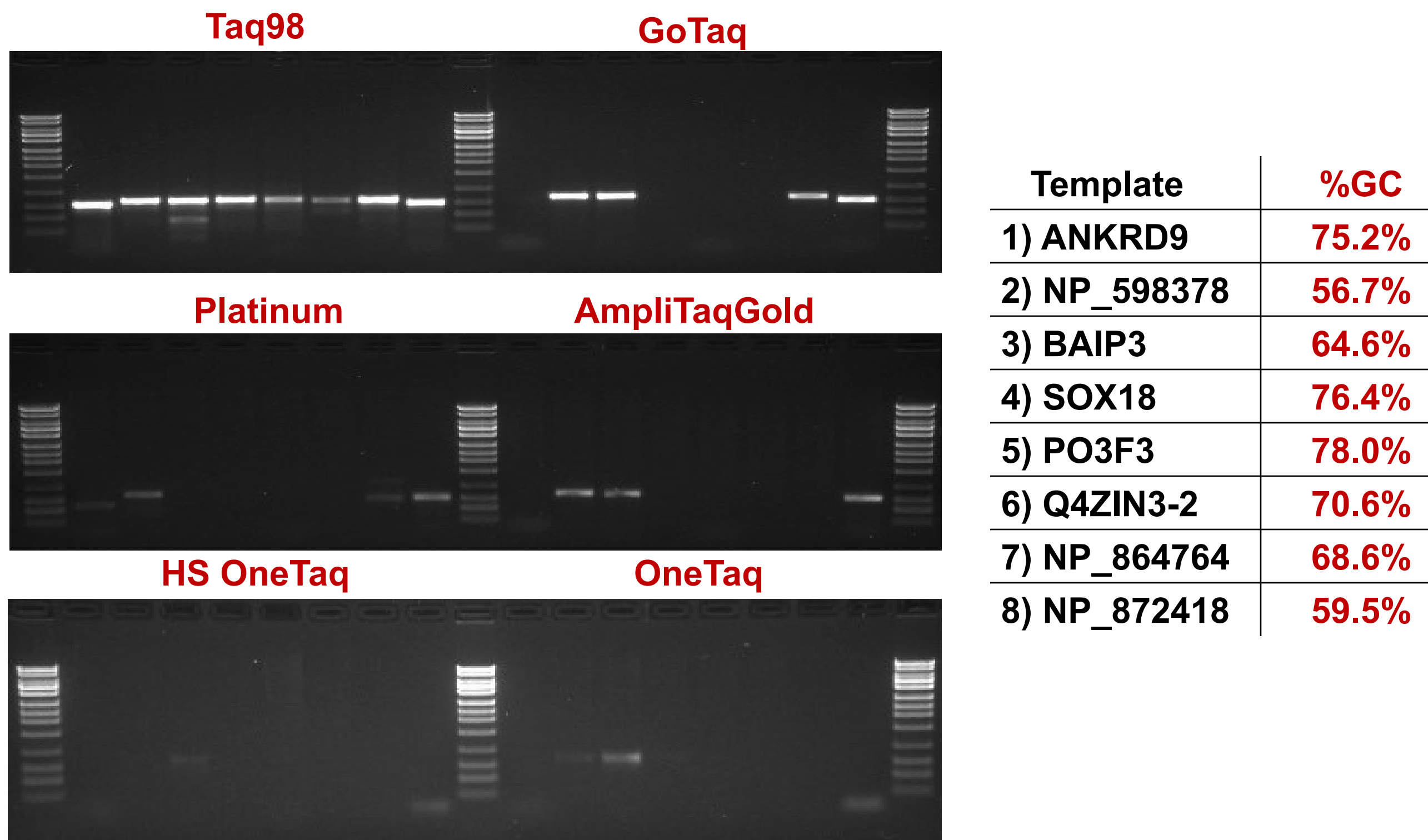


Figure 2. Single colony sequencing. A single bacterial colony containing pUC18 was sequenced directly using Taq98 (upper panel) or AmpliTaq DNAP (lower panel).

PCR AMPLIFICATION OF DIFFICULT HUMAN TEMPLATES

Figure 4. The human target sequences listed were chosen based on an elevated GC content of 60-80%. Sequences were PCR amplified with published primers (Zhang (2009) *Biotechniques* 47:775-8) from purified human genomic DNA using the indicated thermostable polymerases according to manufacturer instructions.



PCR DIRECTLY FROM WHOLE BLOOD

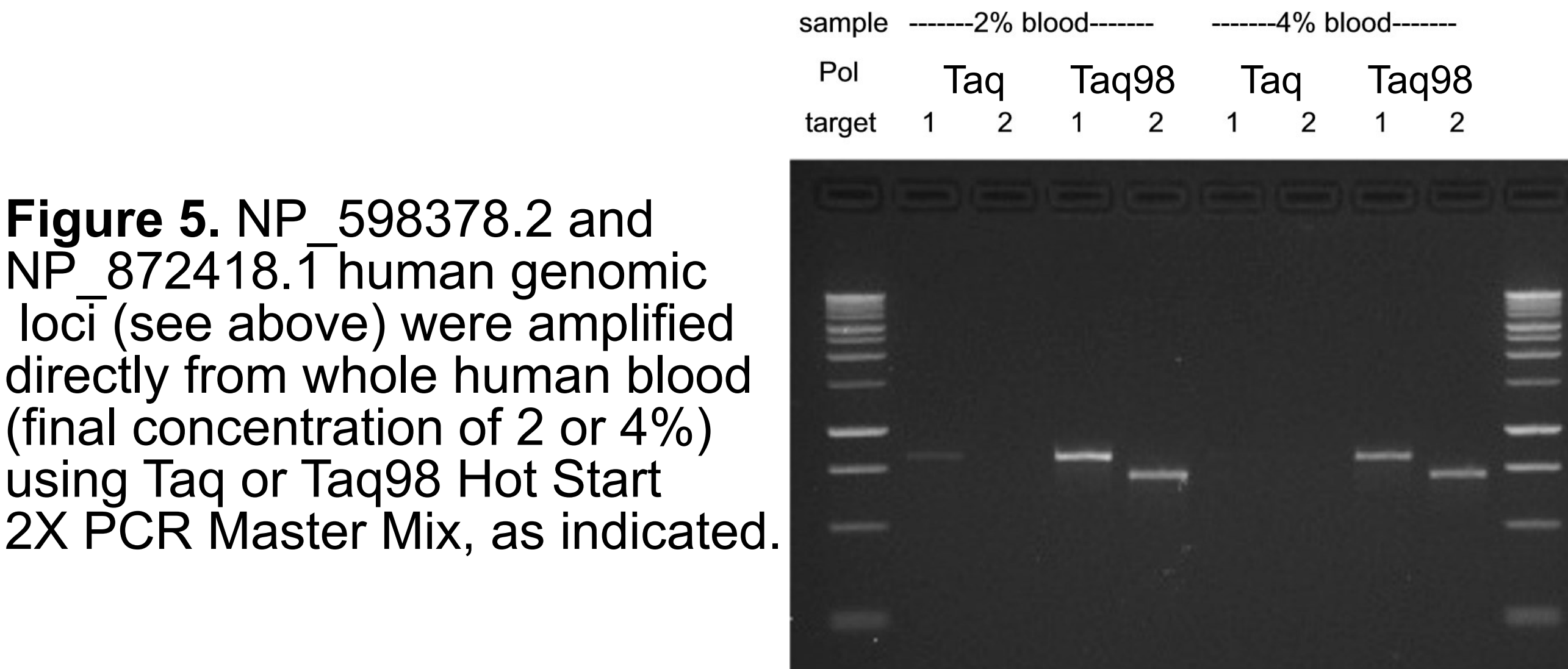
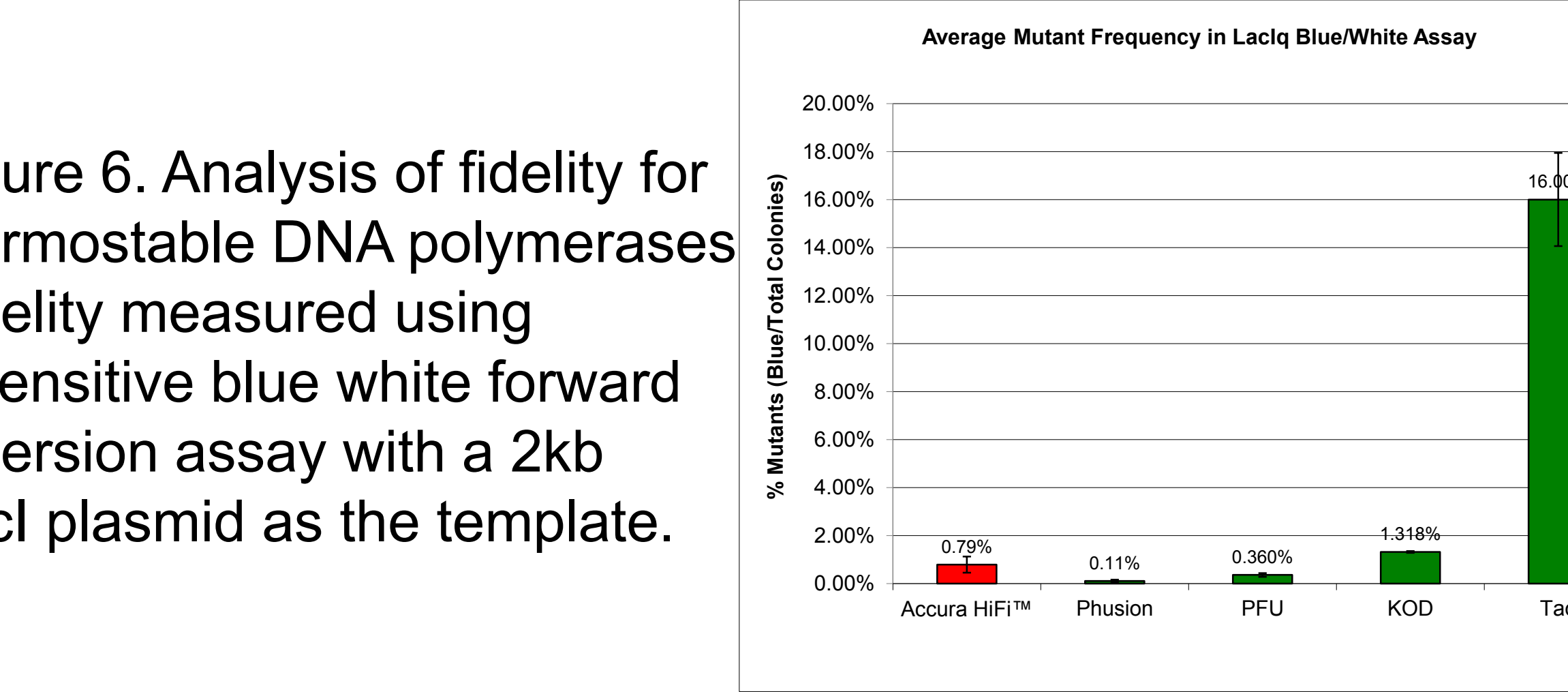


Figure 5. NP_598378.2 and NP_872418.1 human genomic loci (see above) were amplified directly from whole human blood (final concentration of 2 or 4%) using Taq or Taq98 Hot Start 2X PCR Master Mix, as indicated.

Accura High Fidelity DNA Polymerase

20 times higher fidelity than Taq



HIGH SPECIFICITY & FIDELITY PCR OF DIFFICULT HUMAN TEMPLATES

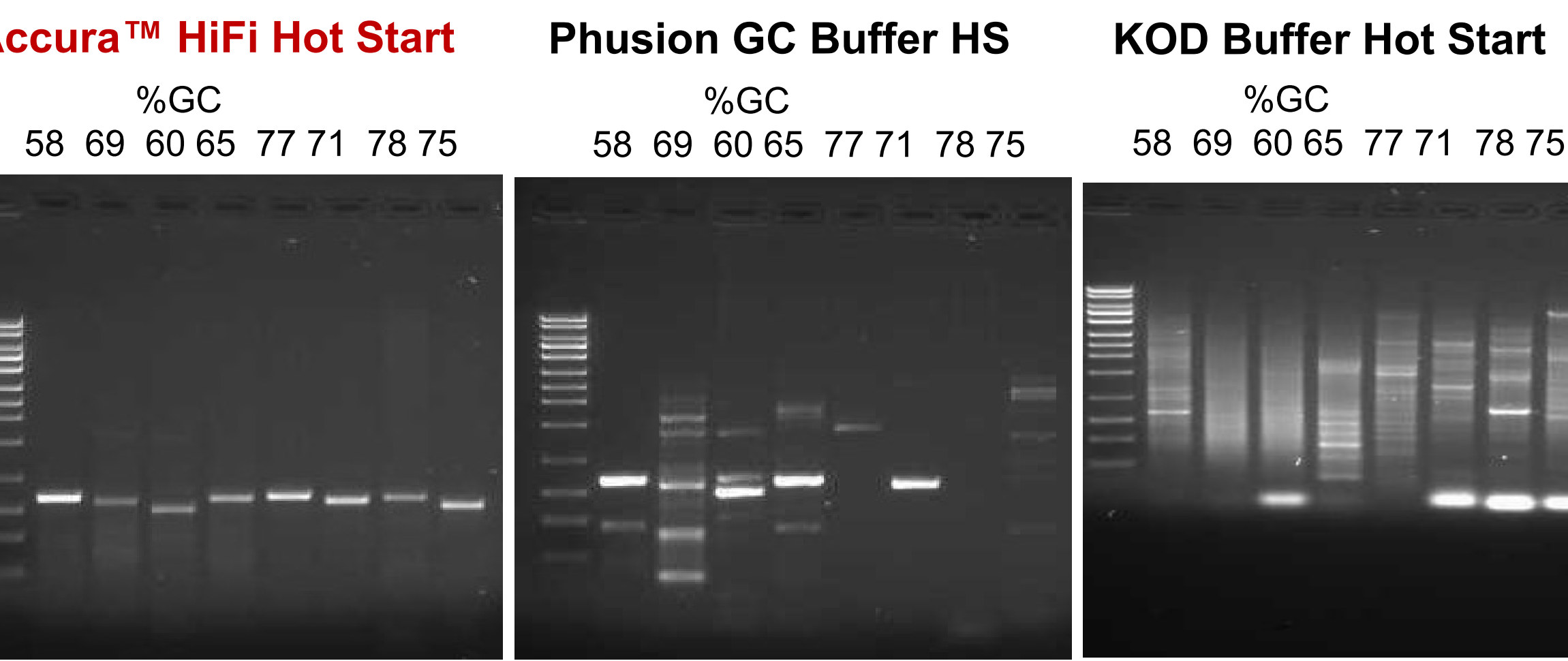
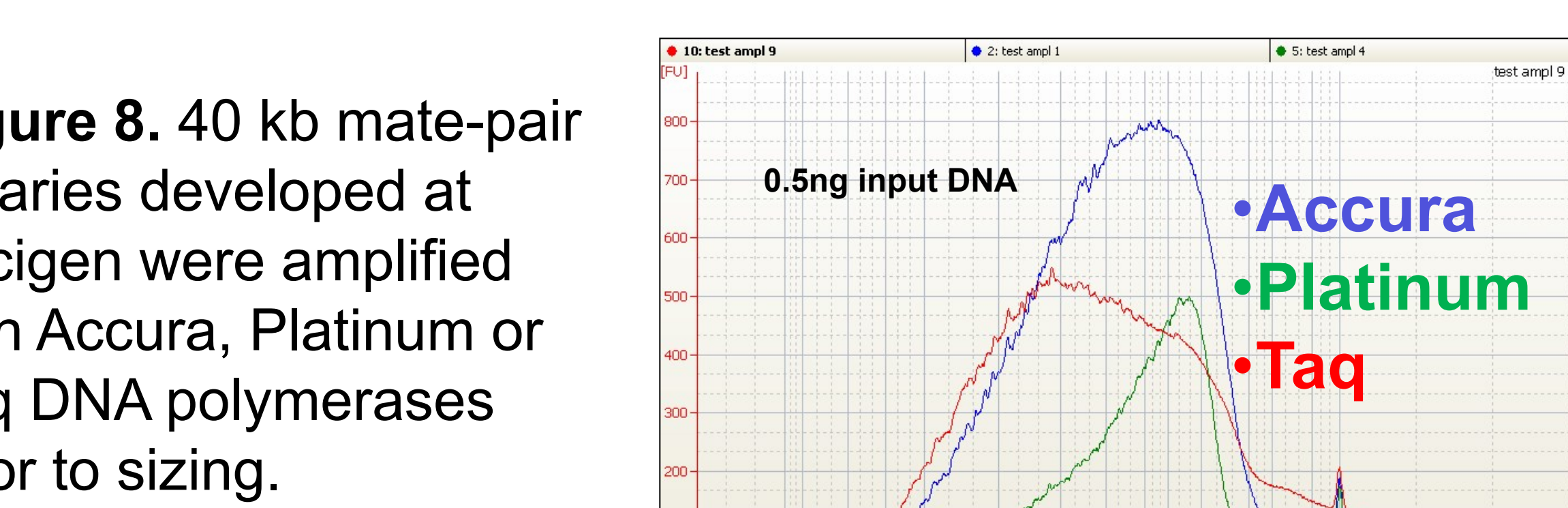


Figure 7. The human target sequences listed in Fig. 4 were chosen based on an elevated GC content of 60-80%. Sequences were PCR amplified from purified human genomic DNA using the indicated thermostable polymerases according to manufacturer instructions.

EVEN, HIGH YIELD AMPLIFICATION OF NGS LIBRARIES



ThermoPhi™ DNA Polymerase

Strand Displacement Amplification with Thermostable High Fidelity Pol

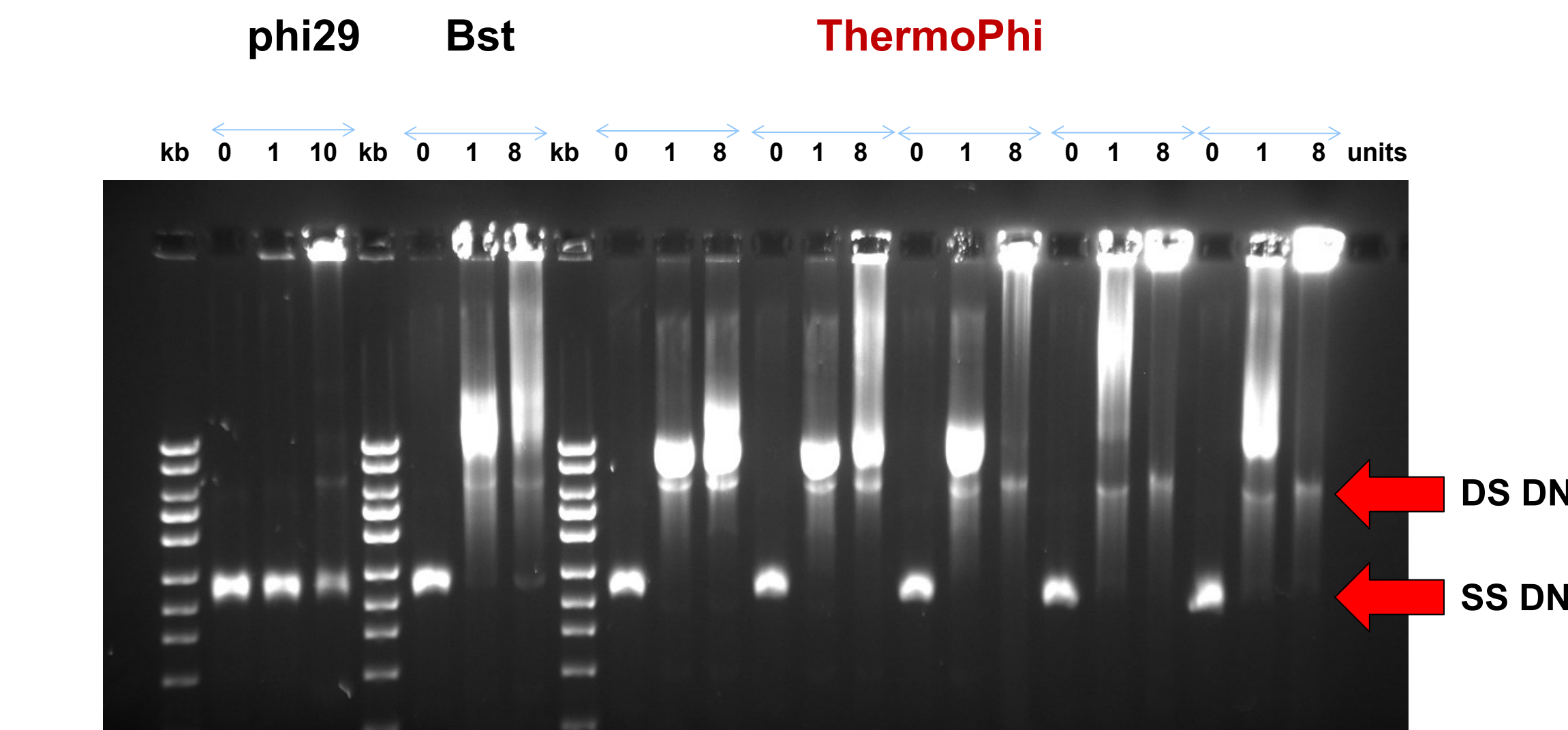
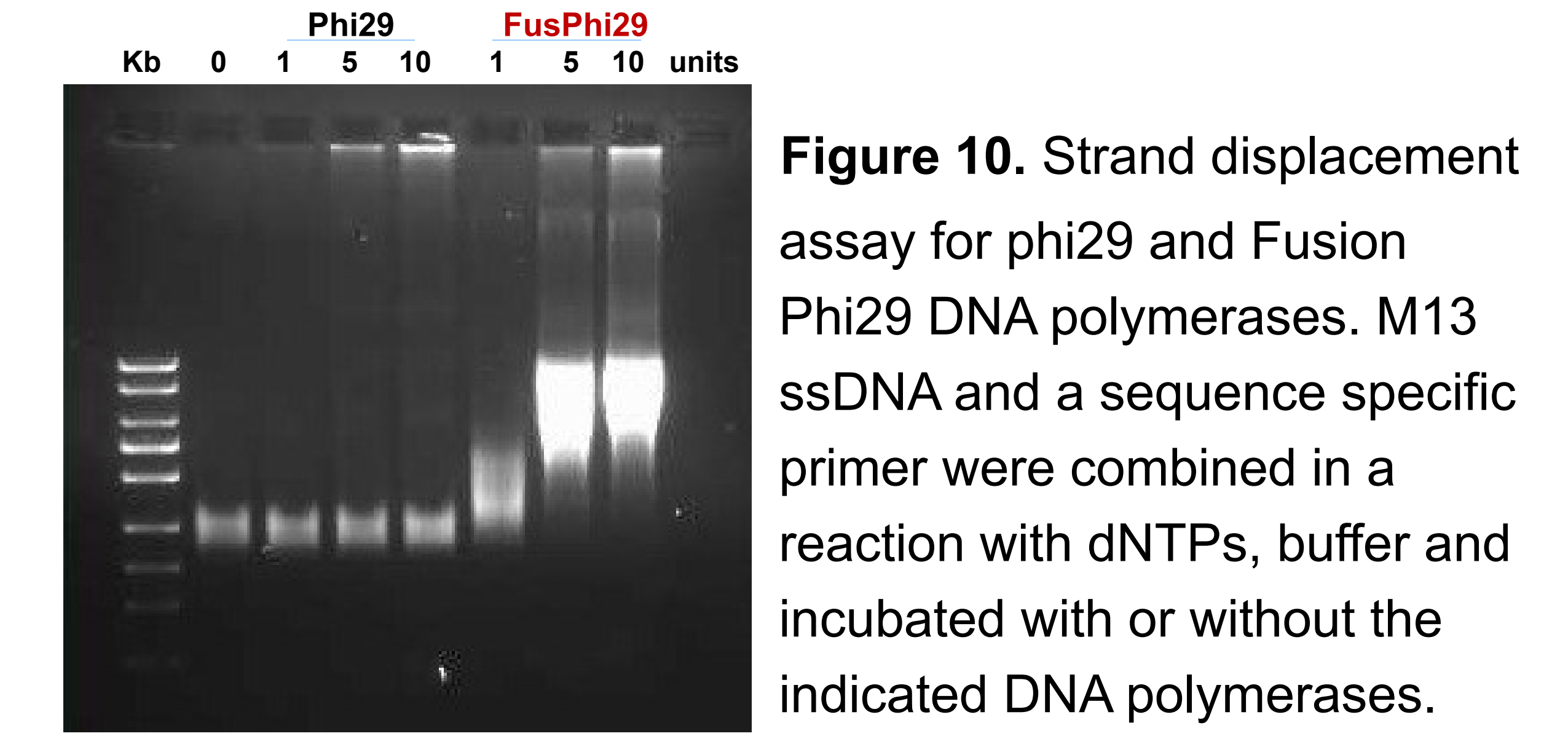


Figure 9. Strand displacement assay for phi29, Bst and ThermoPhi DNA polymerases. M13 ssDNA and a sequence specific primer were combined in a reaction with dNTPs, buffer and incubated with or without the indicated DNA polymerases. The red arrows indicate the position where single stranded (SS) or double stranded (DS) M13 DNA migrates.

Lucigen Meso Polymerases

| Polymerase | 3' Exo- | Temp Optimum | Processive | Strand Displacement |
|------------------|---------|--------------|------------|---------------------|
| Klenow Exo- | N | 37 | L | N |
| Klenow SSHA Exo- | N | 37 | L | N |
| Klenow DSHA Exo- | N | 37 | L | N |
| T4 | Y | 37 | M | Y |
| T4 exo- | N | 37 | M | Y |
| T4 SSHA Exo- | N | 37 | M | Y |
| T4 DSHA Exo- | N | 37 | M | Y |
| T5 DNAP | Y | 37 | H | Y |
| 1773 DNAP | N | 30 | ? | Y |
| Phi29 | Y | 30 | H | Y |
| FUSION Phi29 | Y | 30 | H | Y |

Fusion Phi29 DNA Polymerase



CONCLUSIONS

- Taq98 DNA Pol**
- High affinity DNA polymerase improves Sanger sequencing through secondary structures and from nanogram amounts of unpurified template.
 - Denaturation at 98°C enables amplification through GC rich targets.
 - Higher tolerance for impurities in samples including blood.

- Accura HiFi DNA Pol**
- High fidelity DNA Pol with improved amplification properties.
- ThermoPhi DNA Pol**
- High fidelity DNA Pol with strong strand displacement activity.
- Fusion Phi29 DNA Pol**
- Enhanced amplification of phi29 DNA Pol.

ACKNOWLEDGEMENTS

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