# Cross Site Evaluation of Sanger Sequencing Dye Chemistries

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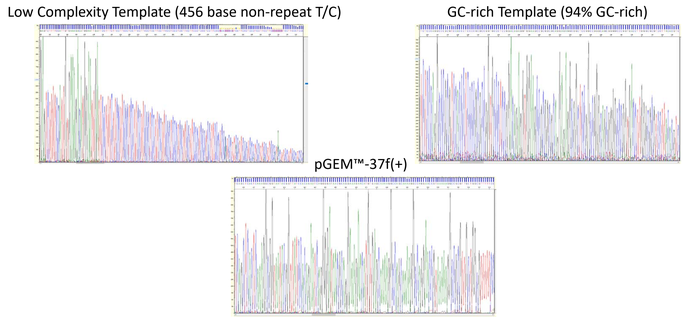
## Abstract

Sanger sequencing remains an essential tool utilized by researchers. Despite competition from commercial providers, many sequencing core facilities continue to offer Sanger sequencing services to their customer base. By reducing costs and providing rapid turnaround times, in-house Sanger sequencing remains a viable core service, often helping to subsidize more costly services such as next generation sequencing. While Applied Biosystems' BigDye™ Terminator chemistry was once the only solution available for Sanger DNA sequencing, several new products employing novel dye chemistries and reaction configurations have entered the market. Currently, it is unclear how these new chemistries perform on various DNA templates, including difficult templates or their amenability to commonly employed cost-saving measures such as dye dilution and reaction miniaturization. With this goal in mind, we compared the quality of Sanger sequencing data produced by kits available from several vendors using control and difficult-to-sequence DNA templates under various reaction conditions. This study will serve as a valuable resource to core facilities conducting Sanger sequencing, providing guidelines on appropriate protocols to use with each kit and determining the most cost effective solutions for Sanger sequencing while maintaining high quality results.

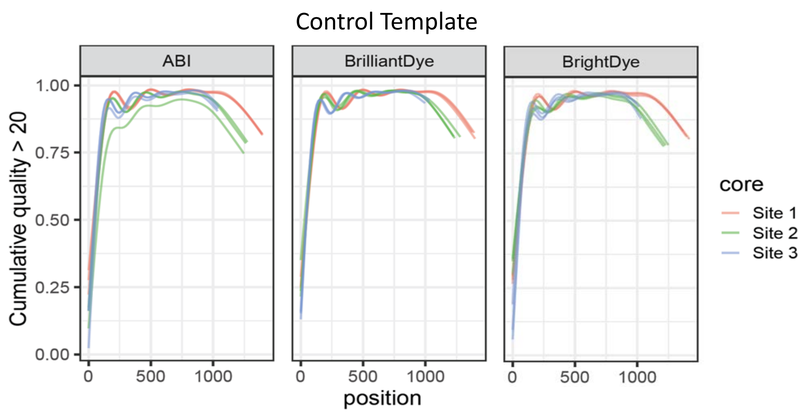
## Experimental Variables




EdgeBio
BrilliantDye™
ThermoFisher SCIENTIFIC
BigDye™
MCLAB
Molecular Cloning Laboratories
BrightDye®

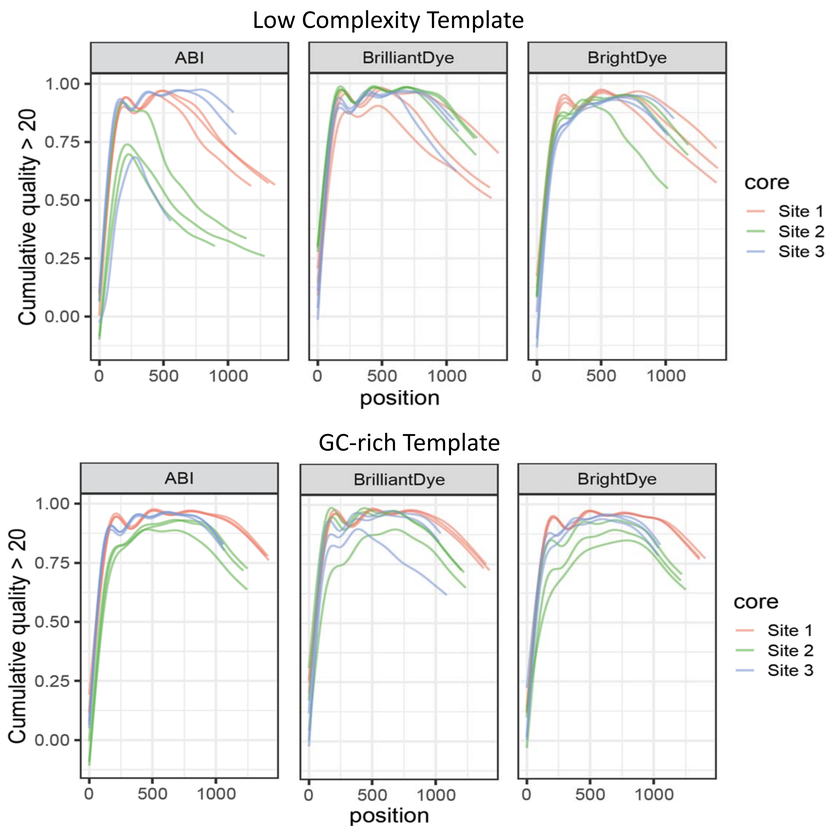
## Benchmarking Results

- Applied Biosystems BigDye™, 0.5X 10ul reaction

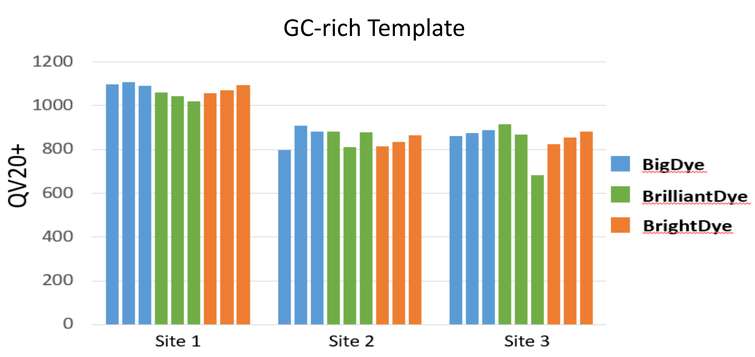



## Difficult to Sequence Templates

- Protocol 1 from Kieleczawa et al*

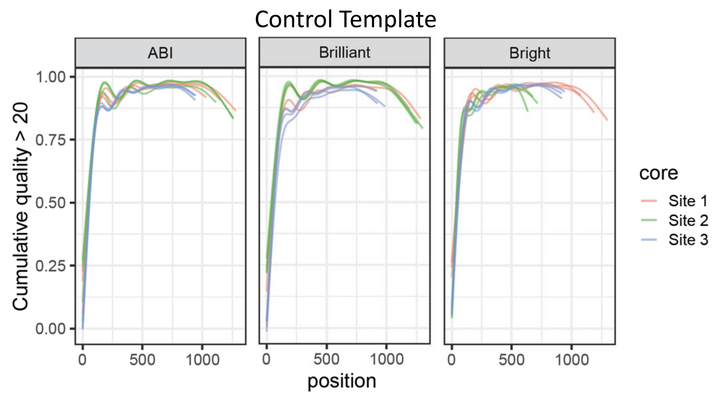


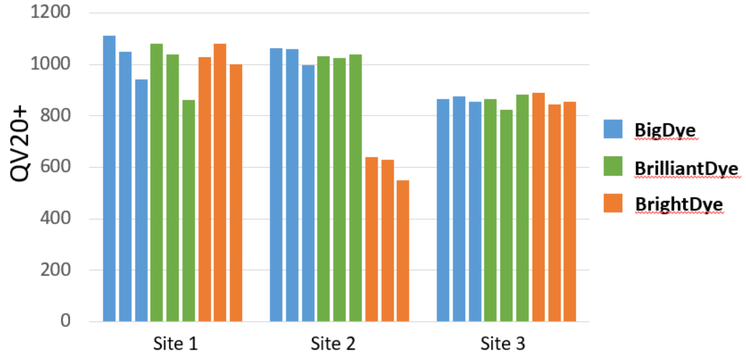





## Drop In Ready

- Each site swapped **ONLY** the dye!
- Each core used their own SOP.




- BrilliantDye and BrightDye can both handle low complexity/high GC templates.

## Conclusions

- All dyes are capable of producing high quality sequence data.
- Dyes work similarly in standard protocol and across sites with pGEM.
- BrilliantDye and BrightDye can both handle low complexity/high GC templates.
- The new chemistries can be dropped into existing core workflows without the need to make major workflow changes.
- When evaluating only pGEM™-37f(+) under standard ABI conditions, adjusting sequencer parameters has a greater impact on read lengths than chemistries.

## Acknowledgements

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* Kieleczawa J, Adam D, Bintzler D, et al. Identification of optimal protocols for sequencing difficult templates: results of the 2008 ABRF DNA Sequencing Research Group difficult template study 2008. J Biomol Tech. 2009;20(2):116-27.