

DSRG-2004 - A Web-based User-Interactive DNA Sequencing Troubleshooting Resource

Heather Lin,^a Helaman Escobar,^b John W. Hawes,^c David S. Needleman,^d Timothy Hunter,^e Emily Jackson-Machelski,^f Kevin L. Knudtson,^g Rashmi Pershad,^h Margaret Robertson,ⁱ and Glenis J. Wiebe^j
^aUniversity of Utah, Salt Lake City, UT, ^bBrigham Young University, Provo, UT, ^cIndiana University School of Medicine, Indianapolis, IN, ^dThomas Jefferson University, Philadelphia, PA, ^eVermont Cancer Center, Burlington, VT, ^fWashington University School of Medicine, St. Louis, MO, ^gUniversity of Iowa, Iowa City, IA, ^hUniversity of Texas M.D. Anderson Cancer Center, Houston, TX, ⁱErnest Galle Clinic and Research Center, UCSF, San Francisco, CA, ^jMax Planck Institute of Molecular Cell Biology & Genetics, Dresden, Germany

Abstract

Automated DNA sequencing has become an essential tool for the molecular biologist. This technology has undergone considerable changes in instrumentation, sequencing chemistry, and analysis software since its development over a decade ago. Users have experienced, and continue to experience, many challenges. As a means of aiding automated DNA sequencing users, the DNA Sequencing Research Group (DSRG) has begun to establish a web-based, user-interactive troubleshooting resource.

Introduction

The DSRG has planned a two year study (2003 - 2005) to develop a troubleshooting resource that is designed to provide a great reference tool for DNA Core Facilities when: (1) trying new sequencing chemistries, (2) evaluating new instrumentation or (3) having instrument or sequencing difficulties. The first part of this study has involved designing the format for this resource and establishing what topics will be included. The second part of the study will be opened out to the sequencing community this year. The DSRG will invite users to submit examples of problems they have encountered and the solutions if any. All before and after chromatograms along with a completed survey sheet describing the problem and details of both runs will be submitted. These examples will then be used to populate the database, which will be available to everyone via the ABRF website. An outline of the above proposal will be presented here. The goal is to provide a searchable database that allows DNA Core Facilities to quickly and easily find solutions to sequencing problems and attracts new members to the ABRF.

Who Will Benefit

- DNA sequencing related core labs worldwide
- Research and biotech companies with in-house sequencing facilities
- Core facility users and customers
- Vendors and manufactures for sequencing reagents and instruments
- ABRF

What is Covered

Instrumentation:
 - ABI 373, 377, 310, 3100, 3700, 3730XL
 - MegaBACE[™] 500, 1000 and 4000
 - Licor
 Chemistry:
 - BigDye Terminator 1.1 and 3.1
 - DYEnamic[™] ET Dye Terminator
 Separation Matrix Material:
 - Performance Optimized Polymer (POP) 4, 5, 6, and 7
 - Linear polyacrylamide (MegaBACE[™] long read matrix)
 - Acrylamide
 Post-amplification Purification Methods
 Loading Reagents
 Others

In What Format

- Search (for known problems and solutions)
- Forum (for posting new problem and solution)
- Contacts (list of participants and contact information)

Example of a search:

- The user enters a query that carries him down a path of logical switch points.
- At each step the search is narrowed enabling the user to pinpoint a specific problem.
- Ultimately, the user will be directed to a solution page based on the search criteria.
- The solution page will contain a description of the problem and where applicable an example chromatogram. Possible remedies will be presented and the user may also be directed to other resource pages or web sites.

Example #1: Waterfall Effect on Capillary Machines

SEARCH: **Step 1**

By instrument
By chemistry
By polymer

Step 2

SEARCH: **Step 3**

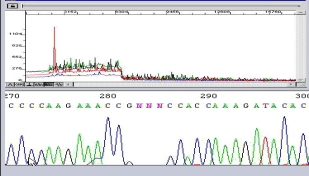
Capillary-based
ABI 3730XL
ABI 3730
ABI 3700
ABI 3100
ABI 310
MegaBACE
Licor

SEARCH: **Step 4**

Potential problems
Run conditions
Effect of Ethanol
Effect of RNA
Bad Capillary
Waterfall Effect
Bad Spectral

Search Result

Search by: Instrument > Capillary-based > ABI3100 > waterfall effect
 Search result: here is a list of data related to your search criteria.



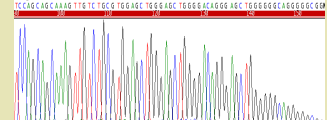
The waterfall effect:
Affects an individual capillary or multiple capillaries. The baseline is elevated at the beginning of the run then drastically drops to a low value for the remainder of the run.

Remedy:
Appears to be a leaching problem into the polymer block.
Wash the blocks in very hot water and repeat this treatment on a weekly basis.

Example #2: Fading Signal due to G Stretch

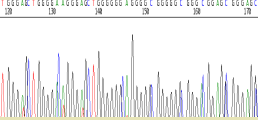
Search by: Chemistry > BigDye > G Stretch

Problem-Poly G drop off



Default Seq. Conditions, BigDye v3.1, 55 degrees annealing

Solution-Alternative sequencing chemistry-dGTP kit



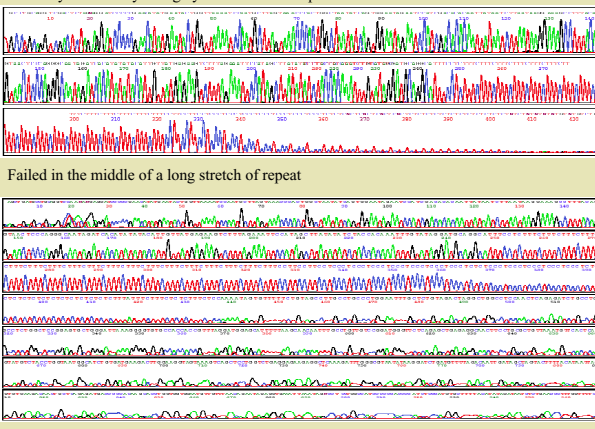
dGTP kit, 55 degrees annealing

Note: BigDye chemistries replace dGTP with dTTP to minimize compressions. dTTP incorporation; however, is less efficient and can terminate in G rich regions.

Note: Use of dGTP chemistry allows efficient extension through G rich regions; this chemistry however causes a characteristic compression seen above.

Example #3: Sequencing Through Difficult Template

Search by: Chemistry > BigDye > Stretch of Repeats



Failed in the middle of a long stretch of repeat

Sequenced successfully through the repeat

Protocol for the successful sequence above on ABI377:

- Hot Start: 96 °C X 2 minutes
- Denaturation: 98 °C X 10 seconds
- Annealing: 55 °C X 15 seconds
- Extension: 60 °C X 4 minutes
- Total 30 cycles

Conclusions

This is a preliminary format for the trouble-shooting resource and the DSRG will finalize the construction and organization of the database within one year. What we need from all ABRF members and those who are interested in DNA sequencing is to help populate the database and continue to contribute after the official resource is established. Please start saving sequencing results of your interests (problematic or successful), or submit your comments to the DSRG through the ABRF forum. Your participation is very welcome and greatly appreciated.