"NewTech" DNA Error Reduction System: Black-box Introduction

Overview

Novici Biotech LLC has recently developed a new technology and corresponding consumable product(s) for reducing errors in synthetic DNA (herein called "NewTech" for purposes of this introduction).

Summarized below are two "black box" applications of NewTech that illustrate the effectiveness of the system for minimizing errors in synthetic DNA and other amplified DNA products.

In these examples, two independent modes of use of NewTech were used to perform DNA error reduction. This technology can be used in additional modes of operation and configuration for compatibility with a variety of standalone commercial applications.

Prototype Error Reduction Test Results

Use Mode 1 example: NewTech-SD

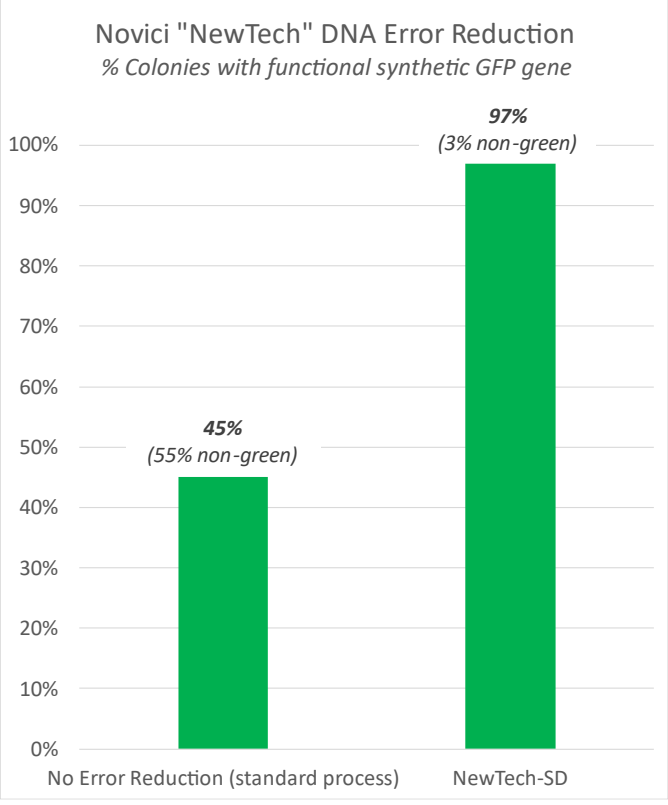
Test Material: Synthetic DNA test fragment encoding a 717bp GFP gene assembled from commercial 60-mer tube oligos. DNA errors within this pool consisted primarily of small indels and base substitutions.

Experiment: The test fragment was taken through a single round of the NewTech-SD process, or through the standard process (no error reduction control).

Scoring: Green (functional GFP) / nongreen (non-functional GFP) bacterial colony counts and sequencing

Results:

- **Negative control:** 45% green, 55% white
- **NewTech-SD:** 97% green, 3% white
- **18-fold reduction** in non-functional genes
- DNA sequencing of colony picks shows a commensurate reduction in actual errors.



In a separate experiment, one round of ErrASE was performed on the synthetic GFP gene, followed by two rounds of NewTech-SD. After these processes, ~98% of the resulting colonies were green. Of 7 non-green colonies that were picked and sequenced, all 7 were empty-vector or other addressable cloning artifacts. Thus, none of the non-green colonies that were analyzed contained any portion of the GFP gene, suggesting that the vast majority of GFP sequences present in the population were functional and that intentional repetition of the process did not generate deleterious artifacts.

Use Mode 2 example: NewTech-SI

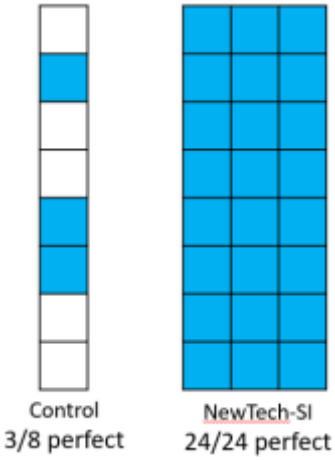
Test Material: DNA fragment encoding a 759bp non-marker test gene deliberately mutagenized by extensive PCR amplification with Taq DNA polymerase. The resulting fragment pool contained primarily single-base substitutions.

Experiment: The fragment was either not subjected to error reduction at all (negative control) or taken through a single round of our NewTech-SI process.

Scoring: DNA sequencing from bacterial colony picks

Results:

- **Negative control**
  - Perfect genes: 3 of 8 picks
  - Errors: average 1 in 1012 bases
- **NewTech-SI**
  - Perfect genes: 24 of 24 picks
  - Errors: None observed in 18,216 bases



NewTech Effectiveness Conclusions

- NewTech incorporation into the DNA synthesis workflow reduced DNA errors by at least 18-fold. In contrast, commercial error reduction products such as Authenticase (NEB), CorrectASE (Life Tech), and ErrASE (Novici) are generally reported to reduce DNA errors by 3 to 10-fold.
- For additive effect, the NewTech use modes described here can also be performed in serial repetition or in combination with additional NewTech operational modes. Also, NewTech can be used in conjunction with other, mechanistically unrelated error reduction strategies such as ErrASE.
- NewTech can be flexibly formatted and performed with standard laboratory equipment.
- Additional use mode embodiments have been tested, and still other modifications envisioned including for effective integration into higher order DNA assembly schemes.

Frequently Asked Questions

- **In the data summarized above, what is the breakdown of correction effects for insertions, deletions, and substitutions?**

    - The target DNAs used in NewTech tests contained both substitutions and insertions/deletions from phosphoramidite oligo pools used in initial synthesis, or they contained primarily substitutions introduced during PCR over-cycling with Taq polymerase.
- **What method was used to obtain sequence data for the experiment described?**
    - All reads were clean, high-quality Sanger PCR reads from individual colony picks.
- **Do you have results for a larger set of sequences; for example, 48-96 constructs of varying complexity?**
    - Data are not available for this type of sequence set for the most advanced NewTech use modes. Sequence complexity and data generation rationale can be addressed for specific application interests in a confidential meeting.
- **What is the substitution pattern per letter before and after the error correction step?**
    - Patterns pre-error correction were typical of the types of target DNAs described. Post-error correction, so few errors were present that it was not possible to discern any particular pattern change without conducting much larger-scale experiments.
- **Does the protocol work for double-stranded DNA or only for plasmid DNA (i.e. cloned double-stranded fragments)?**
    - The protocol presented here involved the insertion of target DNAs into plasmid DNAs.
- **What are the other key requirements in terms of experimental conditions?**
    - These processes can be performed using simple equipment and protocols found in any molecular biology laboratory.
- **What is the legal status of NewTech?**
    - NewTech was invented and developed solely by Novici Biotech LLC members and employees and is protected by a combination of trade secrets and pending patents assigned to Novici. As of May 23, 2024 no licenses have been granted to third parties.
- **Is Novici willing to provide a sample of NewTech reagents with protocols for testing under a non-disclosure agreement?**
    - Novici will provide samples and protocols under a fee-bearing, limited-term, non-exclusive license and material transfer agreement to enable time and scope limited testing.
    - Novici will custom tailor the license scope and limitations to the priority interest of the licensee upon request.

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