Efficient Adaptor Ligation for the Preparation of dsDNA Libraries using the Blunt/TA Ligase Master Mix

Greg Lohman, Ph.D., New England Biolabs, Inc.

Introduction

Numerous applications require the ligation of libraries of dsDNA fragments to a constant "adaptor" dsDNA sequence, including next generation sequencing (NGS) library preparation, high complexity cloning libraries, immobilization of libraries to a surface, or any application that requires the addition of known sequences or modified nucleosides to the ends of a library of random DNA. Typical libraries use fragments that have been blunted (for example, using the Quick Blunting Kit, NEB #E1201) or blunted then dA-tailed (for example, using the NEBNext® dA-Tailing Module, NEB #E6053). For many NGS applications, optimized workflows exist in kitted form that will result in maximized library yields and minimized bias for that particular application (e.g., NEBNext Ultra™ II DNA Library Prep Kit for Illumina®, NEB #E7645). However, for development of new applications or efficient adaptor ligation in other contexts, a stand-alone ligase formulation is required that can rapidly ligate blunt or dT-tailed adaptors onto an insert library with high yield.

The Blunt/TA Ligase Master Mix (NEB #M0367) is a formulation of T4 DNA Ligase pre-mixed with its reaction buffer and proprietary ligation enhancers, in a convenient single-tube 2X mixture (1). In most applications, combining equal volumes of master mix and a solution of dA-tailed insert with a 5-10 fold excess of dT-tailed adaptor will provide rapid ligation and high library yield exceeding that of any other ligase formulation. The Blunt/TA Ligase Master Mix is also suitable for ligation of blunt adaptors onto blunted library fragments, although in this case increased adaptor (20X or greater) may be required to minimize insert concatemers and offset adaptor dimerization.

Here, we demonstrate the use of Blunt/TA Ligase Master Mix for the efficient ligation of adaptors to dA tailed dsDNA fragments. The substrates tested were prepared from short, synthetic oligonucleotides purchased from IDT, and annealed using standard conditions (Figure 1). The 49 base pair, dA-tailed "Insert" molecule is labeled with an internal FAM-dT nucleotide to allow simple quantitation of products via fluorescence, while the 33 base pair, dT-tailed "adaptor" is not fluorescently labeled.

Materials

DNA insert library in nuclease free water
(2 µM in ligatable ends, ~60 ng/µl for 50 bp dsDNA fragments, ~250 ng/µl for 200 bp fragments, or ~1.3 µg/µl for 1,000 bp fragments)

DNA adaptors in nuclease-free water
(10 µM, ~ 300 ng/µl for 50 bp adaptors)

High concentration T4 DNA Ligase
(NEB #M0202 M or T)

T4 DNA Ligase Buffer (10X, included with High Concentration T4 DNA Ligase)

Quick Ligation™ Kit
(NEB #M2200)

Blunt/TA Ligase Master Mix
(NEB #M0367)

Nuclease-free water

Stop solution (50 mM EDTA)

FIGURE 1:
Insert and Adaptor Sequences

In the insert, note that the bolded/underlined T is modified with a fluorescin. 'AM' in the adaptor sequence represents an amino-linker used to block the 3´-hydroxyl.

Insert
5´-pCTTCTAGGTTCCTATGA**T**TCTGGGACTGACCGAGCCTGACTCACAATTGA-3´
3´-AGAAGATCCAAGGATACTAAGACCCTGACTGGCTCGGACTGAGTGTTAACp-5´

5´-pGATCGGAAGAGCACACGTCTGAACTCCAGTCAC-3´AM
3´-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG-5´

Protocol

1. Place master mix on ice prior to reaction setup. Mix tube by flicking before use.
2. Combine dA-tailed dsDNA library in water with 5–10 fold molar equivalents of dT-tailed adaptor.
3. Add an equal volume of Blunt/TA Ligase Master Mix.
4. Mix thoroughly by pipetting up and down 7–10 times or by flicking.
5. Incubate at room temperature (25°C) for 20 minutes. Then, place the tube on ice (incubation times can be extended up to 1 hour if yields are still low after 20 minutes).
6. Stop the reaction by adding 10 µl of stop solution (50 mM EDTA), or by purification using an appropriate method (e.g., Monarch® PCR & DNA Cleanup Kit (NEB #T1030), AMPure® Beads or gel purification). Do not heat inactivate.
7. Purified DNA can be stored at 4°C for use within a few weeks, or frozen at -20°C for prolonged storage.

Results

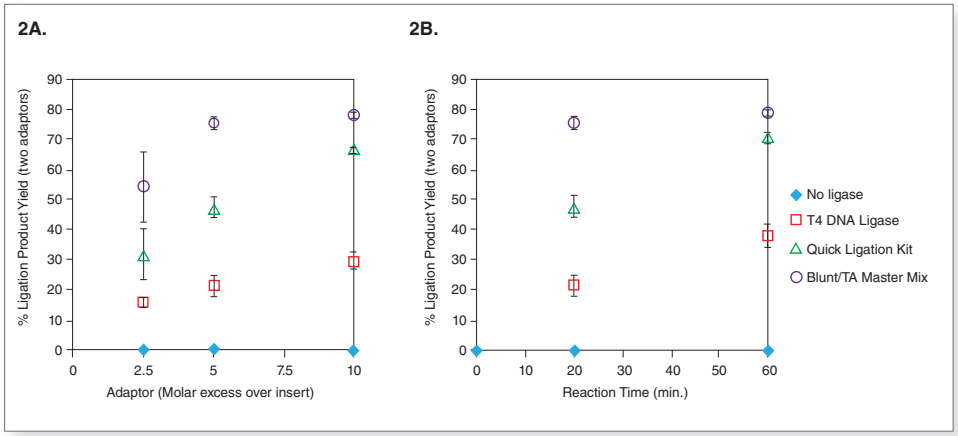
Adaptor ligation using the Blunt/TA Ligase Master Mix (10 µl in a 20 µl reaction volume) was compared to ligation using the NEB Quick Ligation Kit (1 µl Quick Ligase and 10 µl 2X Quick Ligation Buffer in a 20 µl Reaction volume) and High Concentration T4 DNA Ligase (1 µl @ 2,000,000 U/ml and 2 µl 10X T4 DNA Ligase Buffer in a 20 µl reaction volume). The extent of ligation in each reaction was visualized by detection of the FAM-labeled strands by high throughput capillary electrophoresis, which allows for high accuracy in quantitation and product identification (2). The yields of insert with two adaptors ligated using a range of adaptor to insert ratios (2.5, 5 and 10:1) were compared after incubation at 25°C for 20 minutes (Figure 2A). The Blunt/TA Ligase Master Mix shows superior yield of products with two adaptors ligated at even lower (5:1) ratios of adaptor to insert.

Similarly, yield over time was examined at a 5:1 adaptor:insert ratio; the Blunt/TA Ligase Master Mix shows superior yields at 20 minutes, even compared to prolonged incubation with the other ligase formulations.

Lastly, the yield of products after a 20 minute incubation at 25°C and a 10:1 ratio of adaptor to insert was compared between NEB ligases and the ligase products of several competitors (Figure 3). The Blunt/TA Ligase Master Mix shows superior performance over all ligases tested.

FIGURE 2:
Effect of Adaptor Excess and Incubation Time on Ligation Yield

Ligation reactions (20 µl, 50 nM insert) were performed according to provided protocols using NEB T4 DNA Ligase (2,000,000 U/ml), NEB Quick Ligation Kit, or NEB Blunt/TA Ligase Master Mix. In (A), the yields of fully ligated insert (two adaptors) are shown as a function of the molar excess of adaptor in the reaction (2.5, 5 or 10:1 adaptor to insert) incubating for 20 minutes at 25°C. In (B), the yields are shown for a 5:1 adaptor to insert ratio for 20 minute versus 60 minute incubation times. Blunt/TA Ligase Master Mix shows superior yields at lower ratios of adaptor and shorter incubation times over the other formulations.

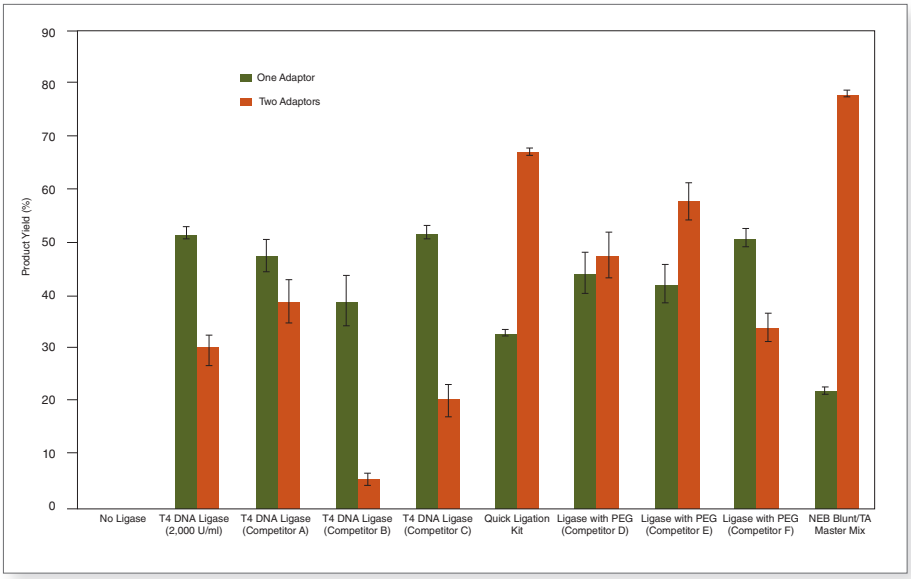


Conclusion

When ligating adaptors to dsDNA, the Blunt/TA Ligase Master Mix offers superior yields over a range of incubation times and adaptor to insert ratios. When high yields are required for these difficult ligations, the Blunt/TA Ligase Master Mix is reccomended.

FIGURE 3:
Comparison of T4 DNA Ligase products in adaptor ligation.

Ligation reactions (20 µl, 50 nM insert, 500 nM adaptor) were performed according to manufacturer's reccomendations using ligases shown above. Reactions were incubated for 20 minutes at 25°C. NEB T4 DNA Ligase and Competitor Ligases A-C were run using 1 µl ligase stock and 2 µl of the supplied 10X buffer in each reaction. NEB Quick Ligation kit and Competitor Ligases D – F are ligation formulations that contain PEG and/or other enhancers, and were run using 1 µl of the supplied ligase stock at a 1X final buffer concentration using the supplied buffer stocks. Yields are reported for species ligated to a single adaptor (green) and ligated to two adaptors (orange). The Blunt T/A Ligase Master Mix demonstrated superior yields over all ligases tested.



References:

1. See also the NEB application note "Joining of Difficult to Ligate dsDNA Fragments with Blunt/TA Ligase Master Mix," http://www.neb.com/tools-and-resources/app-notes/joining-of-difficult-to-ligate-dsdna-fragments-with-blunt-ta-ligase-master-mix.
2. Greenough et. al. (2016) *Nucl. Acids Res.* 44 (2): e15. doi: 10.1093/nar/gkv899.

New England Biolabs, Inc., 240 County Road, Ipswich, MA 01938-2723 Telephone: (978) 927-5054 Toll Free: (USA Orders) 1-800-632-5227 (USA Tech) 1-800-632-7799 Fax: (978) 921-1350 e-mail: info@neb.com