

# Ecoli NightSeq® – More than just a Speed Accelerator

Besides speed, the unique product features of the Ecoli NightSeq® include sequencing robustness, extremely low input DNA amounts as well as its applicability to various microorganisms.

## Introduction

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Ecoli NightSeq® is a groundbreaking new Sanger sequencing service developed by Microsynth. Thanks to Ecoli NightSeq®, users no longer need to isolate their plasmids themselves, but can instead simply pick their *E. coli* colonies and send them directly to Microsynth. And as we handle the DNA preparation and sequencing steps together, sequencing results can be delivered as soon as the next business day\*. This means that all users can proceed directly to processing the correct clones they incubated overnight, gaining a full business day in any downstream process they need to go through. However, this

obvious speed advantage means that insufficient attention is often paid to other very useful features and advantages of Ecoli NightSeq®, which include robustness, low input amounts, and application to different microorganisms. In this tech note, we would like to highlight these lesser-known benefits.

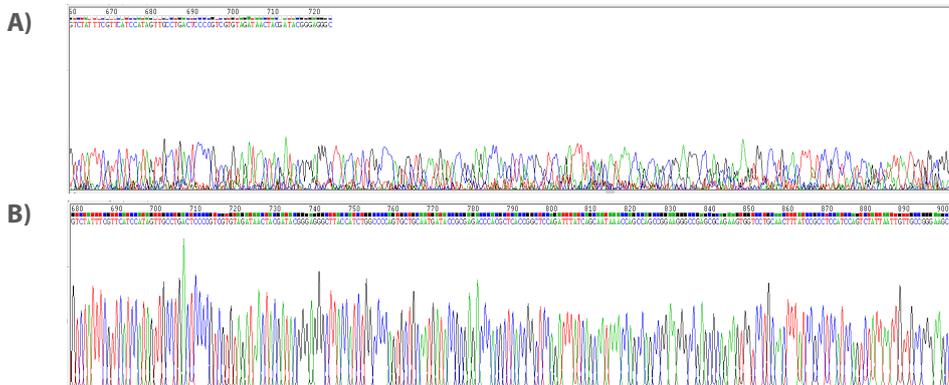
*\*Please note that the speed advantage is only valid for users in Switzerland and Germany who are connected to our same-day courier logistics.*

## Additional Benefits Beyond Accelerating Your Work Process

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**Robustness:** standard sequencing protocols often require large quantities of high-quality DNA (several microliters with concentrations from 40 to 100 ng/μl). This is not always easy to obtain and demands costly plasmid isolation kits/columns, as well as additional work time. Furthermore, spectrophotometrical quantification of double-stranded plasmid DNA is often inaccurate. This is because insufficient RNase treatment and/or chromosomal DNA contaminations lead to an overestimation of plasmid concentration. As a consequence, quite a few reactions end up in low-quality sequencing results or even fail completely when subjected to standard Sanger sequencing services such as Microsynth's Economy Run. In addition, residual ethanol and salt might coelute during plasmid isolation, which will negatively impact your sequencing quality. You should also be aware that variations in DNA quality and quantity can easily occur within a batch of plasmid DNA isolated in parallel from a series of clones, which might consequently lead to heterogeneous quality of sequencing results.

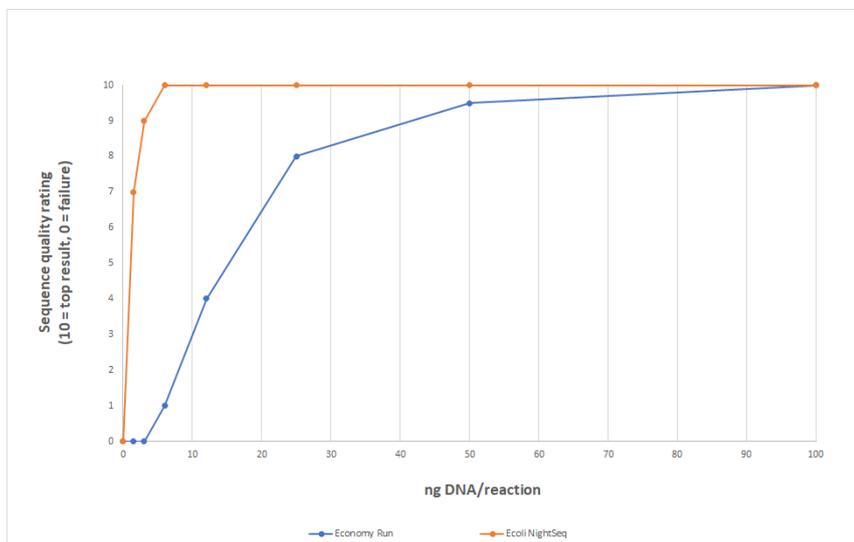
With the Ecoli NightSeq® service, all of these problems have been eliminated. A decisive factor is the innovative and robust plasmid preparation process, which requires only trace amounts of DNA, e.g. from very few bacterial cells. This new process leads to high-quality DNA templates in very defined amounts that are perfectly matched to our Sanger sequencing pipeline. As a result of this, the Ecoli NightSeq® service usually leads to very consistent and reproducible sequencing results for very different input quantities. It also works very well for low-copy vectors, for which you often need to purify your plasmid DNA at midiprep scale. Therefore, we recommend that you no longer spend time worrying about optimal plasmid preparation, but instead outsource plasmid preparation and sequencing to Microsynth via the Ecoli NightSeq®.



**Figure 1:** A) Shows the sequencing of a plasmid DNA with insufficient quantity subjected to Microsynth's Economy Run (read length 724 bps, very low signal intensity). B) Shows the sequencing result of the same sample after using Microsynth's Ecoli NightSeq® service, where only 1  $\mu$ l of the same Miniprep was sufficient to obtain an excellent result (read length 1144 bps, high signal intensity).

**Low-input:** in addition to using living or heat-inactivated bacterial cells as an input, the Ecoli NightSeq® service works perfectly with already isolated plasmid DNA. In this case, it is sufficient to add 1  $\mu$ l of your already isolated plasmid DNA into our tube (in fact, even 1  $\mu$ l of a 1:10 or 1:50 diluted DNA is perfectly acceptable). As the Ecoli NightSeq® approach is very insensitive to varying input DNA quality and quantity, most traces

of plasmid DNA (isolated via e.g. miniprep columns, non-column-based alkaline lysis protocols, or simply supernatants from heat-inactivated bacterial cells) will enable us to produce high-quality sequencing results. Therefore, prior to investing time and money in the laborious retransformation of plasmids into *E. coli*, you might want to use our Ecoli NightSeq® and send us just a tiny fraction of your precious sample.



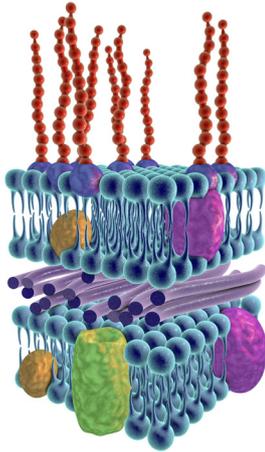
**Figure 2:** Dependence of sequencing quality from the quantity of input plasmid DNA per reaction. Whereas Microsynth's Economy Run service requires minimally 80 ng of input plasmid DNA per sequencing reaction for optimal sequencing results, the Ecoli NightSeq® service requires 10-fold to 50-fold lower quantities. Acceptable results are still possible with quantities as low as 1.5 ng of input DNA.

**Not just restricted to *E. coli*:** as the product name correctly implies, in the majority of cases plasmids from *E. coli* cells will be sequenced. However, this does not mean that the Ecoli NightSeq® service works for *E. coli* cells only. The major restriction for our approach is the efficiency of cell lysis prior to the purification of template DNA. Hence, the Ecoli NightSeq®

service basically works for most gram-negative bacteria. And there is initial evidence that it also works for yeast cells. The possibility of starting not only from *E. coli* provides the following major advantages: if a plasmid has already been transferred into your model organism, for instance, a GFP reporter plasmid in *Agrobacterium*, you don't need to send us your

valuable plasmid stock or start a new plasmid isolation from scratch for sequencing. All you need to do is add your model organism into our pre-filled sequencing tubes and check if your

organism is a good candidate for the Ecoli NightSeq® service.



**Figure 3:** A schematic view of the cell wall of gram-negative bacteria. Basically, most gram-negative bacteria qualify for Microsynth's Ecoli NightSeq® service as long as effective cell lysis can be achieved with our current protocol.

**Less waste:** given that the Ecoli NightSeq® service includes the plasmid preparation step, the user only needs to perform plasmid minipreps for successful clones, hence the need for expensive and harmful plastic material (kit and tip disposables) is significantly reduced. At Microsynth, we've developed a process that only requires very low amounts of plastic tips

for the Ecoli NightSeq® service. The required enzyme and wash solutions are produced on a large scale and they consume very little single-use plastic material. Therefore, the Ecoli NightSeq® can also be described as being innovative in terms of sustainability.

## Concluding Remarks

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Ecoli NightSeq® is a groundbreaking new Sanger sequencing service with obvious speed and labor-saving advantages compared to other current sequencing protocols. Besides the speed and labor savings, Ecoli NightSeq® also has various other unique product features, including its sequenc-

ing robustness, its extremely low input DNA requirements, and its applicability to various microorganisms. Last but not least, this new service requires much less disposable plastic than traditional methods.

## Need More Information?

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