Webinar Q&A: Exonucleases and Endonucleases as Molecular Tools

Q: What enzyme cleaves mismatches in heteroduplexes?

A: Most people use T7 Endonuclease I to detect mismatches in dsDNA. This enzyme works best in indels (single base insertions or deletions) or mismatches with more than two bases.

Q: What are the binding sites for most of these enzymes? Is it the phosphoribose or nucleobase?

A: Since most nucleases do not show base-specificity, the binding site is most likely the phosphoribose.

Q: Is there an exonuclease that cleaves only one nucleotide from a DNA end?

A: We are not aware of any exonucleases or endonucleases we sell that will only cleave one nucleotide from ssDNA or dsDNA. However, some DNA repair enzymes, such as USER Enzyme, will remove the U base from DNA.

Q: Can you tell me about TelN Protelomerase? If I synthesize a double-stranded oligo (56 bp) identical to the TelN recognition site, will it be cleaved (and ligated to hairpins) by the enzyme? I know that some restriction enzymes have difficulty cleaving if their recognition site is positioned close to the end of double stranded DNA. How about TelN?

A: TelN Protelomerase cuts dsDNA at a 56 bp recognition site. The resulting ends are neither blunt nor cohesive, but rather hairpin structures with covalently closed ends that are resistant to digestion by exonucleases.

TelN Protelomerase works best when there are additional base pairs on both sides of the 56 bp recognition site. We have tested a substrate with 10 bp of sequence on one side and no additional sequence on the other side of the recognition sequence and the enzyme showed reduced activity. We have not tested the enzyme on a 56 bp substrate lacking a flanking sequence.

Q: I do plasmid extractions from yeast and microalgae, but we extract genomic DNA with it. Could we use Exonuclease V to eliminate genomic DNA?

A: We do not have a protocol for plasmid extraction from yeast and microalgae using Exonuclease V (RecBCD). Here is a link to a paper where Exo V is used in the purification of mitochondrial DNA. It may contain some useful info for your application. It should work on genomic DNA as long as the genomic DNA appears as a smeared background on an agarose gel.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4344500/

Q: Is *E. coli* Exonuclease I different from Human Exonuclease I? I know that Human Exo I can degrade dsDNA.

A: Yes, these are two totally different enzymes with similar names. E. coli Exonuclease I is a ssDNA exonuclease. We do not supply Human Exonuclease 1 and are unaware of any direct comparisons of that enzyme with *E. coli* Exonuclease I.

Q: Can USER® enzyme be used to make Lenti constructs? For example, can it be used to modify the Lenti vector so it can ligate with the treated insert?

A: USER Enzyme can be used to make any DNA construct. In USER cloning, target DNA molecules and vectors are generated by PCR with 6-10 bases of homology between two adjacent fragments. PCR primers always start with a 5´-dA and contain a single deoxyuracil residue (dU) flanking the 3´-end of the homology region. They can be designed to accommodate multiple fragment/vector assembly, nucleotide substitutions, insertions and deletions. PCR is performed with a dU-compatible DNA polymerase. PCR products are then treated with the USER enzyme to create complementary single-stranded extensions between two adjacent fragments, which then can be annealed or ligated to generate a transformable DNA construct. The optimal assembly is achieved when single-stranded extensions vary from 6-10 nucleotides depending on G/C content. For G/C-rich overhangs, 6-8 nucleotides are optimal for dissociation/annealing; for A/T-rich overhangs, 9-10 nucleotides are recommended. When dU is placed more than 13 nucleotides from the 5´-end, the efficiency drops due to the ineffective dissociation of flanking oligonucleotides from the PCR product. In such cases, multiple dUs in the overhang sequence are recommended.

For detailed USER cloning workflow and references see the following links:
- https://www.neb.com/applications/cloning-and-synthetic-biology/user-cloning/applications-of-user-and-thermolabile-user-ii-enzymes – scroll down for USER Cloning Application
- https://www.neb.com/applications/cloning-and-synthetic-biology/user-cloning

Q: What enzyme would you recommend for eliminating DNA from industrial fermentation products?

A: In general, enzymatic solutions are not used for eliminating DNA in industrial fermentation products, because this solution can be expensive when compared to chromatographic solutions. It also requires the removal of the enzyme after treatment.

Q: Does Lambda Exonuclease only degrade the strand with the 5´ phosphate?

A: Lambda Exonuclease prefers a 5´ phosphate and will degrade that strand with 10-30 fold preference, but it will also degrade the other strand to a much lesser extent.

Q: Do 3´ dideoxynucleotides block nucleases?

A: Different modifications may or may not block exonucleases. Dideoxynucleotides lack a 3´ hydroxyl but they do not block exonucleases.

Q: How do you obtain pure circular DNA and get rid of the degraded nucleotides after using Exonuclease V?

A: Column purify (e.g., using NEB Monarch® cleanup kits), because half of the degradation consists of short oligos.

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Q: Can you tell me more about RNA nucleases and their applications?

A: Several nucleases have are active on RNA, particularly many of the nonspecific endonucleases: Mung Bean Nuclease, Nuclease P1, Nuclease Bal-31 and Micrococcal Nuclease all have good activity on RNA. Additionally, Exonuclease T digests ssRNA with high efficiency. T5 exonuclease also acts on RNA.

The remaining DNA-specific nucleases are strongly inhibited by RNA, but may still act on RNA with reduced activity. Under conditions where DNA-specific exonucleases completely digest DNA targets, some chewing of a few bases may be seen on RNA substrates. The degree of remaining activity on RNA varies from exonuclease to exonuclease and is dependent on buffer conditions and substrate sequence.

In most cases, exonucleases do not change specificity on DNA/RNA hybrid helixes. ssDNA-specific nucleases are blocked by hybridization to an RNA strand, while dsDNA-specific nucleases will digest the DNA strand of DNA/RNA hybrids. Most also result in a slight degradation of the RNA strand. Several nucleases have significant activity on the RNA strand of a DNA/RNA helix: T7 Exo, Exo III and T5 Exo, plus the nonspecific nucleases mentioned above. If it is desirable to digest the DNA strand of a DNA/RNA helix while leaving the RNA portion completely intact, we have found Exo V to be the best nuclease for this application.

When considering a single polynucleotide containing both DNA and RNA regions, the RNA portions of the molecule cannot support initiation of digestion (e.g., a molecule with 3′ RNA and 5′ DNA will not be a substrate for Exonuclease I, which requires a 3′ DNA terminus to initiate). However, digestion typically proceeds 1-3 nt into the RNA portion of the molecule when the nuclease is acting in a processive mode (e.g., a molecule with 5′ RNA and 3′ DNA, the 3′ DNA portion will be digested by Exonuclease I, which will also cleave a few nt into the RNA portion).

Q: Did you say endonucleases only digest from the 5′ end? Are Hae III and Nla III classified as endonucelases?

A: *Hae III* and *Nla III* are restriction endonucleases, which generate a double-stranded break within a specific sequence (recognition site) of a double-stranded specific DNA. The endonucleases that we discussed in the webinar are sequence-independent nucleases.

Q: Do phosphorothioate bonds block exonuclease activity?

A: The answer to this is tricky; it depends on the exonuclease - some are blocked, some are not. Please refer to the following table: https://www.neb.com/tools-and-resources/selection-charts/properties-of-exonucleases-and-nonspecific-endonucleases

Q: What is the best exonuclease for eliminating a hairpin oligonucleotide?

A: We recommend a mixture of Exonuclease III and Exonuclease VII. Exonuclease III will first digest any dsDNA regions, and then Exonuclease VII will then degrade the remaining single-stranded molecules.

Q: Is there a phosphate group at 5′ at the end of every RE digested DNA?

A: Yes. An RE digest leaves a 5′ phosphate and a 3′ hydroxyl.

Q: Does Exonuclease III have endonuclease activity on excess DNA? Does it digest both dsDNA and ssDNA? Is it sequence dependent?

A: Exonuclease III will degrade linear ssDNA about 10-fold less than dsDNA. Circular ssDNA will likely be degraded – it will be nicked, become linear and Exonuclease III will degrade it. You will need to titrate the enzyme to the amount of substrate you have, because it will digest different DNAs at different rates. Exonuclease III is supplied at a very high titration – 1 µl will degrade 30 µg DNA in 30 minutes. Make sure you find the best conditions to get your intended results by using a titration. There are some publications that suggest that Exonuclease III is sequence dependent, but it is difficult to define this. So again, we recommend titrating the enzyme.

Q: What are some methods to block exonucleases?

A: The feature article in NEB Expressions (2019, Issue II) discusses how phosphor modifications will block exonucleases. Most modifications on DNA ends don't block exonuclease activity, but alpha -thioate modifications will block many exonucleases. If you are just looking to inhibit, EDTA will chelate the magnesium to inhibit, but not necessarily block.

Q: Is there also an enzyme capable of keeping supercoiled DNA and getting rid of circular DNA?

A: T5 Exonuclease can initiate at nicks and also degrade linear dsDNA and ssDNA (both linear and circular), leaving only supercoiled DNA.

Q: Is there an enzyme that digests only dsDNA and not ssDNA?

A: We recommend T7 Exonuclease, which preferentially digests dsDNA over ssDNA – it has very little activity on ssDNA and digests both linear and nicked dsDNA, from 5′ to 3′.

Q: Are there any enzymes that recognize nicks and digest nucleotides nearby?

A: Many enzymes will initiate at a nick – Exonuclease III will digest from 3′ to 5′; T7 will digest in the 5′ to 3′ direction. T5 Exonuclease will also initiate at nicks but has ssDNA endonuclease activity so your substrate will be fully degraded. Lambda Exonuclease will also work but is less active than Exonuclease III or T7 Exonuclease. If you want limited digestion, use either Lambda Exonuclease, T7 Exonuclease or Exonuclease III at much reduced concentrations or temperature.

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