**Methylation:**

When bacteria replicate a plasmid, they often methylate specific CpG islands. These are sequences that are often targeted for methylation. Methlyation may block a site from restriction enzyme cleavage. This may give you a headache if the enzyme you wish to use is methylation-sensitive ( it cannot bind recognition sites that are methylated).

There are three different types of [methylation enzymes](https://bitesizebio.com/30630/choosing-right-e-coli-strain/) typically found in laboratory *E. coli* strains: **Dam methylase**, **Dcm methyltransferase** and ***EcoKI*** **methylase**. If you are trying to digest a restriction site that may be methylated, you can use a methylation-incompetent strain of *E. coli* (e.g. [JM11](http://blog.addgene.org/plasmids-101-common-lab-e-coli-strains)) to propagate your plasmid. These *E. coli* strains are incapable of methylating DNA, thus allowing your restriction enzyme to cleave CpG islands. This will help you find strains with the right methylation status.

**Star activity:**

Restriction enzymes actually have a dark side! In the right conditions, they can become promiscuous and digest DNA randomly, rather than at their specific recognition sites. This phenomenon is called **star activity** and is generally caused by long incubation periods (check guidelines for your enzyme) or suboptimal buffer conditions (e.g. pH). Therefore, it is critical to use your desired enzyme with the recommended buffer!

In addition, high glycerol concentration may lead to an in increase star activity. Since most enzymes and their buffers come packaged in glycerol to extend shelf life, you should adequately dilute both the buffer and enzyme. This is why many companies suggest a 20 – 50 µl reaction in their general protocols and provide the buffer as a 10x mix.

## Restriction enzymes need some space:

Always remember that some enzymes are most efficient when they have several base pairs on either side of the recognition site. This is particularly important if you are doing a double digest with the two enzymes very close together or if digesting the ends of a PCR product.

Each manufacturer has specific suggestions for their products, but it is often recommended to ensure that there are at least six base pairs on either side of the recognition site. The easiest way to add more base pairs is to [engineer them](https://bitesizebio.com/20786/overhang-pcr/) via your PCR primers. Just try to avoid sequences carry a risk of primer dimer formation!