Restriction Enzyme Troubleshooting Guide

The following guide can be used for troubleshooting restriction enzyme digestions. You may also be interested in reviewing additional tips for optimizing digestion reactions.

Why is My Restriction Enzyme Not Cutting DNA?

Not getting the cleavage you expected? Let an NEB scientist help you troubleshoot your reaction.

Problem	Possible Cause	Solution
Incomplete or no digestion	Enzyme is inactive	▪ Test enzyme on control DNA with known multiple sites ▪ Enzyme should be stored at -20°C. Enzymes stored at -70°C will freeze, and repeated thaw/freeze cycles may reduce enzyme activity
	Reaction conditions are not optimal	▪ Use recommended buffer supplied with restriction enzyme ▪ Follow double digest recommendations, or try a sequential digest ▪ Repeat with fresh buffer. Additives present in buffer (e.g., DTT, SAM) may degrade over time
	Enzyme concentration is too low	▪ Some plasmids or genomic DNAs may require up to 10–20 units/µg
	Additive is missing	▪ Repeat reaction setup, being sure that enzyme and/or additives (e.g., BSA) are added
	DNA concentration is not optimal	▪ NEB recommends 1 µg of DNA in a 50 µl reaction. ▪ Excess DNA may result in incomplete cleavage.
	Incubation time was too short	▪ Some enzymes can exhibit slower cleavage towards specific sites. In most cases, 1–2 hours are sufficient.
	DNA is contaminated with an inhibitor	▪ Assay substrate DNA in the presence of a control DNA. Control DNA will not cleave if there is an inhibitor present. Miniprep DNA is particularly susceptible to contaminants. ▪ Clean DNA with a spin column, resin or drop dialysis, or increase volume to dilute contaminant.
	Recognition site is not present	▪ Confirm DNA sequence
	Cleavage is blocked by methylation	▪ DNA isolated from a bacterial source may be blocked by Dam and Dcm methylation. DNA should be passed through a *dam-/dcm-* strain (NEB #C2925). ▪ Eukaryotic genomic DNA may be blocked by CpG methylation. This can be overcome by cloning into a bacterial host. ▪ PCR products are not methylated
	DNA may be supercoiled	▪ Restriction enzymes cleave supercoiled DNA with varying efficiency. Additional enzyme may be required.
	Recognition site may be too	▪ As a general rule, add 6 base pairs on either side of the recognition site for efficient cleavage

	close to the end of the DNA fragment	
	Site preference	▪ Enzyme requires two recognition sites for efficient cleavage.
	Quality of DNA	▪ DNA purification procedures that use spin columns can result in high salt levels which inhibits enzyme activity. To prevent this, DNA solution should be no more than 25% of reaction volume.
Unexpected Cleavage Pattern	Determine nature of pattern	▪ Run uncut substrate DNA alongside the digest. A partial digest will show bands found in the uncut, whereas star activity will show bands of unexpected size.
	DNA sample is contaminated	▪ Prepare a new DNA sample
	Additional recognition sites are present in DNA	▪ Confirm DNA sequence
	Star Activity	▪ See tips for avoiding star activity (see page 5) and/or use a High-Fidelity (HF™) Restriction Enzyme (see page 4).
Smearing of DNA on gel	Enzyme has a high binding affinity to DNA and will not dissociate well	▪ Add SDS to the gel loading dye/stop solution to a final concentration of 0.1–0.5% to help dissociate the enzyme from the DNA, or treat with protease before loading
	Nuclease contamination	▪ Care should be taken to avoid cross contamination when setting up reactions
	Agarose running conditions	▪ Use fresh running buffer and appropriate voltage to avoid overheating
	Master Mix containing dye	▪ Use fresh gel
	Agarose gel or running buffer is old when using RE-Mix®.	▪ Use fresh buffer, a fresh gel or add stop dye with EDTA