

## Introduction

Restriction endonucleases, also called restriction enzymes, cut (“digest”) DNA at specific sequences of bases. The restriction enzymes are named for the prokaryotic organism from which they were isolated. For example, the restriction endonuclease EcoRI (pronounced “echo-are-one”) was originally isolated from *E. coli* giving it the “Eco” part of the name. “RI” indicates the particular version on the *E. coli strain* (RY13) and the fact that it was the first restriction enzyme isolated from this strain.

The sequence of DNA that is bound and cleaved by an endonuclease is called the recognition sequence or restriction site. These sequences are usually four or six base pairs long and palindromic, that is, they read the same 5’ to 3’ on the top and bottom strand of DNA. For example the recognition sequence for EcoRI is

5’ GAATTC 3’  
3’ CTTAAG 5’

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**EcoRI cuts between the G and the A on each strand of DNA, leaving a single stranded DNA overhang (also called a “sticky end”) when the strands separate.**

Other restriction enzymes, for example HaeIII, cut in the middle of the palindrome leaving no DNA overhang, called a “blunt end.” HaeIII recognizes

5' GGCC 3'  
3' CCGG 5'

### **HaeIII crystals**

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Before you can use any restriction enzymes on your PCR product you will have to “clean up” your reaction. This is done to remove the Taq polymerase and any residual dNTPs from the product (if you don't remove them they will refill any sticky ends generated by the restriction enzyme!). Another reason to clean up your PCR product is to change the buffer to one more suitable for the restriction enzyme reaction.

Fortunately cleaning up reactions is simple and efficient (though not inexpensive!) using small spin-columns sold by Qiagen, a company you will hear a lot about in the coming weeks.

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### **Qiagen**

The spin-columns have a silica-gel membrane in them to remove salt and contaminants. The membrane binds DNA in the presence of salt and DNA can be efficiently recovered from the membrane once the salt is removed. All the necessary binding and wash buffers are sold with the Qiagen spin-columns and they have unusual and uninformative names like "PB" and "P2". The contents of these buffers are proprietary so we don't know precisely what they contain, but there is some information about them that is included as part of the protocol. This should help you understand the steps and you should think about the instructions rather than blindly follow them.

Today you will use the Qiagen “QIAquick” protocol to clean the DNA you generated by PCR. You will then digest the product as well as some purified plasmid, provided by the teaching faculty, to clone into.