

# 3' Site Before 5' Site on Vector

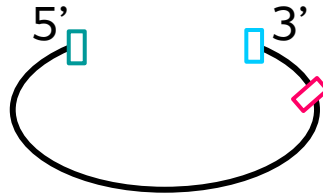
Parent Insert

5' site BglII



3' site BamHI

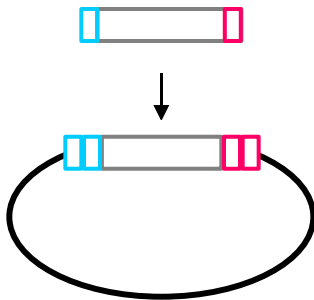
Parent Vector



 BamHI  
 BglII  
 EcoRI

OpenFreezer uses the restriction sites designated on the insert as ligation points in the vector. If the 3' site is found in the vector before the 5' site, OpenFreezer cannot automatically generate the sequence as shown above for this parent vector and insert. User must select different parents, different sites, reverse complement the sites or if possible, create a hybrid site.

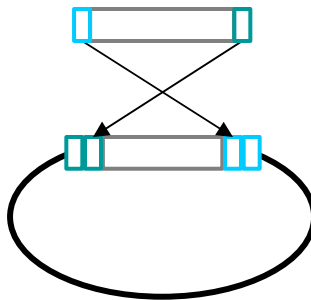
**Choose Different Sites**



**Customize Sites**

5' Vector and Insert – BamHI  
3' Vector and Insert - EcoRI

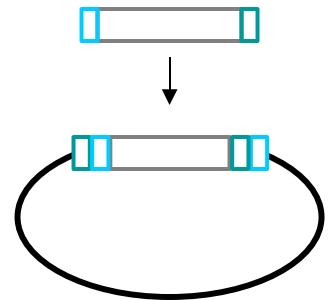
**Reverse Complement**



**Customize Sites**

5' Vector and Insert – BamHI  
3' Vector and Insert - BglII

**Create Hybrid Sites**



**Customize Sites**

5' Vector – BamHI, 5' Insert - BglII  
3' Vector - BglII, 3' Insert - BamHI