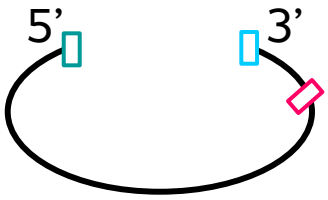
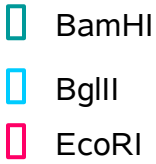
3’ Site Before 5’ Site on Vector

Parent Insert

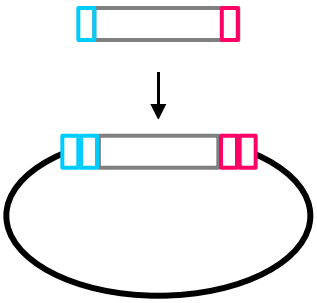
Parent Vector





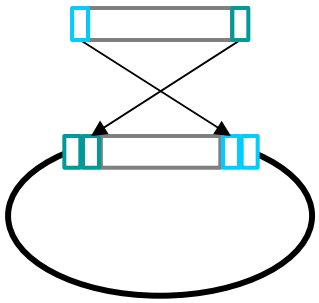
OpenFreezer uses the restriction sites designated on the insert as ligation points in the vector. If the 3’ site is found in the vector before the 5’ site, OpenFreezer cannot automatically generate the sequence as shown above for this parent vector and insert. User must select different parents, different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

Choose Different Sites



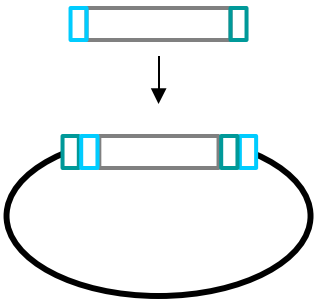
Customize Sites
5’ Vector and Insert – BamHI
3’ Vector and Insert - EcoRI

Reverse Complement



Customize Sites
5’ Vector and Insert – BamHI
3’ Vector and Insert - BglII

Create Hybrid Sites

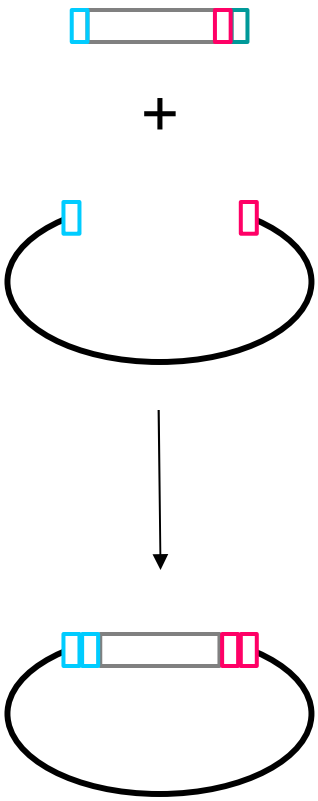


Customize Sites
5’ Vector – BamHI, 5’ Insert - BglII
3’ Vector - BglII, 3’ Insert - BamHI

Customizing Vector

OpenFreezer uses the restriction sites designated on the insert as ligation points in the vector. The user can override this automatic creation by customizing the cloning sites. The user can also select to reverse complement the insert prior to insertion into the vector. Hybrid Sites can be created by selecting different but compatible enzymes for the vector and insert.

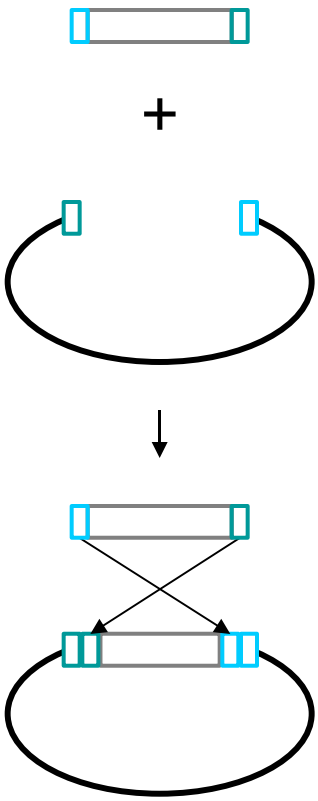
Customize Sites



Customize Sites
5' Vector and Insert – BglII
3' Vector and Insert – EcoRI

(override BamHI as 3' ligation site)

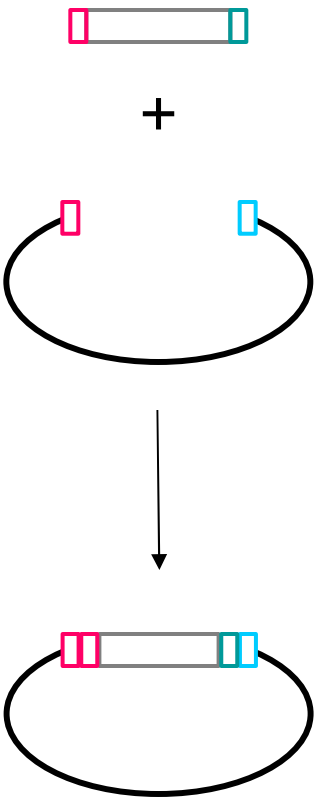
Reverse Complement



Customize Sites
5' Vector and Insert – BamHI
3' Vector and Insert – BglII

(designated 3' BamHI site on Insert is switched to a 5' BamHI site on new Vector)

Create Hybrid Site(s)



Customize Sites
5' Vector and Insert - EcoRI
3' Vector - BglII, 3' Insert – BamHI

(OpenFreezer creates a Hybrid site at the 3' ligaton site)

BamHI

BglII

EcoRI