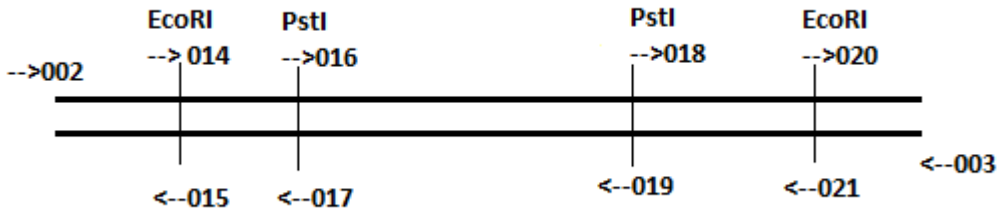
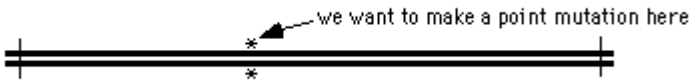
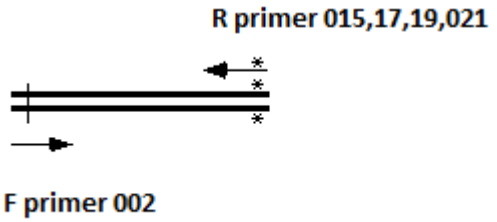
PCR reactions for Site directed mutagenesis of Dxs from *B. subtilis*

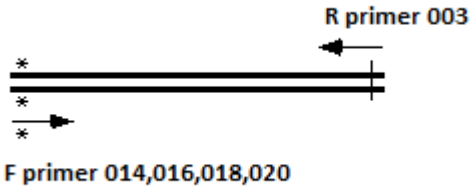
The four restriction sites and primers required for the site directed mutagenesis



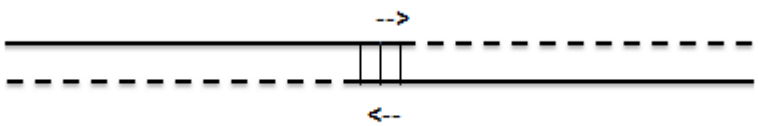


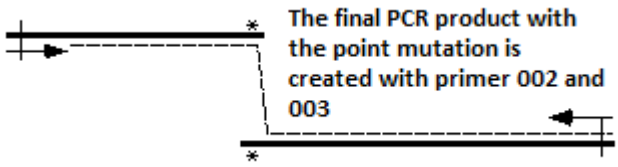
To create a point mutation at a given point we must do 3 PCR reactions, for each restriction site we want to change using Phusion PCR. The 3 reactions are shown below.





The two PCR products will then act as primers for each other enabling the polymerase to create full strands of the Dxs gene. The polymerase can then use the added primers 002 and 003 multiply the product.





For all the PCR reactions use the phusion PCR SOP0010

Removal of EcoRI restriction site at 1067b C→T:

PCR reaction 1:

- Use primer 002 (BsubDXS_F) and 015 (c1067t_antisense) with Dxs from *B.Subtilis* as template
- Run the PCR product on a gel and purify the appropriate band.

PCR reaction 2:

- Use primer 003 (BsubDXS_R) and 014 (c1067t) with Dxs from *B.Subtilis* as template
- Run the PCR product on a gel and purify the appropriate band.

PCR reaction 3:

- Use primers 002 and 003 with the two purified templates from PCR reaction 1 and 2, to get the full Dxs gene with the new mutation.
- Run on a gel and purify the appropriate band.
- Then take some of the purified DNA and do a digestion with EcoRI to see if the restrictionsite has been removed if the mutagenesis has been successful 2 bands should be visible at approximately 1719b and 234b.

If the restriction site is gone the next restriction site can be removed.

Removal of EcoRI restriction site at 1724b C→T:

PCR reaction 4:

- Use primer 002 (BsubDXS_F) and 021(c1724t_antisense) with the purified PCR product from PCR reaction 3
- Run the PCR product on a gel and purify the appropriate band.

PCR reaction 5:

- Use primer 003 (BsubDXS_R) and 020 (c1724t) with the purified PCR product from PCR reaction 3
- Run the PCR product on a gel and purify the appropriate band.

PCR reaction 6:

- Use primers 002 and 003 with the two purified templates from PCR reaction 4 and 5, to get the full Dxs gene with the new mutation.

- Run on a gel and purify the appropriate band.
- Then take some of the purified DNA and do a digestion with EcoRI to see if the restriction site has been removed if the mutagenesis has been successful 1 band should be visible at approximately 1953b.

If the restriction site is gone the next restriction site can be removed.

Removal of PstI restriction site at 1124b A→G:

PCR reaction 7:

- Use primer 002 (BsubDXS_F) and 017(a1124g_antisense) with the purified PCR product from PCR reaction 6
- Run the PCR product on a gel and purify the appropriate band.

PCR reaction 8:

- Use primer 003 (BsubDXS_R) and 016(a1124g) with the purified PCR product from PCR reaction 6
- Run the PCR product on a gel and purify the appropriate band.

PCR reaction 9:

- Use primers 002 and 003 with the two purified templates from PCR reaction 7 and 8, to get the full Dxs gene with the new mutation.
- Run on a gel and purify the appropriate band.
- Then take some of the purified DNA and do a digestion with PstI to see if the restriction site has been removed if the mutagenesis has been successful 2 bands should be visible at approximately 1566b and 387b.

If the restriction site is gone the last restriction site can be removed.

Removal of PstI restriction site at 1571b G→A:

PCR reaction 10:

- Use primer 002 (BsubDXS_F) and 019(g1571a_antisense) with the purified PCR product from PCR reaction 9
- Run the PCR product on a gel and purify the appropriate band.

PCR reaction 11:

- Use primer 003 (BsubDXS_R) and 018(g1571a) with the purified PCR product from PCR reaction 9
- Run the PCR product on a gel and purify the appropriate band.

PCR reaction 12:

- Use primers 002 and 003 with the two purified templates from PCR reaction 10 and 11, to get the full Dxs gene with the new mutation.
- Run on a gel and purify the appropriate band.
- Then take some of the purified DNA and do a digestion with PstI to see if the restriction site has been removed if the mutagenesis has been successful 1 band should be visible at approximately1953b.