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5.36 Biochemistry Laboratory
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Site-directed Mutagenesis and Transformation

I. DNA Site Directed Mutagenesis

   A. PCR primer design

II. Transformation (step 1 of cloning)

   A. PCR

DNA Site-Directed Mutagenesis

- What you have: a wt Abl(229-511)-encoding plasmid

ABL

- What you want: a plasmid encoding an Abl (229-511) mutant.

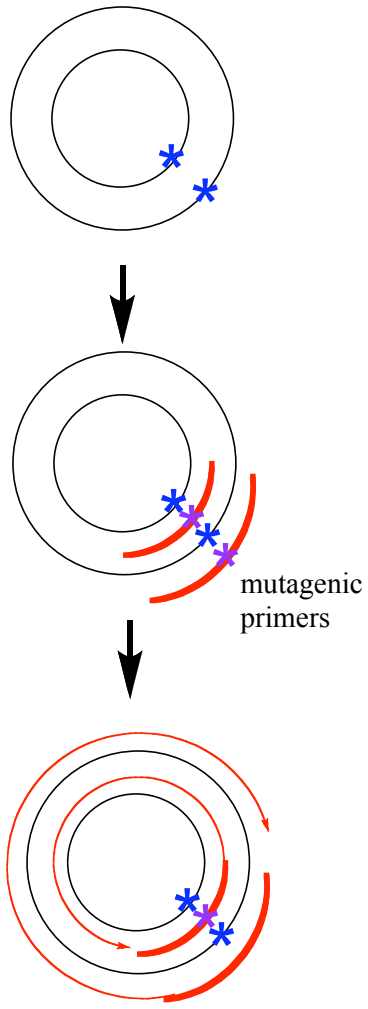
ABL

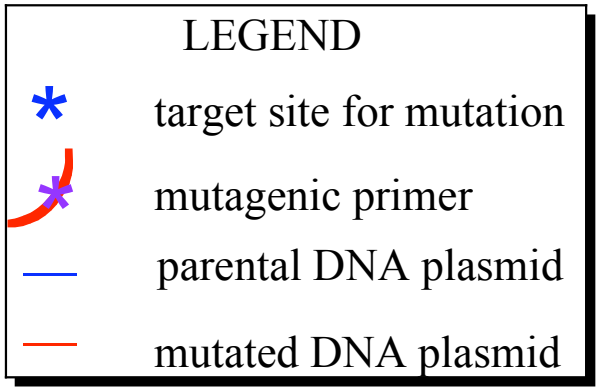
- You will use the Quickchange strategy to generate mutant DNA that encodes the Bcr-Abl protein mutant of your choice.

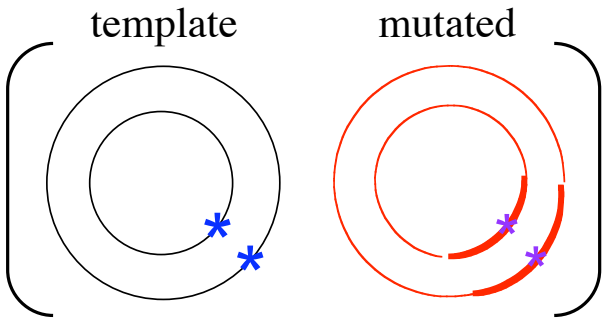
Quikchange overview

Step 1
plasmid preparation

Step 2 (*session 9*)
temperature cycling
(PCR)





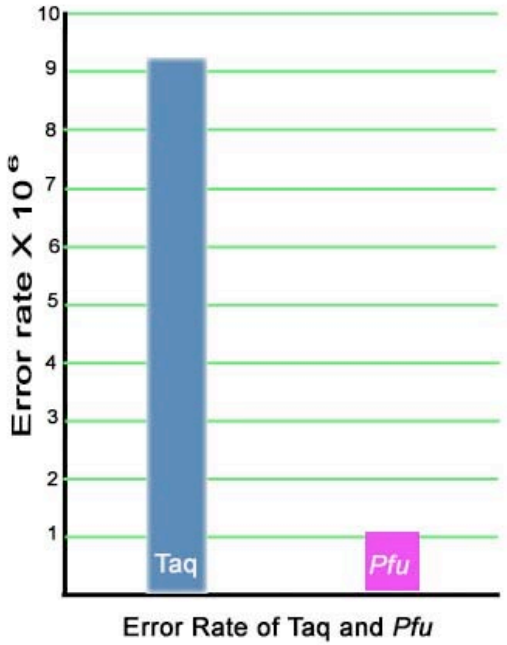


PCR using mutagenic primers

- Instead of replicating just a desired fragment, replicate the entire plasmid
- Need much more powerful polymerase that has higher fidelity than Taq

PCR using mutagenic primers

- Instead of replicating just a desired fragment, replicate the entire plasmid
- Need much more powerful polymerase that has higher fidelity than Taq
- Use Pfu Turbo (isolated from *pyrococcus furiosus*)
- 3' to 5' proofreading gives Pfu Turbo higher fidelity.



http://www.genscript.com/product_001/enzyme/code/E00021/category/enzyme/Pfu_DNA_Polymerase.html?src=google&gclid=CJ__prec0JECFR0yFQodqXZeug

- General PCR protocol for Quickchange Method

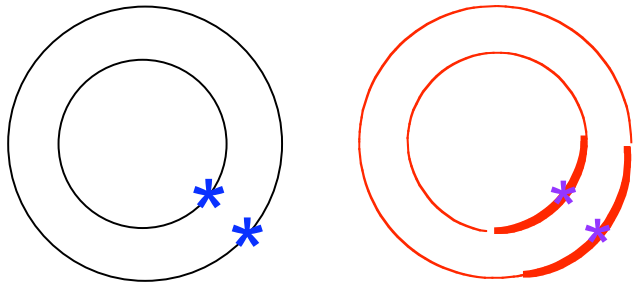
95°C for 30 sec

16-20 cycles of

- 95°C for 30 sec
- 55°C for 1 min
- 65°C for _____ min for our Abl plasmid (2 min per kb)

Hold at 10°C

Quikchange overview

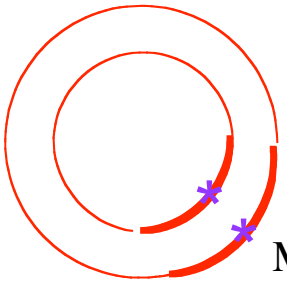


Template plasmid

Mutated plasmid
(PCR product)

Step 3 (*session 10*)

Digest the methylated, non-mutant DNA template with Dpn 1

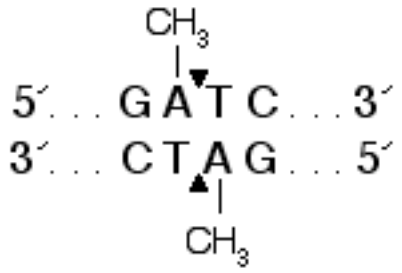


Mutated plasmid (contains nicked cicular strands)

After Dpn 1 digestion is complete, none of the template DNA should remain.

Dpn1

Dpn1 is a restriction enzyme that cleaves at the recognition sequence DNA only when in the methylated form.

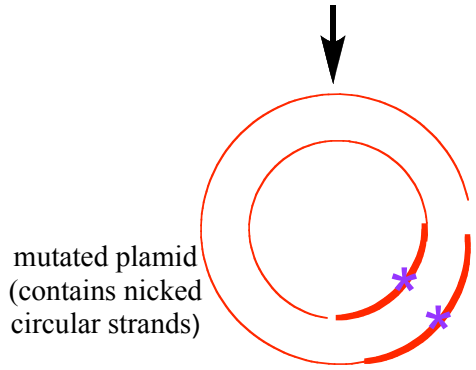


In many bacteria (such as DH5-a *E. coli* cells) methylase enzymes add CH3 groups to specific sequences of DNA.

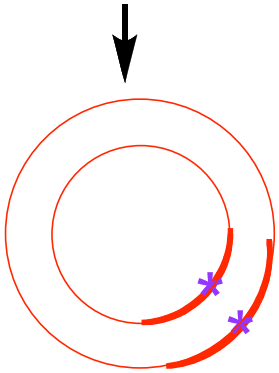
This originated as part of primitive bacteraila immune system. Bacterial methylated its genomic DNA. Foreign DNA is not methylated. Here specific RE can specifically cleave foreign DNA/

Quickchange overview

Step 3 (*session 10*)
Digest the methylated, non-mutant DNA template with Dpn 1



Step 4 (*session 10*)
Transformation



Isolate mutant DNA (*session 11*) and send for sequencing.

Pictures modified from the QuikChange mutagensis handbook (http://www.stratagene.com/manuals/200518.pdf).

Site-directed Mutagenesis and Transformation

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- Quickchange allows you to make point mutants in one day
  - Less than 25 years ago it took months to make point mutants
- PCR, Quickchange, and similar cloning techniques have been instrumental in advancing recombinant technologies and making molecular biology methods much more efficient

Reading Assignment for CI-M lecture next week (Tuesday).

Original nucleotide sequence:
5' ccc ccg ttc tat atc atc act gag ttc atg acc tac ggg 3'

Forward primer:
5' ccc ccg ttc tat atc atc att gag ttc atg acc tac ggg 3'

Reverse primer (the reverse compliment):
3' ggg ggc aag ata tag tag taa ctc aag tac tgg atg ccc 5'
5' ccc gta ggt cat gaa ctc aat gat gat ata gaa cgg ggg 3'

Check: primer should have at least 40% GC content.

T315→I is a c947→t nucleotide point mutation

Design primers that introduce a single point mutation that encodes for the expected aa change.

Original nucleotide sequence:
5' ccc ccg ttc tat atc atc act gag ttc atg acc tac ggg 3'

Forward primer:
5' ccc ccg ttc tat atc atc att gag ttc atg acc tac ggg 3'

Reverse primer (the reverse compliment):
3' ggg ggc aag ata tag tag taa ctc aag tac tgg atg ccc 5'
5' ccc gta ggt cat gaa ctc aat gat gat ata gaa cgg ggg 3'

Check: primer should have at least 40% GC content.

T315→I is a c947→t nucleotide point mutation