QuikChange Mutagenesis

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Abstract

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Guidelines

Primers

I use enzyme x to work with DNA sequences, it is installed on all the macs and has nice translation and reverse complement functions. Many of the same functions are available in a plasmid editor , which has Mac, Windows, and Linux versions. Choose a codon for the new amino acid that is the most similar to the original codon.

-Forward Primer

-identify the bases that code for your residue of interest (eg. 295-297)

-A - Copy the 10 bases before the codon (eg. 285-294)

-B - Write your new codon after the 10 bases you just copied

-C - Copy the 22 bases that follow the codon (eg. 298-319), I always terminate in a G or C your forward primer should be 5'-A-B-C-3'

-Reverse Primer

-D - Copy the ten bases after the codon (eg. 298-307) - **then reverse complement it!**

-E - Write your new codon - **then reverse complement it!**

-F - Copy the 22 bases that precede the codon (eg. 273-294) - **then reverse complement it!** your reverse primer should be 5'-D-E-F-3'

-Order your primers from ElimBio (The newest PO B000177051)

-They should arrive in 200uM concentration, make a 20uM stock by diluting 5ul of Elim Primer with 45ul of dH2O

PCR

Recipe

-Recipe 1.25ul of 20uM Primer F

-1.25ul of 20uM Primer R

-10ul Phusion Buffer (5x) 1ul of 10mM dNTPs

-0.5ul Template DNA (from miniprep, preferably from DH5alpha cells, but BL21 is still dam+ so it should be fine - 5ng starting template is plenty! (note: good range = 50 - 100 ng))

-0.5ul Phusion polymerase

-35ul H20

Cycle

-98C for 30s 98C for 5s (I usually do this step for 10 seconds)

-53C for 20s

-72C for 20s/kb (usually plasmids are ~7kb = 2:20)

-Cycle 16 times - *more cycles are actually bad!*

-72C for 8:00
-4C for hold

If it does not work:

-Miniprep more colonies... ;)
-Order PAGE purified primers if you haven't already. We have a good price from Invitrogen.
-Redesign your primers with netprimer
-For high background of WT sequence, decrease starting amount of template (really, 5ng is plenty), -Get fresh Dpn1 and digest ~24hrs.
-Run a gradient of annealing temperatures (try 52-62).

Split reaction protocol (for high primer-dimer problems):

Recipe F

-1ul of 20uM Primer F
-5ul Phusion Buffer (5x)
-0.5ul of 10mM dNTPs
-0.5ul Template DNA (from miniprep, preferably from DH5alpha cells, but BL21 is still dam+ so it should be fine) 0.5ul Phusion polymerase
-17.5 ul H20

Recipe R

-1ul of 20uM Primer F
-5ul Phusion Buffer (5x)
-0.5ul of 10mM dNTPs
-0.5ul Template DNA (from miniprep, preferably from DH5alpha cells, but BL21 is still dam+ so it should be fine)
-0.5ul Phusion polymerase
-17.5 ul H20

Cycle each F and R in separate tubes for:

-98C for 30s
-98C for 5s (I usually do this step for 10 seconds)
-53C for 20s
-72C for 20s/kb (usually plasmids are ~7kb = 2:20)
-Cycle 10 times
-72C for 8:00
-4C for hold

Mix tubes F and R and cycle:

-98C for 30s
-98C for 5s (I usually do this step for 10 seconds)
-53C for 20s
-72C for 20s/kb (usually plasmids are ~7kb = 2:20)
-Cycle 10 times
-72C for 8:00
-4C for hold

Follow transformation, DpnI digest as above

Degenerator: http://andersonlab.qb3.berkeley.edu/Software/Degenerator.html

NB: Not to be confused with this: http://www.imdb.com/title/tt0100449/

Materials

QIAquick PCR Purification Kit 28104 by Qiagen

Protocol

PCR

Step 1.
Prepare PCR mixture.

PROTOCOL

. PCR Mixture for QuickChange Mutagenesis

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Recipe

Step 1.1.
125ul of 20uM Primer F.

Recipe

Step 1.2.
1.25ul of 20uM Primer R.

Recipe

Step 1.3.
10ul Phusion Buffer (5x).

Recipe

Step 1.4.
1ul of 10mM dNTPs.

Recipe

Step 1.5.
0.5ul Template DNA.

NOTES

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From miniprep, preferably from DH5alpha cells, but BL21 is still dam+ so it should be fine - 5ng starting template is plenty! (note: good range = 50 - 100 ng).

Recipe

Step 1.6.
0.5ul Phusion polymerase.

Recipe

Step 1.7.

35ul H20.

PCR

Step 2.

Perform the PCR using the following cycling conditions:
-98C for 30s
-98C for 5s (I usually do this step for 10 seconds)
-53C for 20s
-72C for 20s/kb (usually plasmids are 7kb = 2:20)
-Cycle 16 times - more cycles are actually bad!
-72C for 8:00
-4C for hold

DpnI and transformation

Step 3.

After PCR, add 1ul of DpnI to each pcr tube.

DpnI and transformation

Step 4.

Incubate 1 hour-O/N at 37C.

DURATION
15:00:00

NOTES
James Fraser 27 Jul 2015
I always do this O/N or for at least 4 hours (Avi).
James Fraser 27 Jul 2015
I got 95% efficiency when I DpnI ~24hrs (Cat).

DpnI and transformation

Step 5.

PCR Purify using Qaigen kit.

REAGENTS
QIAquick PCR Purification Kit 28104 by Qiagen

DpnI and transformation

Step 6.

Add 5ul of PCR reaction into 25ul DH5alpha or TG1 cells.

NOTES
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I eluted in 27ul EB and transformed all 25ul elution in 50ul TG1 cells + 25ul KCM (Cat).

DpnI and transformation

Step 7.

Incubate on ice for 5 minutes.

DURATION
00:05:00

NOTES
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If you have time, incubate for 30min - 1hr (Avi).

DpnI and transformation

Step 8.
Heatshock at 42C for 45 seconds.

DURATION
00:00:45

Dpnl and transformation

Step 9.
Recover on ice for 2 minutes.

DURATION
00:02:00

Dpnl and transformation

Step 10.
Add 200ul LB (if in 96 well or 8 strip format) to each reaction.

NOTES
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350ul SOC, plate 50ul (Cat).

Dpnl and transformation

Step 11.
Shake at 37C for 1 hour.

DURATION
01:00:00

Dpnl and transformation

Step 12.
Plate on warmed antibiotic plates.