QuikChange Primer Design

? Help

The QuikChange® Primer Design Program supports mutagenic primer design for your QuikChange mutagenesis experiments. Using primer design guidelines described in QuikChange manuals, this program calculates/designs the appropriate primer sequences with the optimal melting temperature. Read Help for more information about the program.

Expand Help

1. It is recommended that you clear this form prior to loading each new sequence:

Clear Input

2. Select QuikChange® mutagenesis kit that you are using:

QuikChange® Multi — Help in choosing a mutagenesis kit

3. Find your DNA sequence by pressing

Load a file from your hard drive:

Choose File No file chosen

Or, paste plain text or FASTA-formatted DNA sequence in the box below:

```
atggaaactgaacagccagaagaaaccttccctaacactgaa
accaatggtgaatttggtaaacgccctgcagaagatatggaa
gaggaacaagcatttaaaagatctagaaacactgatgagatg
gttgaattacgcattctgcttcagagcaagaatgctggggca
gtgattggaaaaggaggcaagaatattaaggctctccgtaca
```

Warning: QuikChange® Multi kit is required for multiple mutations. Note: only primers specific for one DNA strand (sense or antisense) are needed.

4. Load it.

Upload now - or - Upload translated

or specify a DNA region to translate from ____ to ____ Upload translated region

View protein sequence (translation F1):

```
METEQPEETFPNTETNGEFGKRPAEDMEEEQAFKRSR
NTDEMVELRILLQSKNAGAVIGKGGKNIKALRTDYNA
SVSVPDSSGPERILSISADIETIGEILKKIIPTLEEG
LQLPSPTATSQLPLESDAVECLNYQHYKGSDFDCELR
LLIHQSLAGGIIGVKGAKIKELRENTQTTIKLFQECC
```

5. Select up to seven amino acids that you want to change:

	site 1	site 2	site 3	site 4	site 5	site 6	site 7
Protein: change amino acid(s) to:	D(asp)	D(asp)	-*-	-*-	-*-	-*-	-*-

or

Delete a region between two checked amino acids (NOTE: two checked residues will not be deleted)

1 M	2 E	3 T	4 E	5 Q	6 P	7 E	8 E	9 T	10 F
11 P	12 N	13 T	14 E	15 T	16 N	17 G	18 E	19 F	20 G
21 K	22 R	23 P	24 A	25 E	26 D	27 M	28 E	29 E	30 E
31 Q	32 A	33 F	34 K	35 R	36 S	37 R	38 N	39 T	40 D
41 E	42 M	43 V	44 E	45 L	46 R	47 I	48 L	49 L	50 Q

Finally, obtain your designed primer sequences.

Design primers

Clear this form to load a new sequence:

Clear Input

Primer sequences:

Primer Name	Primer Sequence (5' to 3')
S284D	5'-tctagaagagattatgatgatatggaccctcgtcgaggacc-3'
S284D_antisense	5'-ggtcctcgacgagggtccatatcatcataatctcttctaga-3'
S353D	5'-ctctgcaatagatacatgggacccatcagaatggcagatg-3'
S353D_antisense	5'-catctgccattctgatgggtcccatgtatctattgcagag-3'

Oligonucleotide information:

Primer Name	Length (nt.)	Tm	Duplex Energy at 65°C	Energy Cost of Mismatches
S284D	41	78.16°C	-47.24 kcal/mole	8.4%
S284D_antisense	41	78.16°C	-49.54 kcal/mole	8.2%
S353D	40	78.08°C	-49.32 kcal/mole	8.1%
S353D_antisense	40	78.08°C	-46.10 kcal/mole	8.7%

Primer-template duplexes:

Primer Name	Primer-Template Duplex
S284D	5'-tctagaagagattatgatgatatggaccctcgtcgaggacc-3' |||||||||||||||||||||||| |||||||||||||| ggtagatcttctctaatactactatactcgggagcagctcctggtgg
S284D_antisense	ccatctagaagagattatgatgatatgagccctcgtcgaggaccacc |||||||||||||||||||||||| |||||||||||||| 3'-agatcttctctaatactactatacctgggagcagctcctgg-5'
S353D	5'-ctctgcaatagatacatgggacccatcagaatggcagatg-3' ||||||||||||||||||| |||||||||||||||||| cctgagacgttatctatgtacctcgggtagtcttaccgtctaccga
S353D_antisense	ggactctgcaatagatacatggagcccatcagaatggcagatggct ||||||||||||||||||| |||||||||||||||||| 3'-gagacgttatctatgtaccctgggtagtcttaccgtctac-5'

Clear this form to load a new sequence:

Clear Input

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