

Northeast Structural Genomics Consortium Primer Prim'er – PCR Primer Design Software

http://www.nesg.org/primer_primer

Primer Prim'er (Everett et al., 2004) is a PCR primer design tool that completely automates the primer design process. Primer Prim'er generates vector specific PCR primer sets designed to amplify and insert DNA targets into laboratory vectors. Both restriction endonuclease and viral recombination cloning strategies are supported. It is implemented under Adobe's flash player

Primer Prim'er possesses a rich interactive interface designed to be both a teaching tool as well as a powerful tool for structural genomic efforts (Figure 1). The software introduces endonuclease restriction and viral recombination sites into calculated primer sets and adds additional nucleotides in order to preserve frame with vector based fusions. Primer Prim'er is very customizable. Users can readily define and use any set of vectors, select from a variety of annealing temperature formulas, design mutagenic primer sets and automatically create series of primer sets designed to vary the boundaries of targeted regions of DNA. Calculated primer sets are reported in both a graphical format and several text based formats for ease of ordering. Calculated primer sets can be sorted upon a variety of criteria such as expected PCR product length and agarose gels can be simulated to demonstrate how the expected PCR products would appear in the laboratory.

The software has been used by the NESG protein sample production pipeline (Acton et al., 2005) to design PCR primers for more than 20,000 expression vectors.

Everett, J.K.; Acton, T.B.; Montelione, G.T. *J. Struct. Funct. Genomics* 2004, 5: 13-21. Primer Prim'r: A web based server for automated primer design.

Acton, T.B.; Gunsalus, K.C.; Xiao, R.; Ma, L-C.; Aramini, J.M.; Baran, M.C.; Chiang, Y-W.; Climent, T.; Cooper, B.; Denissova, N.; Douglas, S.M; Everett, J.K.; Ho, C.K.; Macapagal, D.; Paranjji, R.K.; Shastry, R.; Shih, L-Y.; Swapna, G.V.T.; Wilson, M.; Wu, M.; Gerstein, M.; Inouye, M.; Hunt, J.F.; Montelione, G.T. *Meth. Enzymology* 2005, 394:210-243. Robotic cloning and protein production platform of the Northeast Structural Genomics Consortium.

The screenshot displays the Primer Prim'er web interface. On the left is a navigation menu with 'Home', 'Start', 'Vectors', 'Options', and 'Help'. Below this is a 'Target control' section with 'previous target', 'next target', and 'automate' buttons. The main area shows 'Target number 2 / 3' and 'Target name WR2'. A legend indicates that a blue triangle represents a '5' restriction site marker' and a red triangle represents a '3' restriction site marker'. Below this, four vector options are listed: pET 14-15A, pET 14-15B, pET 14-15C, and pET 14-15d. Each option shows a graphical representation of the vector with restriction sites and a corresponding amino acid sequence (N nnr and C nnr). For example, pET 14-15B has a 5' site of NdeI and a 3' site of XhoI, with an N nnr sequence of S H M A N S and a C nnr sequence of —.

Figure 1. Primer Prim'er possess a rich interactive interface. The non-native amino and carboxy terminal that will be expressed dynamically update as the user selects different cloning sites by clicking and dragging the 3' and 5' site markers.