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Generation of deletion and insertion mutants

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Method Article

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Abstract

Introduction

Site specific mutagenesis can be used to generate mutants in specific residues. The method used here was originally developed by Kunkel \(see ref. 1).

Procedure

1) Clone AtAMT1;1 into pDRf1-GW using GATEWAY^{TM} technology. Generate all amino acid substitutions by site-directed mutagenesis of the wild type AtAMT1;1 sequence[1]. Also generate AtAMT1;1 variants by site-directed mutagenesis. 2) For the C-terminal truncation mutants, use single strand DNA of pDRf1-AtAMT1;1 as a template to insert a short DNA fragment coding for a stop codon and carrying XhoI restriction site \(5'tgagtcgac). 3) Remove the remaining part of the Cterminus by XhoI digestion. 4) For all insertion mutants, use single stranded DNA from pDRf1-AtAMT1;1 carrying a 3x cmyc-tag at the very end of the AtAMT1;1 C-terminus as template. 5) Insert the C-terminal insertion of 5'atcgatggacgt, encoding IDGR, at positions 443, 444, 445, 446, 448, 455 or 456 into the AMT1;1 protein. For the L3-4 \(Ins150) and L5-6 \(Ins 231) insertions, insert a 5'ggttggagctcccgggggcgcc DNA fragment, encoding GGAPGGA, at position 150 or 231 into the AMT1;1 protein. **Insertions:** Ins150: …IAER_GGAPGGA_TQFV… Ins231: … FDN_GGGAPGGA_GRAI… Ins443: …ILKK_IDGR_LLRI… Ins444: …LKKM_IDGR_KLLR… Ins445: … KKMK_IDGR_LLRIS… Ins446: …KMKL_IDGR_LRIS… Ins448: …KLLR_IDGR_SSED… Ins455: … EDEM_IDGR_AGMD… Ins456: …DEMA_IDGR_GMD…

References

\(1) Kunkel, T. A. Rapid and efficient site-specific mutagenesis without phenotypic selection. _Proc. Natl. Acad. Sci. USA_ **82**, 488-492 \(1985).