|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **K368ASGEVSKHLYKVWK382** | | **Forward primer**  **(5’-3’)** | | **Reverse primer**  **(5’-3’)** | |
| **Common cloning primers (AfeI/BsiWI: RE sites)** | | CTGAAGCAGCGCTGATGTACG | | CTTTCTCCCGTACGTATGTGATGG | |
| **Ala mutations (Charge neutral mutations)** | | | | | |
| K375H376-A | KASGEVSAALYKVWK | | GCCTCTGGTGAAGTGTCTGCCGCTTTGTATAAAGTGTGGAAG | | CTTCCACACTTTATACAAAGCGGCAGACACTTCACCAGAGGC |
| K375/379/382-A | KASGEVSAHLYAVWA | | GCCTCTGGTGAAGTGTCTGCCCACTTGTATGCCGTGTGGGCCAAGATTGGGATTTGGAAC | | GTTCCAAATCCCAATCTTGGCCCACACGGCATACAAGTGGGCAGACACTTCACCAGAGGC |
| Y378V380W381-A | KASGEVSKHLAKAAK | | GAAGTGTCTAAACACTTGGCTAAAGCGGCCAAGAAGATTGGGATTTGG | | CCAAATCCCAATCTTCTTGGCCGCTTTAGCCAAGTGTTTAGACACTTC |
| K375HLYKVWK382-8A | KASGEVSAAAAAAAA | | GCCTCTGGTGAAGTGTCTGCCGCTGCAGCTGCAGCCGCAGCTAAGATTGGGATTTGGAAC | | GTTCCAAATCCCAATCTTAGCTGCGGCTGCAGCTGCAGCGGCAGACACTTCACCAGAGGC |
| **Glu mutations (Charge reversal mutations)** | | | | | |
| K375H376-E | KASGEVSEELYKVWK | | GCCTCTGGTGAAGTGTCTGAGGAATTGTATAAAGTGTGGAAG | | CTTCCACACTTTATACAATTCCTCAGACACTTCACCAGAGGC |
| H376-E | KASGEVSKELYKVWK | | GCCTCTGGTGAAGTGTCTAAAGAATTGTATAAAGTGTGGAAG | | CTTCCACACTTTATACAATTCTTTAGACACTTCACCAGAGGC |
| K375/379/382H376-E | KASGEVSEELYEVWE | | GCCTCTGGTGAAGTGTCTGAGGAATTGTATGAGGTGTGGGAGAAGATTGGGATTTGGAAC | | GTTCCAAATCCCAATCTTCTCCCACACCTCATACAATTCCTCAGACACTTCACCAGAGGC |
| K368/375/379/382H376-E | EASGEVSEELYEVWE | | AAAGAGGAAGGAACTGAAGAGGCCTCTGGTGAAGTGTCT | | AGACACTTCACCAGAGGCCTCTTCAGTTCCTTCCTCTTT |
| K368-E | EASGEVSKKLYKVWK | | AAAGAGGAAGGAACTGAAGAGGCCTCTGGTGAAGTGTCT | | AGACACTTCACCAGAGGCCTCTTCAGTTCCTTCCTCTTT |

**Figure 4-table supplement 1.** GluK1-1aATD splice mutants.The various mutants used in the study and the primer sequences to make charge-neutral and charge-reversal mutants in GluK1-1a are tabulated.