|  |  |  |
| --- | --- | --- |
| **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.