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| **Knockouts** |
| **nAChR subunit** | **gRNA target name** | **gRNA sequence (NGG)** | **oligonucleotides name (Forward/Reverse)** | **oligonucleotides sequence** |
| *nAChRα1* | Da1\_111(+) | 5’CGGAGATGTAGTAGTCCTGCAGG3’ | 41\_Da1\_111\_F | 5’GTCGCGGAGATGTAGTAGTCCTGC3’ |
|  |   |   | 42\_Da1\_111\_R | 5’AAACGCAGGACTACTACATCTCCG3’ |
|  | Da1\_126(-) | 5’CCTGCAGGTCGATGCCCACCTCG3’  | 43\_Da1\_126\_F | 5’GTCGCGAGGTGGGCATCGACCTGC3’ |
|  |   |   | 44\_Da1\_126\_R | 5’AAACGCAGGTCGATGCCCACCTCG3’ |
| *nAChRα2* | Da2\_99 (+) | 5’GCTCCTCTGCGAAACCGTTCAGG3’  | 45\_Da2\_99\_F | 5’GTCGCTCCTCTGCGAAACCGTTC3’ |
|  |   |   | 46\_Da2\_99\_R | 5’AAACGAACGGTTTCGCAGAGGAG3’ |
| *nAChRα3* | Da3\_18(+) | 5’GTCCGGACGCCAGATGTGATCGG3’ | 49\_Da3\_18\_F | 5’GTCGTCCGGACGCCAGATGTGAT3’ |
|  |   |   | 50\_Da3\_18\_R | 5’AAACATCACATCTGGCGTCCGGA3’ |
| *nAChRα4* | Da4\_19(+) | 5’TTGTTGCGACGAACCATACTTGG3’ | 53\_Da4\_19\_F | 5’GTCGTTGTTGCGACGAACCATACT3’ |
|  |   |   | 54\_Da4\_19\_R | 5’AAACAGTATGGTTCGTCGCAACAA3’ |
| *nAChRα5* | Da5\_232(-) | 5’CCGGGGATCTTCAAGTCGACGTG3’ | 57\_Da5\_232\_F | 5’GTCGCACGTCGACTTGAAGATCCC3’ |
|  |   |   | 58\_Da5\_232\_R | 5’AAACGGGATCTTCAAGTCGACGTG3’ |
|  | Da5\_251(+) | 5’CGTGCAAGATCGACATCACGTGG3’  | 59\_Da5\_251\_F | 5’GTCGCGTGCAAGATCGACATCACG3’ |
|  |   |   | 60\_Da5\_251\_R | 5’AAACCGTGATGTCGATCTTGCACG3’ |
| *nAChRα6* | Da6\_70(+) | 5’CGTATTCTTCTTTCCCGGCATGG3’ | 61\_Da6\_70\_F | 5’GTCGCGTATTCTTCTTTCCCGGCA3’ |
|  |   |   | 62\_Da6\_70\_R | 5’AAACTGCCGGGAAAGAAGAATACG3’ |
| *nAChRα7* | Da7\_1226(+) | 5’CATTGACCACCGGACGCTCCAGG3’ | 63\_Da7\_1226\_F | 5’GTCGCATTGACCACCGGACGCTCC3’ |
|  |   |   | 64\_Da7\_1226\_R | 5’AAACGGAGCGTCCGGTGGTCAATG3’ |
| *nAChRβ1* | Db1\_2(+) | 5’TGGAGTCTTCCTGCAAATCCTGG3’ | 67\_Db1\_2\_F | 5’GTCGTGGAGTCTTCCTGCAAATCC3’ |
|  |   |   | 68\_Db1\_2\_R | 5’AAACGGATTTGCAGGAAGACTCCA3’ |
| *nAChRβ2* | Db2\_955(+) | 5’TCAGACCTAACCAAACCGTCAGG3’ | 71\_Db2\_955\_F | 5’GTCGTCAGACCTAACCAAACCGTC3’ |
|  |   |   | 72\_Db2\_955\_R | 5’AAACGACGGTTTGGTTAGGTCTGA3’ |
| *nAChRβ3* | Db3\_466(+) | 5’CTTTGAAGTCCAGCGAGGTCTGG3’ | 75\_Db3\_466\_F | 5’GTCGCTTTGAAGTCCAGCGAGGTC3’ |
|  |   |   | 76\_Db3\_466\_R | 5’AAACGACCTCGCTGGACTTCAAAG3’ |
| **C-terminal tagging** |
| **nAChR subunit** | **gRNA target name** | **gRNA sequence (NGG)** | **oligonucleotides name (Forward/Reverse)** | **oligonucleotides sequence** |
| *nAChRα6* | Da6\_181(+) | 5’TTGCACGATTATGTGCGGAGCGG3’ | 131\_Da6\_181\_F | 5’GTCGTTGCACGATTATGTGCGGAG3’ |
|   |   |   | 132\_Da6\_181\_R | 5’AAACCTCCGCACATAATCGTGCAA3’ |
|   | Da6\_176(+) | 5’CCTTATTGCACGATTATGTGCGG3’ | 133\_Da6\_176\_F | 5’GTCGCCTTATTGCACGATTATGTG3’ |
|   |   |   | 134\_Da6\_176\_R | 5’AAACCACATAATCGTGCAATAAGG3’ |

## Supplementary Figure 6. List of gRNAs and oligonucleotides used for cloning.