

ASK2J001 78_SNCA_A30P_A12.2_AB 3409 bp
$5^{\prime} \quad$ CAGGTCTCTGGGAGGTGAGTACTTGTCCCTTTGGGGAGCCTAAGGAAAGAGACTTGACCTGGCTTTCGTCCTGCTTCTGA GTCCAGAGACCCTC СACTCATGAACAGGGAAACCCCTCGGATTCCTTTCTCTGAACTGGACCGAAAGCAGGACGAAGACT

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TATTCCCTTCTCCACAAGGGCTGAGAGATTAGGCTGCTTCTCCGGGATCCGCTTTTCCCCGGGAAACGCGAGGATGCTCC +НبННبН ب ATAAGGGAAGAGGTGTTCCCGACTCTCTAATCCGACGAAGAGGCCCTAGGCGAAAAGGGGCCCTTTGCGCTCCTACGAGG SNCA

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ATGGAGCGTGAGCATCCAACTTTTCTCTCACATAAAATCTGTCTGCCCGCTCTCTTGGTTTTTCTCTGTAAAGTAAGCAA + + +ب+ TACCTCGCACTCGTAGGTTGAAAAGAGAGTGTATTTTAGACAGACGGGCGAGAGAACCAAAAAGAGACATTTCATTCGTT

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GCTGCGTTTGGCAAATAATGAAATGGAAGTGCAAGGAGGCCAAGTCAACAGGTGGTAACGGGTTAACAAGTGCTGGCGCG
 CGACGCAAACCGTTTATTACTTTACCTTCACGTTCCTCCGGTTCAGTTGTCCACCATTGCCCAATTGTTCACGACCGCGC

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GGGTCCGCTAGGGTGGAGGCTGAGAACGCCCCCTCGGGTGGCTGGCGCGGGGTTGGAGACGGCCCGCGAGTGTGAGCGGC
 CCCAGGCGATCCCACCTCCGACTCTTGCGGGGGAGCCCACCGACCGCGCCCCAACCTCTGCCGGGCGCTCACACTCGCCG

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GCCTGCTCAGGGTAGATAGCTGAGGGCGGGGGTGGATGTTGGATGGATTAGAACCATCACACTTGGGCCTGCTGTTTGCC НبН CGGACGAGTCCCATCTATCGACTCCCGCCCCCACCTACAACCTACCTAATCTTGGTAGTGTGAACCCGGACGACAAACGG

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TGAGTTTGAACCACACCCCGAGTGAGCAGTTAGTTCTGTTGCCTACGCCTTTCCACCATCAACCTGTTAGCCTTCTTCTG
 ACTCAAACTTGGTGTGGGGCTCACTCGTCAATCAAGACAACGGATGCGGAAAGGTGGTAGTTGGACAATCGGAAGAAGAC

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GGATTCATGTTAAGGATACCCCTGACCCTAAGCCTCCAGCTTCCATGCTTCTAACTCATACTGTTACCCTTTAGACCCCG
 CCTAAGTACAATTCCTATGGGGACTGGGATTCGGAGGTCGAAGGTACGAAGATTGAGTATGACAATGGGAAATCTGGGGC

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[^0]ATCCGTGGTTAGGTGGCTAGACTTTTACTAGCCAAGATGGATGGGAGATGCTAAATTTTTAATGCCAGAGCTAAAAATGT


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\begin{aligned}
& \text { Sanger Sequencing Primer } \\
& \text { cacactttggagggtttctc }
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$$

CTGCTTTGTCCAATGGTTAAATGAGTGTACACTTAAAAGAGTCTCACACTTTGGAGGGTTTCTCATGATTTTTCAGTGTT
 GACGAAACAGGTTACCAATTTACTCACATGTGAATTTTCTCAGAGTGTGAAACCTCCCAAAGAGTACTAAAAAGTCACAA
SNCA $\gg$

TTTTGTTTATTTTTCCCCGAAAGTTCTCATTCAAAGTGTATTTTATGTTTTCCAGTGTGGTGTAAAGGAATTCATTAGCC
 AAAACAAATAAAAAGGGGCTTTCAAGAGTAAGTTTCACATAAAATACAAAAGGTCACACCACATTTCCTTAAGTAATCGG $\begin{array}{r}\hline \text { SNCA } \\ \hline \text { SNCA-201 }\end{array}$

ATGGATGTATTCATGAAAGGACTTTCAAAGGCCAAGGAGGGAGTTGTGGCTGCTGCTGAGAAAACCAAACAGGGTGTGGC
 TACCTACATAAGTACTTTCCTGAAAGTTTCCGGTTCCTCCCTCAACACCGACGACGACTCTTTTGGTTTGTCCCACACCG


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AGTGATGGGTTAGGATAATCAATACTCTAAATGCTGGTAGTTCTCTCTCTTGATTCATTTTTGCATCATTGCTTGTCAAA
 TCACTACCCAATCCTATTAGTTATGAGATTTACGACCATCAAGAGAGAGAACTAAGTAAAAACGTAGTAACGAACAGTTT

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Donor Template WT -> SNV
tcact
Donor Template WT ->SNV

AAGGTGGACTGAGTCAGAGGTATGTGTAGGTAGGTGAATGTGAACGTGTGTATTTGAGCTAATAGTAAAAAATGCGACTG
 TTCCACCTGACTCAGTCTCCATACACATCCATCCACTTACACTTGCACACATAAACTCGATTATCATTTTTTACGCTGAC


TTTGCTTTTCCAGATTTTTAATTTTGCCCTAATATTTATGACTTTTTAAAAATGAATGTTTCTGTACCTACATAATTCTA
 AAACGAAAAGGTCTAAAAATTAAAACGGGATTATAAATACTGAAAAATTTTTACTTACAAAGACATGGATGTATTAAGAT


TTTCAGAGAACAGTTTTAAAAACTCATAGTCTTTTAAAAAATAATCAAGAATATTCTTAAGAATCAAAATCATTGATGGA
 AAAGTCTCTTGTCAAAATTTTTGAGTATCAGAAAATTTTTTATTAGTTCTTATAAGAATTCTTAGTTTTAGTAACTACCT
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TCTGTGATTTCTTTTACCATCATGAAAAATGTTTGTCAATTTTAATCCATTCTGATTTTTAAAATATGACTTTGATATGC
 AGACACTAAAGAAAATGGTAGTACTTTTTACAAACAGTTAAAATTAGGTAAGACTAAAAATTTTATACTGAAACTATACG


CCCTGTGATGTGTATAAAGAGACCTATTTGTGGCCCTAAAATGGAAAGAACAGATTAGTCTTTGATAGAGTTACTTCATG
 GGGACACTACACATATTTCTCTGGATAAACACCGGGATTTTACCTTTCTTGTCTAATCAGAAACTATCTCAATGAAGTAC


TGATCATTTGGTCTCTGTGAACACTGAGGACAGAGAAAAGTGCTTGAGGGCTGCTACTAATCTCTCAGAAACATTTGTAT
 ACTAGTAAACCAGAGACACTTGTGACTCCTGTCTCTTTTCACGAACTCCCGACGATGATTAGAGAGTCTTTGTAAACATA $\begin{array}{r}\hline \text { SNCA } \\ \hline \text { SNCA-201 }\end{array}$
 TCAAGTAGGTAGTTTACTGTGTGTATGATTTTCTTATTTCTTTAACTACGAATAATGGATGAACAAGGATTTCAAGGTGG SNCA SNCA-201


TTGGGGTATACACCCAAACTCTGACTCTCTTTTCTGTAACTTGAACTGTATTCAATTGAGTGTTATTTTACAAACCACTT

 AACCCCATATGTGGGTTTGAGACTGAGAGAAAAGACATTGAACTTGACATAAGTTAACTCACAATAAAATGTTTGGTGAA | SNCA |
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TGAATTCCTTGGAAAAGAATAGACACACACTCTCATCCACAGGCATAGACACACACACTCAACACAGACACATTGCCCAT
 ACTTAAGGAACCTTTTCTTATCTGTGTGTGAGAGTAGGTGTCCGTATCTGTGTGTGTGAGTTGTGTCTGTGTAACGGGTA


TCTTCCTCTCTTCTTTCTCCTCTGAGCTTTTTCACATTCTCTGGTGGCAACTATAGCAGTAAGAGTCACAGGATGAACAG
 AGAAGGAGAGAAGAAAGAGGAGACTCGAAAAAGTGTAAGAGACCACCGTTGATATCGTCATTCTCAGTGTCCTACTTGTC

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TCAGGTGGAGGATGACCACATTGAGTTGCCTAGCTGAAACATGTGCTCCGTCTATGTCTGCAAAGTGAAAGAAAGCTACA
 AGTCCACCTCCTACTGGTGTAACTCAACGGATCGACTTTGTACACGAGGCAGATACAGACGTTTCACTTTCTTTCGATGT $\begin{array}{r}\hline \text { SNCA } \\ \hline \text { SNCA-201 } \\ \hline\end{array}$

CTATCTCTTCAACATAGATCAGTGGGGGAAATTTTATACTTGGGATGATTTATATGAATGCATCTCATCAAAGTTCACAA
 GATAGAGAAGTTGTATCTAGTCACCCCCTTTAAAATATGAACCCTACTAAATATACTTACGTAGAGTAGTTTCAAGTGTT

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CACATTTTTTTTTCAGTTTTTTATTTTCAGTTTTTAGAGTCAGGGCCTTGCTCTGTCGCCCAGGCTGGACTGCAGTGATG
 GTGTAAAAAAAAAGTCAAAAAATAAAAGTCAAAAATCTCAGTCCCGGAACGAGACAGCGGGTCCGACCTGACGTCACTAC

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CTATCATAGCTCACTGCATCCTTGAATTCCTGGGCTCAAGTCATGCCCCCACCTCAGCCTCCTGAGTAGCCAGGATTATA
 GATAGTATCGAGTGACGTAGGAACTTAAGGACCCGAGTTCAGTACGGGGGTGGAGTCGGAGGACTCATCGGTCCTAATAT

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TCCGTTTGTCCTCAGATTTCCAAGTTCTGCAAGCACAAGTTTCTTTGACTTAGTTACCTTTTAACTGTTCATTGAAATCA + + $+\boldsymbol{+}$

TTTTCAATGTCTCTCATGGCATTTAACACATAGCACATTCTATAAATTTTTTATTGGTTACATTCTGAGTTCTAATTGAG
 AAAAGTTACAGAGAGTACCGTAAATTGTGTATCGTGTAAGATATTTAAAAAATAACCAATGTAAGACTCAAGATTAACTC $\begin{array}{r}\hline \text { SNCA } \\ \hline \text { SNCA-201 } \\ \hline\end{array}$

AGTTGAACTTACACACAGAATTTAAGATAAAAAATGACCATGTGAAGACACAATAGTATAGTCCAGGGATTGGCAAAATT
 TCAACTTGAATGTGTGTCTTAAATTCTATTTTTTACTGGTACACTTCTGTGTTATCATATCAGGTCCCTAACCGTTTTAA SNCA

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TTGGGTAAGGAATCAGATAGCACGTATTTTAAGCCATGAGATCTATGTCTTGGCCAGGTGCCGTGGCTCAGGTCTTTAAT + + +ب+ AACCCATTCCTTAGTCTATCGTGCATAAAATTCGGTACTCTAGATACAGAACCGGTCCACGGCACCGAGTCCAGAAATTA


CCCAGCACTTTGAGAGCCCGAGGCTGGTGGATCACTTGAGCCCAGGGGTTTGAGACCAGCCTGGGCCACATGGTGAAACC
 GGGTCGTGAAACTCTCGGGCTCCGACCACCTAGTGAACTCGGGTCCCCAAACTCTGGTCGGACCCGGTGTACCACTTTGG


CTGTGTCTACAAACAACGCAAAAATTAGCCGGGTATGGTAGCATGCATGTGTATTGCCAGCTACCCAGGAGGCTGAGGTA + $+\boldsymbol{+}+\boldsymbol{+} \boldsymbol{+}$ GACACAGATGTTTGTTGCGTTTTTAATCGGCCCATACCATCGTACGTACACATAACGGTCGATGGGTCCTCCGACTCCAT

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GCTTGCTGACTACTGATAGAGTGGAAAGAACATGCACTAATCACACAAACCAAAGTTTTAGTTGAGACTACATCACTTAT
 CGAACGACTGATGACTATCTCACCTTTCTTGTACGTGATTAGTGTGTTTGGTTTCAAAATCAACTCTGATGTAGTGAATA

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CACCTTTAGGGTCTTGGGGAAGCGTACTTAACATCTCTGAGCATCACTT ب+ $+\boldsymbol{+} \mid+\boldsymbol{+}$ GTGGAAATCCCAGAACCCCTTCGCATGAATTGTAGAGACTCGTAGTGAA

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|  | Primer | Length | 0 | Binding | Sites | $\rightleftharpoons$ | Tm | Date Added |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | PCR Forward | 25-mer |  | 661 .. | 685 | $\rightarrow$ | $58^{\circ} \mathrm{C}$ | Apr 24, 2023 |
|  | $\begin{aligned} \text { /sequence }= & \text { atcttttcatgcaactccacttctg } \\ & 40 \% \mathrm{GC} / 7518.0 \mathrm{Da} \end{aligned}$ |  |  |  |  |  |  |  |
| $\checkmark$ | Sanger Sequencing Primer | 20-mer |  | 845 .. | 864 | $\rightarrow$ | $56^{\circ} \mathrm{C}$ | Apr 24, 2023 |
|  | $\begin{aligned} \text { /sequence }= & \text { cacactttggagggtttctc } \\ & 50 \% \mathrm{GC} / 6099.0 \mathrm{Da} \end{aligned}$ |  |  |  |  |  |  |  |
| $\checkmark$ | Donor Template WT ->SNV | 100-mer |  | 1026 .. 1 | 1125 | - | $75^{\circ} \mathrm{C}$ | Apr 24, 2023 |
|  | $\begin{aligned} \text { /sequence }= & \text { tcactcatgaacaagcaccaaactga } \\ & 46 \% \mathrm{GC} / 30,514.8 \mathrm{Da} \end{aligned}$ | ggggttta |  | CTACATAGA | AGAACA |  | CTTTTG | CTTTCCTGGTGCTTCTGCCACACCCTGTTTG |
| $\checkmark$ | gRNA Protospacer | 20-mer |  | 1044 .. 1 | 1063 | $\rightarrow$ | $56^{\circ} \mathrm{C}$ | Apr 24, 2023 |
|  | $\begin{aligned} \text { /sequence }= & \text { AGCAGCAGGAAAGACAAAAG } \\ & 45 \% \mathrm{GC} / 6226.2 \mathrm{Da} \end{aligned}$ |  |  |  |  |  |  |  |
| $\checkmark$ | PCR Reverse | 25-mer |  | 1539 .. 1 | 1563 | - | $57^{\circ} \mathrm{C}$ | Apr 24, 2023 |
|  | $\begin{aligned} \text { /sequence }= & \text { cattttagggccacaaataggtctc } \\ & 44 \% \mathrm{GC} / 7641.0 \mathrm{Da} \end{aligned}$ |  |  |  |  |  |  |  |


[^0]:    GGAATTTAAAAAAGGGGTTAATCTTTTCATGCAACTCCACTTCTGAAATGCAGTAATAACAACTCAGAGGATTCATCCTA HبН

