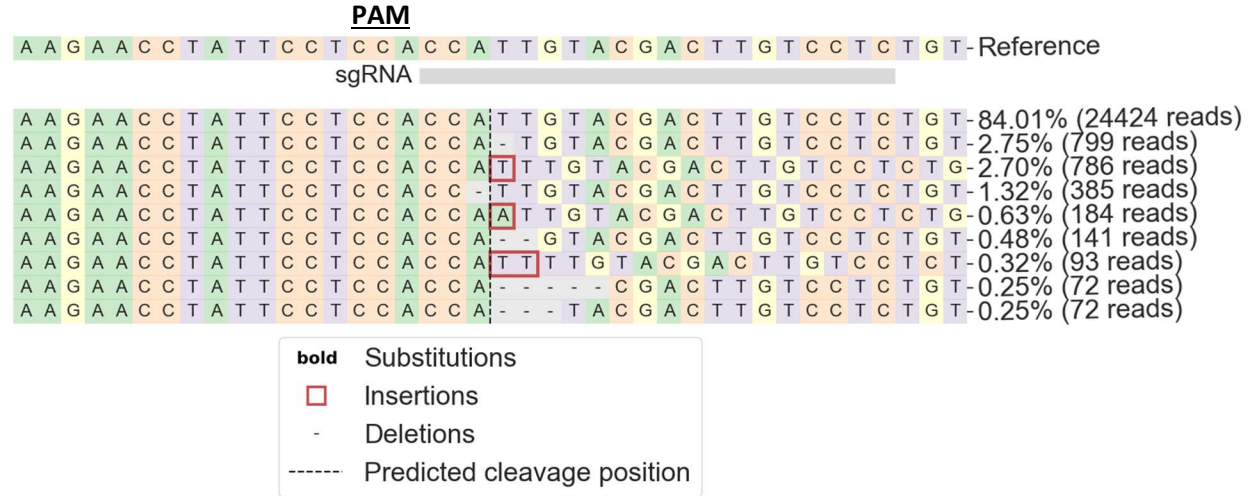


Targeting DNA Polymerase to DNA double-strand breaks reduces DNA deletion size and increases templated insertions generated by CRISPR/Cas9

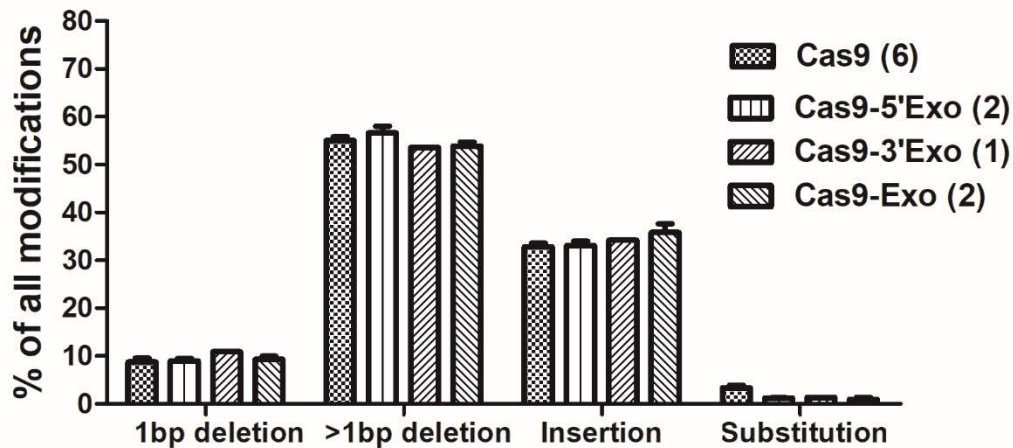
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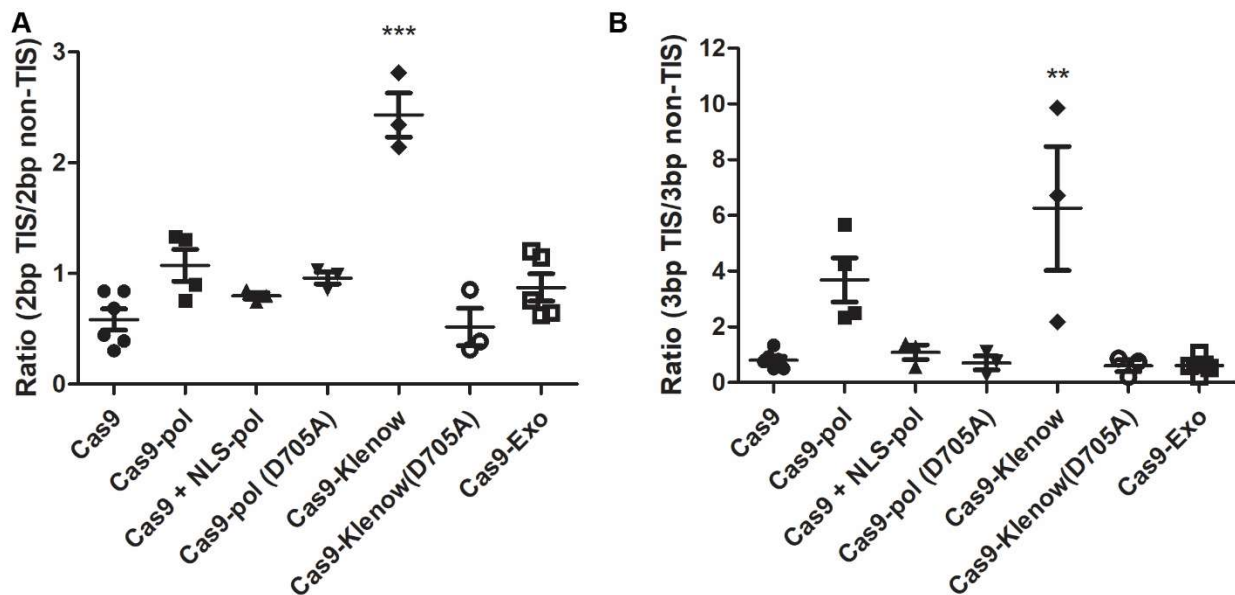
²Department of Cancer Biology, Wake Forest University Health Sciences, Winston-Salem, NC, 27157



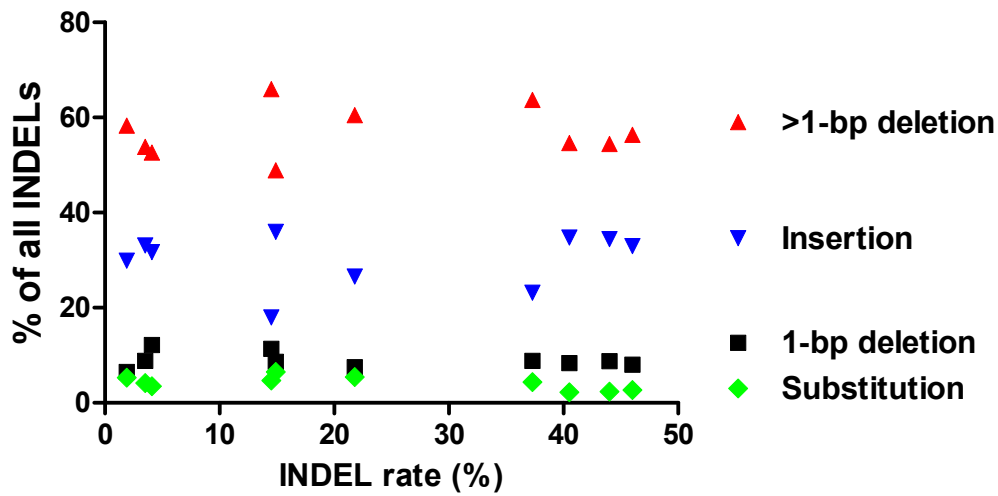
Supplementary Figure S1. NGS analysis of the integrated target sequence in GFP-reporter cells treated with CLCN5 sgRNA and Cas9-pol. Data were analyzed by CRISPResso2.



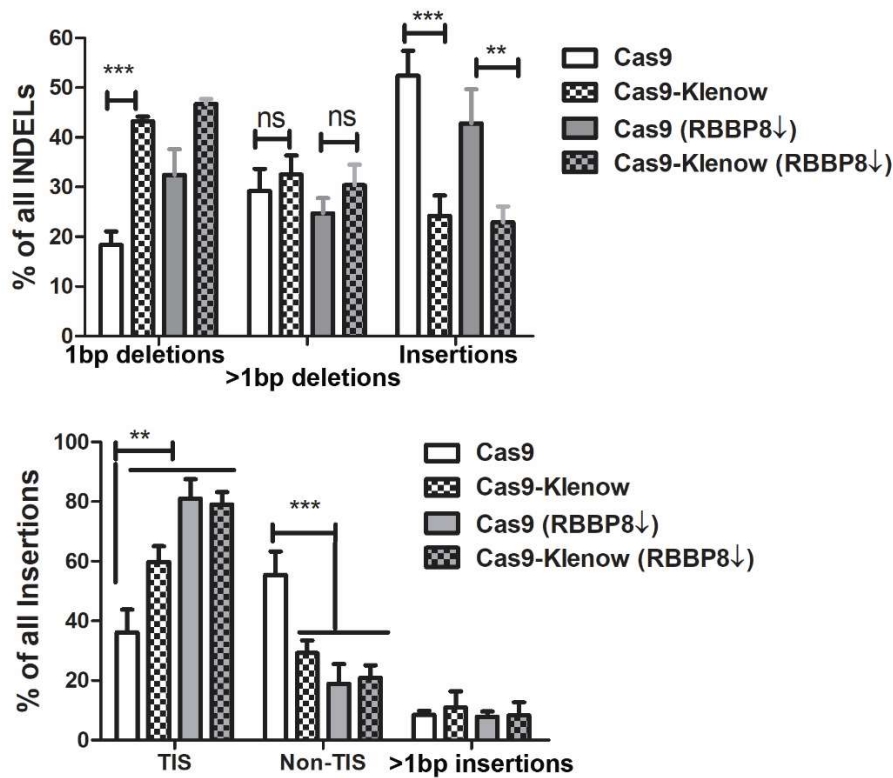
Supplementary Figure S2. Cas9 and various exonuclease fusions had similar mutation profiles as Cas9. Numbers in parentheses indicate replicate numbers. Data were analyzed by CRISPResso2.



Supplementary Figure S3. Effects of different pol I domains and mutants on 2-bp (A) and 3-bp (B) TIS. The data for exo, 3' exo and 5' exo fusion proteins targeting *CLCN5* in HEK293T cells were pooled into one group. Each dot indicates one datum point. ** and *** indicate $p < 0.05$ and $p < 0.001$ compared with Cas9 group. Tukey's Multiple Comparison Test was performed following one-way ANOVA. Cas9-pol showed a trend of increase but did not reach statistical significance due to large intra group variation.

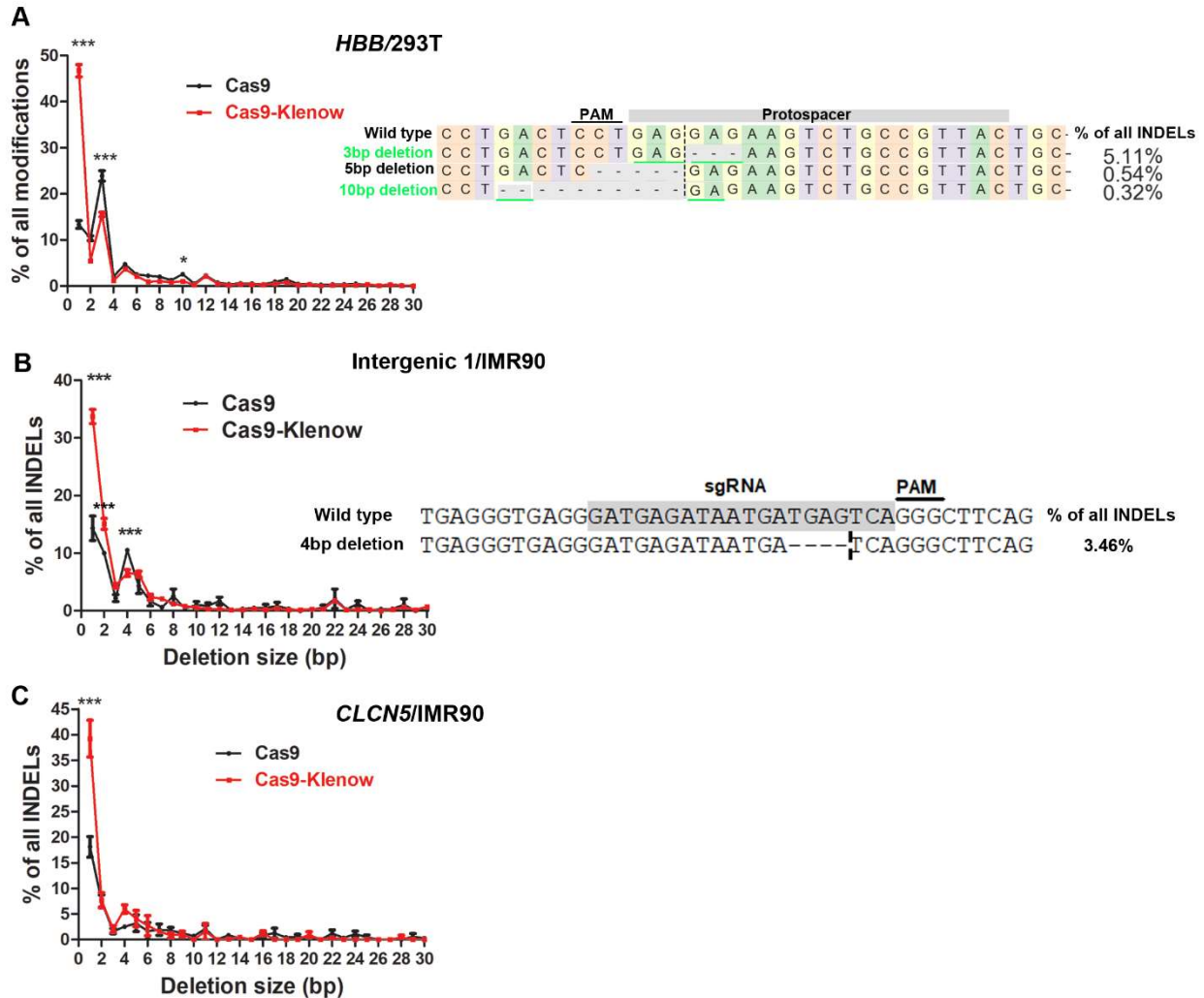


Supplementary Figure S4. No effects of overall INDEL rates on the percentages of different mutation types. Data were analyzed by CRISPResso2.



Supplementary Figure S5. Effects of *RBBP8* Knockdown on *CLCN5* mutation profiles in IMR90 cells. Top image: effects on deletions and overall insertions. Bottom image: Effects on 1-bp TIS and 1-bp non-TIS. Each group had 3 biological replicates. Two-way ANOVA was

followed by Bonferroni posttests. ** and *** indicate $p < 0.01$ and $p < 0.001$ between the indicated groups.

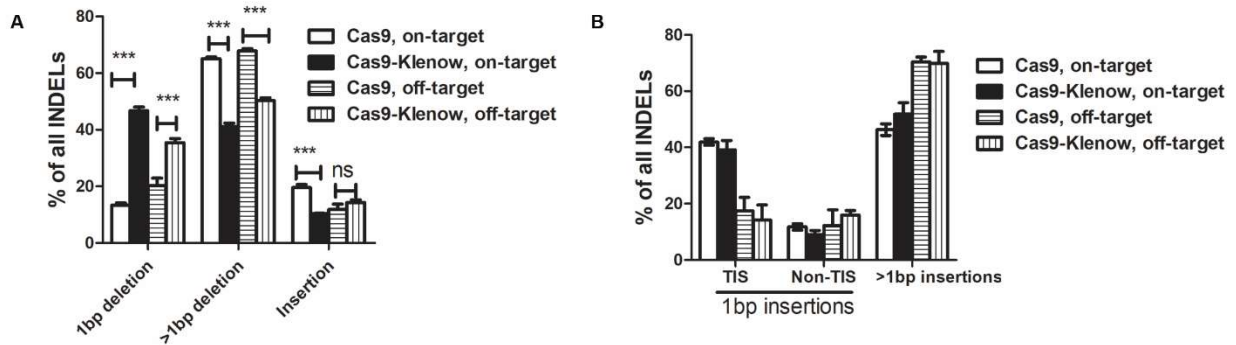


Supplementary Figure S6. Deletions decreased by Cas9-Klenow and their sequences. A. Targeting *HBB* in HEK293T cells. B. Targeting Intergenic 1 in IMR90 cells. C. Targeting *CLCN5* in IMR90 cells. The left images show the percentages of deletion sizes. The right images show the sequences of the most commonly observed deletions. No sequence could be listed for *CLCN5/IMR90* since no major deletion peaks were observed. The regions underlined with green lines indicate microhomology at the predicted cleavage site (vertical dashed lines). Two-way ANOVA was followed by Bonferroni posttests. * and *** indicates $p < 0.05$ and $P < 0.001$ between the two groups.

Target
PAM

HBB : GTAACGGCAGACTTCTCCTCAGG
HBD : TTGACAGCAGTCTTCTCCTCAGG
OT-4 : ATACTTAAGGACTTCTCCTCCAT
OT-5 : GGAGGGGCAGGCTTCTCCTCTGG
OT-8 : TCACAGGCAGACTTCTCCACGGG

Supplementary Figure S7. Sequences of off-targets examined. PAMs are underline. Red nucleotides are those of mismatch.



Supplementary Figure S8. Effects of fusing Klenow fragment to Cas9 on the *HBB* on-target and *HBB* “off-target” site in HEK293T cells. **A. Deletions and insertions. **B.** TIS and non-TIS. *** indicates $P < 0.001$ between the two groups (Bonferroni posttests following two-way ANOVA).**

Supplementary Table S1. Constructs

No.	Name	Purpose	Generation strategy
1	pspCas9-POLI-Tetra-com vector	Vector plasmid for expressing spCas9-POLI fusion protein and sgRNA. There are two copies of HBB 3' UTR in the 3'UTR of spCas9-POLI to enhance expression. The Tetraloop of the sgRNA scaffold was replaced by com-patamer to enable Cas9 RNP encapsulation into viral capsids via com-COM interaction.	The DNA sequence of <i>E. coli</i> DNA POLI (encoded by POLA) was optimized for human and mouse expression as “GGATCCTCTGAGACACCAGGCACCTCCGAGTCTGCCACCCCTGA GGGAGGCAGCGGAGGCAGCGGCTCCGTGCAGATCCCACAGAAC CCCCTGATCCTGGTGGACGGCAGCTCCTACCTGTATCGGGCCTAC CACGCCCTTCCCACCTCTGACAAACTCCGCCGGAGAGCCAACCGG AGCCATGTATGGCGTGCTGAATATGCTGAGGAGCCTGATCATGCA GTACAAGCCTACACACGCCCGCGTGGTGTGTTGATGCCAAGGGCAA GACCTTCCGCGACGAGCTGTTTGAGCACTACAAGAGCCACAGGC CACCAATGCCTGACGATCTGAGGGCACAGATCGAGCCACTGCAC GCAATGGTGAAGGCCATGGGCCTGCCTCTGCTGGCCGTGAGCGG AGTGGAGGCCGACGATGTGATCGGCACACTGGCAAGGGAGGCAG AGAAGGCAGGCCGCCCAGTGCTGATCTCCACCGGCGACAAGGAT ATGGCCCAGCTGGTGACACCAAACATCACCTGATCAACACCATG ACAAATACCATCCTGGGCCCGAGGAGGTGGTGAATAAGTATGGC GTGCCTCCAGAGCTGATCATCGATTTCTGGCCCTGATGGGCGAC TTAGCGATAACATCCCTGGAGTGCCAGGAGTGGGAGAAAAGAC CGCACAGGCCCTGCTGCAGGGCCTGGGAGGCCTGGACACCCTGT ACGCCGAGCCAGAGAAGATCGCCGCGCTGTCCCTTAGGGGGCC AAGACAATGGCCGCAAGCTGGAGCAGAATAAGGAGGTGGCCTA CCTGTCTTATCAGCTGGCCACAATCAAGACCGACGTGGAGCTGGA GCTGACCTGCGAGCAGCTGGAGGTGCAGCAGCCTGCAGCAGAGG AGCTGTGGGCTGTTCAAGAAGTACGAGTTTAAGAGATGGACAG CCGATGTGGAGGCCGCAAGTGGCTGCAGGCAAAGGGAGCAA GCCAGCAGCAAAGCCACAGGAGACAAGCGTGGCAGACGAGGCAC

No.	Name	Purpose	Generation strategy
			<p>CAGAGGTGACAGCCACCGTGATCTCCTACGATAACTATGTGACAA TCCTGGACGAGGAGACTGAAGGCCTGGATCGCCAAGCTGGAG AAGGCCCCCGTGTTCGCCTTTGATACAGAGACAGACAGCCTGGAT AACATCTCCGCCAATCTGGTGGCCCTGTCTTTCCGCAATCGAGCCT GGCGTGGCCGCCTATATCCAGTGGCCACGACTACCTGGATGC CCCCGACCAGATCAGCAGGGAGAGAGCCCTGGAGCTGCTGAAGC CTCTGCTGGAGGATGAGAAGGCCCTGAAGGTCGGCCAGAACCTG AAGTATGACAGGGGCATCCTGGCCAATTACGGCATCGAGCTGAGA GGCATCGCCTTTGACACCATGCTGGAGTCTTATATCCTGAATAGC GTGGCAGGCCGGCACGACATGGATTCCCTGGCCGAGAGGTGGCT GAAGCACAAGACAATCACCTTCGAGGAGATCGCCGGCAAGGGCA AGAACCAGCTGACCTTCAACCAGATCGCCCTGGAGGAGGCAGGC AGGTACGACAGCAGAGGACGACGATGTACCCTGCAGCTGCACCT GAAGATGTGGCCAGATCTGCAGAAGCACAAGGGCCCCCTGAACG TGTTCCGAGAAATATCGAGATGCCCTGGTGCCTGTGCTGAGCCGGA TCGAGCGCAACGGCGTGAAGATCGACCCTAAGGTGCTGCACAAT CACTCCGAGGAGCTGACCCTGAGACTGGCCGAGCTGGAGAGAA GGCCACGAGATCGCCGGCGAGGAGTTCAACCTGTCTCTACAA AGCAGCTGCAGACCATCCTGTTTGAGAAGCAGGGGCATCAAGCCCC TGAAGAAAACCCCTGGAGGAGCACCATCTACCAGCGAGGAGGTG CTGGAGGAGCTGGCCCTGGATTATCCCTGCCTAAAGTATGCTCG GAGTACCGGGGCTGGCCAAGCTGAAGTCTACATATACCGACAAG CTGCCCCCTGATGATCAACCCTAAGACAGGAAGGGTGCACACCAGC TACCACCAGGCAGTGACAGCAACCGGCCGCCTGAGCTCCACCGA TCCAAACCTGCAGAATATCCCCGTGAGGAATGAGGAGGGCAGGA GAATCAGACAGGCCCTTCATCGCCCCCGAGGATTATGTGATCGTGT CCGCCGACTACTCTCAGATCGAGCTGAGGATCATGGCCCACCTGT CCAGAGATAAGGGCCTGCTGACAGCCTTCGCCGAGGGCAAGGAC ATCCACAGGGCAACCGCAGCAGAGGTGTTTGGCCTGCCTCTGGA GACAGTGACCTCCGAGCAGCGGCCTCTGCCAAGGCCATCAACT TCGGCCTGATCTATGGCATGTCTGCCTTTGGCCTGGCCAGGCAGC TGAATATCCCTAGAAAGGAGGCCAGAAAGTACATGGACCTGTATT TCGAGAGGTACCCAGGCGTGCTGGAGTACATGGAGAGGACAAGG GCACAGGCAAAGGAGCAGGGCTATGTGGAGACACTGGATGCGAG GAGACTGTACCTGCCAGACATCAAGTCTAGCAACGGAGCAAGGA GGGCAGCAGCAGAGAGGGGCCCATCAATGCCCCCATGCAGGG CACAGCCCGGATATCATCAAGAGAGCCATGATCGCAGTGGACG CATGGCTGCAGGCAGAGCAGCCAAGGGTGAGAATGATCATGCGAG GTGCACGATGAGCTGGTGTGTTGAGGTGCACAAGGACGATGTGGA CGCCGTGGCCAAGCAGATCCACCAGCTGATGGAGAAGTGTACCA GGCTGGATGTGCCACTGCTGGTGGAAAGTGGGCAGCGGAGAGAAT TGGGACCAGGCCACTGAGGATCC” and was inserted into BamH1 site of pspCas9-Tetra-com vector. The peptide sequence is: MDYKDHGDYKDHIDIDYKDDDDKMAPKKRKRKVIHGVPAADKKYSI GLDIGTNSVGVAVITDEYKVPKFKVLGNDRHSIKKNIIGALLFDS GETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRL ESFLVEEDKHERHPIFGNIVDEVAYHEKYPTIYHLRKLVDSTDKADL RLIYLALAHMIKFRGHFLIEGDLNPDNSDVKLFIQLVQTYNQLFEENPI NASGVDAKAILSARLSKSRLENLIAQLPGEKKNLFGNLIASLGLTP NFKSNFDLAEDAKLQLSKDYYDDLDNLQAIGDQYADFLAAKNLSD AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYK EIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKNLRE DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILTF RIPYYVGPLARGNSRFAMTRKSEETITPWNFEVVDKGASAQSFIE RMTNFDKNLPNEKVLPHSLLYEYFTVYNELTKVKYVTEGMRKPAFL SGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFN ASLGTYHDLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKY AHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKS GFANRNFMQLIHDDSLTFKEDIQKAQVSGQDLSLHEHIANLAGSPA KGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGGQKNSRERM KRIEIGIKELGSQILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELD INRLSDYDVDHIVPQSFLKDDSIDNKVLRSDKNRGSNDNVPSEEVVK RMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRLVET RQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQY KVREINNYHHAHDAYLNAVVGTAIIKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRLIETNGETG EIVWDKGRDFATVRKVLSPQVNVKTEVQTGGFSKESILPKRNSD KLIARKKDWDPKYGFDSPVAVSVLVVAKVEKGSKSLKSVKELL</p>

No.	Name	Purpose	Generation strategy
			<p>GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML ASAGELQKGNELALPSKYVNFLLYASHYEKLGSPEDNEQKQLFVEQ HKHYLDEIIEQISEFSKRVLADANLDKVL SAYNKHRDKPIREQAENIIHL FTLNLGAPAAFYFDTTIDRKRYTSTKEVLDTLIHQSIITGLYETRIDL SQLGGDKRPAATKKAGQAKKKKGSSETPGTSESATPEGGSGGSGS VQIPQNPLILVDGSSYLYRAYHAFPLTNSAGEPTGAMYGVNLMLRSL IMQYKPTHAAVFDAGKTFRDELFEHYKSHRPPMPDDLRAQIEPLH AMVKAMGLPLLAVSGVEADDVIGTLAREAEKAGRPVLISTGDKDMAQ LVTNITLINTMTNTILGPEEVVNKYGVPELIIDFLALMGDSSDNIPIGV PGVGEKTAQALLQGLGGLDTLYAEPEKIAGLSFRGAKTMAAKLEQNK EVALYSYQLATIKTDVELELTCEQLEVVQPPAAEELLGLFKKYEFRWT ADVEAGKWLQAKGAKPAKPQETSVADEAPEVTATVISYDNYVTILD EETLKAWIAKLEKAPVFAFDTETDSDLNISANLVGLSFAIEPGVAAIYIPV AHDYLDAPDQISRERALELLKPLLEDEKALKVGNLKYDRGILANYGIE LRGIAFDTMLESYILNSVAGRHDMSLAERWLKHKITITFEEIAGKGN QLTFNQIALEEAGRYAAEDADVTQLHLKMWPDQKHKGPLNVFENI EMPLVPVLSRIERNVGVKIDPKVLHNSHSEELTLRLAELEKKAHEIAGEEF NLSSTKQLQTLFEKQGIKPLKKTTPGGAPSTSEEVLEELALDYPLPKVIL EYRGLAKLKSTYTDKLP.LMINPKTGRVHTSYHQAVTATGRLSSTDPNL QNIPVRNEEGRRIQAFIAPEDYVIVSADYSQIELRIMAHLSRDKGLLT AFAEGKDIHRATAAEVFGLEPLTVTSEQRRSAKAINFGLIYGM5AFL ARQLNIPRKEAQKYMDFERYPGVLEYMERTRAQAKEQGYVETLD GRRLYL.PDIKSSNGARRAAAERAANAPMQGTAADIKRAMIAVDLAWL QAEQPRVRMIMQVHDELVEVHKDDVDVAQKIQHQLMENCTRLDVP LLVEVSGGENWDQAH (Color Key: Cas9-linker-Polymerase)</p>
2	pspCas9-poli-Tetra-com-clcn5-sp-g1	Vector plasmid for expressing spCas9-poll fusion protein and sgRNA targeting human CLCN5 gene.	<p>The annealed product of hCLCN5-sp-g1-F (ACCGGAGGACAAGTCGTACAATGG) and hCLCN5-sp-g1-R (AAACCCATTGTACGACTTGTCTC) was inserted between the BbsI sites of pspCas9-POLI-Tetra-com vector by T4 DNA ligase.</p>
3	pspCas9-Klenow-Tetra-com-CLCN5-sp-g1	Plasmid for expressing spCas9-Klenow fusion protein and sgRNA targeting human CLCN5 gene.	<p>Use pspCas9-POLI-tetra-com-hCLCN5-sp-g1 as the template and Klenow-F (GTCTGCCACCCCTGAGGGAGGAGCACCAGAGGTGACAGCCAC) and Klenow-R (TTGTGCAGCACCTTAGGGTTCG) as the primers to amplify a 735 bp fragment. This DNA was inserted between the two Bsu36I sites of pspCas9-POLI-tetra-com-hCLCN5-sp-g1 by Infusion reaction.</p> <p>The end result is the removal of the DNA coding for the 5' exonuclease activity of E. coli POLI. Nucleic acid sequence:</p> <p>ATGGACTATAAGGACCACGACGGAGACTACAAGGATCATGATATT GATTACAAAGACGATGACGATAAGATGGCCCCAAAGAAGAAGCGG AAGGTCGGTATCCACGGAGTCCCAGCAGCCGACAAGAAGTACAG CATCGGCCTGGACATCGGCACCAACTCTGTGGGCTGGGCCGTGA TCACCGACGAGTACAAGGTGCCAGCAAGAAATCAAGGTGCTGG GCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGAGCC CTGCTGTTGACAGCGGCGAAACAGCCGAGGCCACCCGGCTGAA GAGAACCGCCAGAAGAAGATACACCAGACGGAAGAACCAGGATCT GCTATCTGCAAGAGATCTTCAGCAACGAGATGGCCAAAGGTGGACG ACAGCTTCTCCACAGACTGGAAGAGTCTTCTGTTGGAAGAGG ATAAGAAGCACGAGCGGCACCCCATCTTCGGCAACATCGTGGAC GAGGTGGCCTACCACGAGAAGTACCCACCATCTACCACCTGAGA AAGAACTGGTGGACAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGC CTATCTGGCCCTGGCCACATGATCAAGTTCGGGGCCACTGCT GATCGAGGGCGACCTGAACCCGACAACAGCGACGTGGACAAGC TGTTATCCAGCTGGTGCAGACCTACAACCAGCTGTTCCGAGGAAA ACCCCATCAACGCCAGCGGCGTGGACGCCAAGGCCATCCTGTCT GCCAGACTGAGCAAGAGCAGACGGCTGGAAAATCTGATCGCCCA GCTGCCCGGCGAGAAGAAGAATGGCCTGTTCCGAAACCTGATTG</p>

No.	Name	Purpose	Generation strategy
			<p>CCCTGAGCCTGGGCTGACCCCAACTTCAAGACAACTTCGACC TGGCCGAGGATGCCAACTGCAGCTGAGCAAGGACACCTACGAC GACGACCTGGACAACCTGCTGGCCAGATCGGCCAGCAGTACGC CGACCTGTTTCTGGCCGCAAGAACCCTGTCCGACGCCATCCTGCT GAGCGACATCCTGAGAGTGAACACCGAGATCACCAAGGCCCC TGAGCGCCTCTATGATCAAGAGATACGACGAGCACCACCAGGAC TGACCCTGCTGAAAGCTCTCGTGCGGCAGCAGCTGCCTGAGAAG TACAAAGAGATTTTCTCGACCAGAGCAAGAACGGCTACGCCGGC TACATTGACGGCGGAGCCAGCCAGGAAGGTTCTACAAGTTCATC AAGCCATCCTGAAAAGATGGACGGCACCGAGGAAGTCTCGT GAAGCTGAACAGAGAGGACCTGCTGCGGAAGCAGCGGACCTTCG ACAACGGCAGCATCCCCACCAGATCCACCTGGGAGAGCTGCAC GCCATTCTGCGGGCGGAGGAAGATTTTTACCCATTCTGAAGGAC AACCGGAAAAGATCGAGAAGATCCTGACCTCCGCATCCCCTAC TACGTGGGCCCTTGGCCAGGGGAAACAGCAGATTCCGCTGGAT GACCAGAAAGAGCGAGGAAACCATCACCCCTGGAAGTTCGAGG AAGTGGTGGACAAGGGCGCTTCCGCCAGAGCTTCCAGGAGCG ATGACCAACTTCGATAAGAACCTGCCAACGAGAAGGTGCTGCC AAGCACAGCCTGCTGTACGAGTACTTACCGTGTATAACGAGCTG ACCAAAGTGAATACGTGACCGAGGGAATGAGAAAGCCCGCTTC CTGAGCGGCGAGCAGAAAAAGGCCATCGTGGACCTGCTGTTCAA GACCAACCGGAAAAGTACCGTGAAGCAGCTGAAAGAGGACTACT CAAGAAAAATCGAGTGTTCGACTCCGTGGAATCTCCGGCGTGG AGATCGGTTCAACGCCTCCCTGGGCACATACCACGATCTGCTGAA AATTATCAAGGACAAGGACTTCTGGACAATGAGGAAAACGAGGA CATTCTGGAAGATATCGTGCTGACCTGACACTGTTTGAGGACAG AGAGATGATCGAGGAACGGCTGAAAACCTATGCCACCTGTTGGA CGACAAAGTGATGAAGCAGCTGAAGCGGGCGGAGATACACCGGT GGGCAGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAA GCAGTCCGGCAAGACAATCCTGGATTTCCTGAAGTCCGACGGCT CGCCAACAGAACTTCATGCAGCTGATCCACGACGACAGCCTGAC CTTTAAAGAGGACATCCAGAAAGCCAGGTGTCCGGCCAGGGCG ATAGCCTGCACGAGCACATTGCCAATCTGGCCGGCAGCCCCGCC ATTAAGAAGGGCATCCTGCAGACAGTGAAGGTGGTGGACAGGCT CGTGAAGTGATGGGCCGGCACAAGCCCCGAGAATCGTGATCG AAATGGCCAGAGAGAACCAGACCACCCAGAAGGGACAGAAGAAC AGCCGCGAGAGAATGAAGCGGATCGAAGAGGGGCATCAAAGAGCT GGCAGCCAGATCCTGAAAGAACACCCCGTGGAAAAACCCAGC TGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAATGGGCCG GATATGTACGTGGACCAGGAAGTGGACATCAACCGGTGTCCGAC TACGATGTGGACCATATCGTGCCCTCAGAGCTTTCTGAAGGACGAC TCCATCGACAACAAGGTGCTGACCAGAAGCGACAAGAACCAGG CAAGAGCGACAACCTGCCCCTCCGAAGAGGTCTGGAAGAAGTGA AGAACTACTGGCGGACGCTGCTGAACGCCAAGCTGATTACCCAGA GAAAGTTCGACAATCTGACCAAGGCCGAGAGAGGGCGGCTGAGC GAACTGGATAAAGCCGGCTTCATCAAGAGACAGCTGGTGGAAAC CCGGCAGATCACAAGCACGTGGCACAGATCCTGGACTCCCGGA TGAACACTAAGTACGACGAGAATGACAAGCTGATCCGGGAAGTGA AAGTGATCACCTGAAGTCCAAGCTGGTGTCCGATTTCCGGAAGG ATTTCCAGTTTTACAAAGTGCAGGAGATCAACAATACCACCACGC CCACGACGCTACCTGAACGCCGTGCTGGGAACCGCCCTGATCA AAAAGTACCCTAAGCTGGAAAGCGAGTTGCTGTACGGCGACTACA AGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAA ATCGGCAAGGCTACCGCAAGTACTTCTTCTACAGCAACATCATG AATTTTTCAAGACCGAGATTACCCTGGCCAACGGCGAGATCCGG AAGCGGCTCTGATCGAGACAAACGGCGAAACCGGGGAGATCGT GTGGGATAAGGGCCGGGATTTGCCACCGTGCAGAAAGTGTGA GCATGCCCAAGTGAATATCGTGAAGAAAGACCGAGGTGCAGACAG GCGGCTTCAGCAAAGAGTCTATCCTGCCAAGAGGAACAGCGATA AGCTGATCGCCAGAAAGAAGGACTGGGACCCTAAGAAGTACGGC GGCTTCGACAGCCCCACCGTGGCCTATTCTGTGCTGGTGGTGGC CAAAGTGGAAAAGGGCAAGTCCAAGAACTGAAGAGTGTGAAGA GCTGCTGGGGATCACCATCATGGAAGAAGCAGCTTCGAGAAGAA TCCCATCGACTTTCTGGAAGCCAAGGGCTACAAAGAAGTGAAGAA GGACCTGATCATCAAGCTGCCTAAGTACTCCCTGTTTCGAGCTGGA AAACGGCCGGAAGAGAATGCTGGCCTCTGCCGGCAACTGCAGA AGGGAAACGAACTGGCCCTGCCCTCCAAATATGTGAAGTTCCTGT ACCTGGCCAGCCACTATGAGAAGCTGAAGGGCTCCCCGAGGAT</p>

No.	Name	Purpose	Generation strategy
			<p>AATGAGCAGAAAACAGCTGTTTGTGGAACAGCACAAGCACTACCTG GACGAGATCATCGAGCAGATCAGCGAGTTCTCCAAGAGAGTGATC CTGGCCGACGCTAATCTGGACAAAGTGTGTCCGCCTACAACAAG CACCGGGATAAGCCCATCAGAGAGCAGGCCGAGAATATCATCCA CCTGTTTACCCTGACCAATCTGGGAGCCCCTGCCGCCTTCAAGTA CTTTGACACCACCATCGACCCGAAGAGGTACACCAGCACCAAAGA GGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCCGCCCTGT ACGAGACACGGATCGACCTGTCTCAGCTGGGAGGCCGACAAAAGG CCGGCGGCCACGAAAAAGGCCGGccaggcaaaaaagaaaaaggGATCC TCTGAGACACCAGGCACCTCCGAGTCTGCCACCCTGAGGGAGG AGCACCAGAGGTGACAGCCACCGTGTCTCCTACGATAACTATGT GACAATCCTGGACGAGGAGACACTGAAGGCCTGGATCGCCAAGC TGGAGAAGGCCCCCGTGTTCGCCTTGTACAGAGACAGACAGCC TGGATAACATCTCCGCCAATCTGGTGGGCCTGTCTTTCGCCATCG AGCCTGGCGTGGCCGCCTATATCCCAGTGGCCACGACTACCTG GATGCCCCCGACCAGATCAGCAGGGAGAGAGCCCTGGAGCTGTCT GAAGCTCTGTGGAGGATGAGAAGGCCCTGAAGTCCGGCCAGA ACCTGAAGTATGACAGGGGCATCCTGGCCAATTACGGCATCGAGC TGAGAGGCATCGCCTTGGACACCATGCTGGAGTCTTATATCCTGA ATAGCGTGGCAGGCCGGCACGACATGGATTCCTGGCCGAGAGG TGGCTGAAGCACAAAGACAATCACCTTCGAGGAGATCGCCGAA GGGCAAGAACCAGCTGACCTTCAACCAGATCGCCCTGGAGGAGG CAGGCAGGTACGCAGCAGAGGACGCAGATGTGACCCTGCAGCTG CACCTGAAGATGTGGCCAGATCTGCAGAAGCACAAGGGCCCCCT GAACGTCTTTGAGAATATCGAGATGCCCTGGTGCCTGTGCTGAG CCGGATCGAGCGCAACGGCGTGAAGATCGACCTAAGGTGCTGC ACAATCACTCCGAGGAGCTGACCCTGAGACTGGCCGAGCTGGAG AAGAAGGCCACGAGATCGCCGGCGAGGAGTTCAACCTGTCTCT TACAAAGCAGCTGCAGACCATCCTGTTTGAAGAAGCAGGCCATCA GCCCCGAAGAAAAACCCCTGGAGGAGCACCATCTACCAGCGAGG AGGTGCTGGAGGAGCTGGCCCTGGATTATCCCCTGCCTAAAGTGA TCCTGGAGTACCGGGGCCCTGGCCAAGCTGAAGTCTACATATACCG ACAAGCTGCCCTGATGATCAACCCTAAGACAGGCCGGGTGCACA CCAGCTACCACCAGGCAGTGACAGCAACCGGCCCGCTGAGCTCC ACCGATCCAAACCTGCAGAATATCCCCGTGAGGAATGAGGAGGG CAGGAGAATCAGACAGGCCTTCATCGCCCCGAGGATTATGTGAT CGTGTCCGCCGACTACTCTCAGATCGAGCTGAGGATCATGGCCCA CCTGTCCAGAGATAAAGGGCCTGCTGACAGCCTTCGCCGAGGGCA AGGACATCCACAGGGCAACCGCAGCAGAGGTGTTTGGCCTGCCT CTGGAGACAGTGACCTCCGAGCAGCGGCCTCTGCCAAGGCCAT CAACTTCGGCCTGATCTATGGCATGTCTGCCTTTGGCCTGGCCAG GCAGCTGAATATCCCTAGAAAGGAGGCCCAGAAGTACATGGACCT GTATTTTCGAGAGATAACCCCGCGTGTGGAGTACATGGAGAGGA CACGCGCACAGGCAAAGGAGCAGGGCTATGTGGAGACACTGGAT GGCAGGAGACTGTACCTGCCAGACATCAAGTCTAGCAACGGAGC AAGGAGGGCAGCAGCAGAGAGGGCCGCCATCAATGCCCCCATGC AGGGCAGCCCGGATATCATCAAGAGAGCCATGATCGCAGTG GACGCCTGGCTGCAGGCAGAGCAGCCAAGGGTGAAGAATGATCAT GCAGGTGCACGATGAGCTGGTGTGTTGAGGTGACAAGGACGATG TGGACGCCGTGGCCAAGCAGATCCACCAGCTGATGGAGAACTGT ACCCGCCTGGATGTGCCACTGCTGGTGAAGTGGGAAGCGGAGAG GAATTGGGACCAGGCCACTGAG. Peptide sequence: MDYKDHGDYKDHDIKDDDDKMAPKKRKGVIHGVPAADKKYSI GLDIGTNSVGVAVITDEYKVPSKFKVLGNTDRHSIKKNLIGALLFDS GETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRL ESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVSTDKADL RLIYLALAHMIKFRGHFLIEGDLNPDNSDVDFLFIQLVQTYNQLFEENPI NASGVDAKAILSARLSKSRLENLIAQLPGEKKNLFGNLIASLGLTP NFKSNFDLAEDAKLQLSKDTYDDDLNLLAQIGDQYADFLAAKNLSD AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYK EIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEILLVKNRE DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILTF RIPYVVGPLARGNSRFAMTRKSEETITPWNFEVVDDKGASAQSFIE RMTNFDKNLPNEKVLPHKSLLEYFTVYNELTKVKYVTEGMRKPAFL SGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFN ASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTFEDREMIERLKY AHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKS GFANRNFMLIHDDSLTFKEDIQKAQVSGQGDLSLHEHIANLAGSPAIK</p>

No.	Name	Purpose	Generation strategy
			<p>KGILQTVKVVDELVKVMGRHKPENIVEMARENQTTQKGQKNSRERM KRIEEGIKELGSQILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELD INRLSDYDVDHIVPQSFLKDDSIDNKVLRSDKNRKGSDNVPSEEVVK KMKNYWRQLLNAKLITQRKFDNLTKAERGGSELKAGFIKRLQVET RQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFY KVREINNYHHAHDAYLNAVVG TALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETG EIVWDKGRDFATVRKVL SMPQVNIKKTEVQTGGFSKESILPKRNSD KLIARKKDWDPKYGGFDSPTVAYSVLVAKVEKGSKLLKSVKELL GITIMERSSEKPNIDFLEAKGYKEVKDLIILPKYSLFEENGRKRM ASAGELQKGNELALPSKYVNFY LASHYEKLGSPEDNEQKQLFVEQ HKHYLDEIIEQISEFSKRVLADANL DKVLSAYNKHRDKPIREQAENIHL FTLNLGAPAAFKYFDTTIDRKRYTSTKEVLDTLHQSITGLYETRIDL SQLGGDKRPAATKKAGQAKKKKGSSETPGTSESATPEGGAPEVTAT VISYDNYVTILDEETLKAWIAKLEKAPVFAFDTE TDSLNDISANLVGLSF AIEPGVAAYIPVAHDYLDAPDQISRERALELLKPLLEDEKALKVGGQNLK YDRGILANYGIELRGIAFDTMLESYILNSVAGRHDMSLAERWLKHKTI TFEEIAGKGNQLTFNQIALEEAGRYAAEDADVTLQLHLKMWPDQK HKGPLNVFENIEMPLVPVLSRIERNGVKIDPKVLHNSHEELTLRLAELE KKAHEIAGEEFNLSSTKQLQTLFEKQGIKPLKKTGGAPSTSEEVLEE LALDYPLPKVILEYRGLAKLKSTYTDKPLMINPKTGRVTSYHQAVTA TGRLSSTDPNLQNI PVRNEEGRRIRQAFIAPEDYVIVSADYSQIELRIM AHLSRDKGLLTAFAEGKDIHRATAAEVGLPLET VTSEQRRSAKAINF GLIYGMSAFLARQLNIPRKEAQKYM DLYFERYPGVLEYMERTRAQA KEQGYVETLDGRRLYLPDIKSSNGARRAAAERAANAPMQGTAADIK RAMIAVDAWLQAEQPRVRMIMQVHDELVFEVHKDDVDVAVAKQHQL MENCTRLDVPLLVEVGS GENWDQAH (Color Key: Cas9-linker- Klenow)</p>
4	pspCas9-3exo-Tetra-com-CLCN5-sp-g1	Plasmid for expressing a fusion protein of spCas9 and the 3' exonuclease domain of POLI, and sgRNA targeting human CLCN5 gene.	<p>Use pspCas9-exo-tetra-com-hCLCN5-sp-g1 as the template and Klenow-F (GTCTGCCACCCCTGAGGGAGGAGCACCAGAGGTGACAGCCAC) and 3exo-R (CtaggaatttctagactaGTGCTTCTGCAGATCTGGCCAC) to amplify a 650 bp fragment. This fragment was inserted between the Bsu36I and XbaI sites of pspCas9-exo-tetra-com-hCLCN5-sp-g1 Infusion reaction. Nucleic acid sequence: ATGGACTATAAGGACCACGACGGAGACTACAAGGATCATGATATT GATTACAAAGACGATGACGATAAGATGGCCCCAAAGAAGAAGCGG AAGGTCGGTATCCACGGAGTCCCAGCAGCCGACAAGAAGTACAG CATCGGCCTGGACATCGGCACCAACTCTGTGGCTGGGCGTGA TCACCGACGAGTACAAGGTGCCAGCAAGAAATTCAAGGTGCTGG GCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGAGCC CTGCTGTTTCGACAGCGGCGAAACAGCCGAGGCCACCCGGCTGAA GAAACCGCCAGAAGAAGATACACCAGACGGAAGAACCGGATCT GCTATCTGCAAGAGATCTTCAAGCAACGAGATGGCCAAGGTGGAC ACAGCTTCTTCCACAGACTGGAAGAGTCTTCTGCTGGAAGAGG ATAAGAAGCACGAGCGGCACCCCATCTTCGGCAACATCGTGGAC GAGGTGGCCTACCACGAGAAGTACCCACCATCTACCACCTGAGA AAGAAACTGGTGGACAGCACCGACAAGGCCGACCTGCGCTGAT CTATCTGGCCCTGGCCACATGATCAAGTTCGGGGCCACTTCTCT GATCGAGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGC TGTTCAACAGCTGGTGCAGACCTACAACCAGCTGTTCCGAGGAAA ACCCCATCAAGCCAGCGGCGTGGACGCCAAGGCCATCTGTCT GCCAGACTGAGCAAGAGCAGACGGCTGGAAAATCTGATCGCCCA GCTGCCCGGCGAGAAGAAGATGGCCTGTTCCGAAACCTGATTG CCCTGAGCCTGGGCTGACCCCAACTTCAAGAGCAACTTGACC TGCCCGAGGATGCCAAACTGCAGCTGAGCAAGGACACTACGAC GACGACCTGGACAACCTGCTGGCCAGATCGGCGACCAAGTACGC CGACCTGTTTCTGGCCGCAAGAACCTGTCCGACGCCATCTGCT GAGCGACATCTGAGAGTGAACACCGAGATACCAAGGCCCCCC TGAGCGCTCTATGATCAAGAGATACGACGAGCACCACGAGGAC TGACCCTGCTGAAAGCTCTCGTGGCGCAGCAGCTGCCTGAGAAG TACAAAGAGATTTTCTTCGACCAGAGCAAGAACGGCTACGCCGGC TACATTGACGGCGGAGCCAGCCAGGAAGAGTTCTACAAGTTCATC</p>

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			<p>AAGCCCATCTGGAAAAGATGGACGGCACCAGGAAGTCTGCTGCT GAAGCTGAACAGAGAGGACCTGCTGCGGAAGCAGCGGACCTTCG ACAACGGCAGCATCCCCACCAGATCCACCTGGGAGAGCTGCAC GCCATTCTGCGGGCAGGAAGATTTTTACCCATTCTGAAAGGAC AACCGGAAAAGATCGAGAAGATCCTGACCTCCGCATCCCCTAC TACGTGGGCCCTCTGGCCAGGGGAAACAGCAGATTCGCTGGAT GACCAGAAAGAGCGAGGAAACCATCACCCCTGGAACCTCGAGG AAGTGGTGGACAAGGGCGCTTCCGCCAGAGCTTCATCGAGCGG ATGACCAACTTCGATAAGAACCTGCCAACGAGAAGGTGCTGCC AAGCACAGCCTGCTGTACGAGTACTTACCCTGTATAACGAGCTG ACCAAAGTGAATACGTGACCGAGGGAATGAGAAAGCCCCGCCTC CTGAGCGGCGAGCAGAAAAAGGCCATCGTGGACCTGCTGTTCAA GACCAACCGAAAAGTGACCGTGAAGCAGCTGAAAGGACTTACTT CAAGAAAATCGAGTCTCGACTCCGTGGAATCTCCGGCGTGGA AGATCGGTTCAACGCCTCCCTGGGCACATACCACGATCTGCTGAA AATTATCAAGGACAAGGACTTCTGGACAATGAGGAAAAACGAGGA CATTCTGGAAGATATCGTGTGACCTGACACTGTTTGGAGCAG AGAGATGATCGAGGAACGGCTGAAAACCTATGCCACCTGTTCGA CGACAAAGTGATGAAGCAGCTGAAGCGGGCGGAGATACACCGGCT GGGGCAGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAA GCAGTCCGGCAAGACAATCCTGGATTTCTGAAGTCCGACGGCTT CGCCAACAGAACTTCATGCAGCTGATCCACGACGACAGCCTGAC CTTTAAAGAGGACATCCAGAAAGCCAGGTGTCCGGCCAGGGCG ATAGCCTGCACGAGCACATTGCCAATCTGGCCGGCAGCCCGCC ATTAAGAAGGGCATCCTGACAGCAGTGAAGGTGGTGAGCAGCT CGTGAAGTGATGGCCCGGCACAAGCCCGAGAATCGTGAATCG AAATGGCCAGAGAGAACCAGACCACCCAGAAGGGACAGAAGAAC AGCCGCGAGAGAATGAAGCGGATCGAAGAGGGGCATCAAGAGCT GGCAGCCAGATCTGAAAGAACACCCCGTGGAAAAACCCACGC TGCAGAACGAGAAGCTGTACTGTACTACTGCAGAATGGGCGG GATATGTACGTGGACCAGGAAGTGGACATCAACCGGCTGTCCGAC TACGATGTGGACCATATCGTGCCTCAGAGCTTTCTGAAGGACGAC TCCATCGACAACAAGGTGCTGACCAGAAGCGACAAGAACCAGGGG CAAGAGCGACAACCTGCCCTCCGAAGAGGTCTGAAGTCCGACGTA AGAATACTGGCGCAGCTGCTGAACGCCAAGCTGATTACCCAGA GAAAGTTCGACAATCTGACCAAGGCCGAGAGAGGGCGGCTGAGC GAACTGGATAAAGCCGGCTTCATCAAGAGACAGCTGGTGGAAAC CCGGCAGATCACAAGCACGTGGCACAGATCCTGGACTCCCGGA TGAACACTAAGTACGACGAGAATGACAAGCTGATCCGGGAAGTGA AAGTATCACCTGAAGTCCAAGCTGGTGTCCGATTTCCGGAAGG ATTTCCAGTTTTACAAAGTGCAGGAGATCAACAATACCACCACGC CCACGACCCCTACCTGAACGCCGTCTGGGAACCCCTGATCA AAAAGTACCCCTAAGCTGGAAGCGAGTTCTGTACGGCGACTACA AGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAA ATCGGCAAGGCTACCGCCAAGTACTTCTTCTACAGCAACATCATG AACTTTTTCAAGACCGAGATTACCCTGGCCAACGGCGAGATCCGG AAGCGCCTCTGATCGAGACAACGGCGAAACCCGGAGATCGT GTGGGATAAGGGCCGGGATTTTGCACCGTGGGAAAAGTGTGA GCATGCCCAAGTGAATATCGTGAAGAACCGAGGTGCAGACAG CGGGCTTCAGCAAAGAGTCTATCCTGCCCAAGAGGAACAGCGATA AGCTGATCGCCAGAAAGAAGGACTGGGACCCTAAGAAGTACGGC GGCTTCGACAGCCCCACCGTGGCCTATTCTGTGCTGGTGGTGGC CAAAGTGGAAAAGGGCAAGTCCAAGAACTGAAGAGTGTGAAGA GCTGCTGGGGATCACCATCATGAAAGAAGCAGCTTCGAGAAGAA TCCCATCGACTTTCTGGAAGCCAAGGGCTACAAAGAAGTGA GGACCTGATCATCAAGCTGCCTAAGTACTCCCTGTTGAGCTGGA AAACGGCCGGAAGAGAATGCTGGCCTCTGCCGGCGAACTGCAGA AGGGAAACGAACTGGCCCTGCCCTCCAAATATGTGAATTCCTGT ACCTGGCCAGCCACTATGAGAAGCTGAAGGGCTCCCCGAGGAT AATGAGCAGAAACAGCTGTTTGTGGAACAGCACAAGCACTACTG GACGAGATCATCGAGCAGATCAGCGAGTTCTCCAAGAGAGTGATC CTGGCCGACGCTAATCTGGACAAAGTGTGTCCGCCTACAACAAG CACCGGGATAAGCCATCAGAGAGCAGGCCGAGAATATCATCCA CCTGTTTTACCTGACCAATCTGGGAGCCCTGCCGCTTCAAGTA CTTTGACACCACCATCGACCGGAAGAGGTACACCAGCACCAAGA GGTGTGACGCGCCACCCTGATCCACCAGAGCATCACCGGCTGT ACGAGACACGGATCGACCTGTCTCAGCTGGGAGGCGACAAAAGG CCGGCGCCACGAAAAAGGCCGGccaggcaaaaagaaaaggGATCC</p>

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			<p>TCTGAGACACCAGGCACCTCCGAGTCTGCCACCCTGAGGGAGG AGCACCAGAGGTGACAGCCACCGTATCTCCTACGATAACTATGT GACAATCCTGGACGAGGAGACACTGAAGGCCTGGATCGCCAAGC TGGAGAAGGCCCCCGTGTTCGCCTTTGATACAGAGACAGACAGCC TGGATAACATCTCCGCCAATCTGGTGGGCTGTCTTTCGCCATCG AGCCTGGCGTGGCCGCTATATCCCAGTGGCCACGACTACCTG GATGCCCCCGACCAGATCAGCAGGGAGAGGCCCTGGAGCTGCT GAAGCCTCTGCTGGAGGATGAGAAGGCCCTGAAGGTCGGCCAGA ACCTGAAGTATGACAGGGGCATCCTGGCCAATTACGGCATCGAGC TGAGAGGCATCGCCTTTGACACCATGCTGGAGTCTTATATCCTGA ATAGCGTGGCAGGCCGGCAGACATGGATCCCTGGCCGAGAGG TGGCTGAAGCACAAAGACAATCACCTTCGAGGAGATCGCCGAGAA GGGCAAGAACCAGCTGACCTTCAACCAGATCGCCCTGGGGAGG CAGGCAGGTACGCAGCAGAGGACGCAGATGTGACCCTGCAGCTG CACCTGAAGATGTGGCCAGATCTGCAGAAGCActag. Peptide sequence: MDYKDHDGDYKDHDIDYKDDDDKMAPKKRKRKVGIHGVAADKKYSI GLDIGTNSVGVAVITDEYKVPSSKFKVLGNTDRHSIKNLIGALLFDS GETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRL ESFLVEEDKKHERHPFGNIVDEVAYHEKYPTIYHLRKKLVSTDKADL RLIYLALAHMIKFRGHFLIEGDLNPDNSDVDFLQIQLVQTYNQLFEENPI NASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFNLIALSLGLTP NFKSNFDLAEDAKQLSKDYYDDDLNLLAQIGDQYADLFLAAKNLSD AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYK EIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKNRE DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPLKDNREKIEKILTF RIPYYVGPLARGNSRFAMTRKSEETITPWNFEVVDPKASQSFIE RMTNFDKNLPNEKVLPHSLLYEYFTVYNELTKVKYVTEGMRKPAFL SGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFN ASLGTYHDLLKIKDKDFLDNEENEDILEDIVLTLTFEDREMIERLKY AHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKS GFANRNFMLIHDDSLTFKEDIQKAQVSGQGDLSLHEHIANLAGSPA KIGILQTVKVVDELVKVMGRHKPENIVEMARENQTTQKQKNSRERM KRIEEGIKELGSQILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELD INRLSDYDVDHIVPQSFLKDDSIDNKVLRSDKNRGKSDNVPSEVVK KMKNYWRQLLNAKLITQRKFDNLTKAERGGSELKAGFIKQRLVET RQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFY KVREINNYHHADAYLNAVVGTAIKKYPKLESEFVYGDYKYVDVRK MIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRLIETNGETG EIVWDKGRDFATVRKVLSPQVNIKKTEVQTGGFSKESILPKRNSD KLIARKKDWDPKYGDFDPTVAYSVLVAVKVEKGSKLLKSVKELL GITIMERSSEKPNIDFLEAKGYKEVKKDLIILPKYSLFELENRGRKML ASAGELQKGNELALPSKYVNFLYLASHYEKLGSPEDNEQKQLFVEQ HKHYLDEIIEQISEFSKRVLADANLDKVL SAYNKHRDKPIREQAENIHL FTLNLGAPAAFKYFDTTIDRKRYTSTKEVLDTLHQSIITGLYETRIDL SQLGGDKRPAATKKAGQAKKKKGSSETPGTSESATPEGGAPEVTAT VISYDNYVTILDEETLKAWIAKLEKAPVFAFDTEITDSDLNISANLGLSF AIEPGVAAYIPVAHDYLDAPDQISRERALELLKPLLEDEKALKVGNL YDRGILANYGIELRGIAFDTMLESYILNSVAGRHDMDSLAERWLKHKTI TFEEIAGKGNQLTFNQIALEEAGRYAAEDADVTLQLHLKMWPDLQK H(Color Key: Cas9-linker-3' exo)</p>
5	pspCas9-Exo-tetra-com-hCLCN5-sp-g1	Plasmid for expressing a fusion protein of spCas9 and the 5' and 3' exonuclease domain of POLI, and sgRNA targeting human CLCN5 gene.	<p>Cut pspCas9-POLI-tetra-com-hCLCN5-sp-g1 with Accl+NheI to remove the DNA coding for the DNA polymerase activity. Treat the DNA with the Klenow fragment of DNA POLI to make blunt ends. Then use T4 DNA ligase to ligate the ends.</p> <p>Nucleic acid sequence: ATGGACTATAAGGACCACGACGGAGACTACAAGGATCATGATATT GATTACAAAGACGATGACGATAAGATGGCCCCAAAGAAGAAGCGG AAGGTCGGTATCCACGGAGTCCCAGCAGCCGACAAGAAGTACAG CATCGGCCTGGACATCGGCACCAACTCTGTGGGCTGGCCGCTGA TCACCGACGAGTACAAGGTGCCAGCAAGAAATTCAAGGTGCTGG GCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGAGCC CTGCTGTTTCGACAGCGGCGAAACAGCCGAGGCCACCCGGCTGAA GAGAACCGCCAGAAGAAGATACACCAGACGGAAGAACCAGCTCT GCTATCTGCAAGAGATCTTCAGCAACGAGATGGCCAAGGTGGACG ACAGCTTCTTCCACAGACTGGAAGAGTCTTCTGCTGGTGAAGAGG ATAAGAAGCACGAGCGGCACCCCATCTTCGGCAACATCGTGGAC GAGGTGGCCTACCACGAGAAGTACCCACCATCTACCACCTGAGA</p>

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			<p>AAGAACTGGTGGACAGCACCGACAAGGCCGACCTGCGGCTGAT CTATCTGGCCCTGGCCACATGATCAAGTTCCGGGGCCACTTCT GATCGAGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGC TGTTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCCGAGGAAA ACCCCATCAACGCCAGCGGCGTGGACGCCAAGGCCATCTGTCT GCCAGACTGAGCAAGAGCAGACGGCTGAAAAATCTGATCGCCCA GCTGCCCGGCGAGAAGAAGAATGGCCTGTTCCGAAACCTGATTG CCCTGAGCCTGGGCCTGACCCCAACTTCAAGAGCAACTTCGACC TGGCCGAGGATGCCAAACTGCAGCTGAGCAAGGACACCTACGAC GACGACCTGGACAACCTGCTGGCCAGATCGGCGACCAGTACGC CGACCTGTTTCTGGCCGCCAAGAACCTGTCCGACGCCATCTGCT GAGCGACATCCTGAGAGTGAACACCGAGATCACAAGGCCCCCC TGAGCGCCTCTATGATCAAGAGATACGACGAGCAACACCGAGGAC TGACCCTGCTGAAAGCTCTCGTGCGGCAGCAGCTGCCTGAGAAG TACAAAGAGATTTTCTTCGACCAGAGCAAGAACGGCTACGCCGGC TACATTGACGGCGGAGCCAGCCAGGAAGAGTTTACAAGTTCATC AAGCCATCCTGAAAAAGATGGACGGCACCGAGGAACCTCGT GAAGCTGAACAGAGAGGACCTGCTGCGGAAGCAGCGGACCTTCG ACAACGGCAGCATCCCCACCAGATCCACCTGGGAGAGCTGCAC GCCATTCTGCGGCGGAGGAAGATTTTTACCCATTCTGAAGGAC AACCGGAAAAAGATCGAGAAGATCCTGACCTTCCGATCCCCCTAC TACGTGGGCCCTCTGGCCAGGGGAAAACAGCAGATTGCGCTGGAT GACCAGAAAGAGCGAGGAAACCATCACCCCTGGAACCTTCGAGG AAGTGGTGGACAAGGGCGCTTCCGCCAGAGCTTCATCGAGCGG ATGACCAACTTCGATAAGAACCTGCCAACGAGAAGTGTGCC AAGCACAGCCTGCTGTACGAGTACTTCAACCGTGTATAACCGCTG ACCAAAGTAAATACGTGACCGAGGGAATGAGAAAGCCCGCCTTC CTGAGCGGGCAGCAGAAAAAGGCCATCGTGACCTGCTGTTCAA GACCAACCGGAAAGTGACCGTGAAGCAGTGAAGAGGACTACTT CAAGAAAAATCGAGTGCTTCCGACTCCGTGGAATCTCCGGCTGGA AGATCGGTTCAACGCCTCCCTGGGCACATACCACGATCTGCTGAA AATTATCAAGGACAAGGACTTCTGACAAATGAGGAAAAACGAGGA CATTCTGGAAGATATCGTGCTGACCCTGACACTGTTTGAGGACAG AGAGATGATCGAGGAACGGCTGAAAACCTATGCCACCTGTTTCGA CGACAAAGTGATGAAGCAGCTGAAGCGGGCGGAGATACACCGGCT GGGGCAGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAA GCAGTCCGGCAAGACAATCCTGGATTTCTGAAGTCCGACGGCTT CGCCAACAGAACTTTCATGCAGCTGATCCACGACGACAGCCTGAC CTTTAAAGAGGACATCCAGAAAGCCAGGTGTCCGGCCAGGGCG ATAGCCTGCACGACATTGCCAATCTGGCCGGCAGCCCGCC ATTAAGAAAGGCATCCTGCAGACAGTGAAGGTGGTGGACGAGCT CGTGAAGTGATGGGCCGCAAGCCCGAGAACATCGTATCG AAATGGCCAGAGAGAACCAGACCACCCAGAAGGGACAGAAAC AGCCGCGAGAGAATGAAGCGGATCGAAGAGGGCATCAAAGAGCT GGGCAGCCAGATCCTGAAAGAACACCCCGTGGAAAAACACCCAGC TGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAATGGGCGG GATATGTACGTGGACCAGGAAGTGGACATCAACCGGCTGTCCGAC TACGATGTGGACCATATCGTGCCCTCAGAGCTTTCTGAAGGACGAC TCCATCGACAACAAGGTGCTGACCAGAAGCGACAAGAACCAGGGG CAAGAGCGACAACGTGCCCTCCGAAGAGGTCTGGAAGAAGATGA AGAACTACTGGCGGACGCTGCTGAACGCCAAGCTGATTACCCAGA GAAAGTTCGACAATCTGACCAAGGCCGAGAGAGGGCGGCTGAGC GAACTGGATAAGGCCGGCTTTCATCAAGAGACAGCTGGTGGAAAC CCGGCAGATCACAAGCAGTGGCACAGATCCTGGACTCCCGGA TGAACACTAAGTACGACGAGAATGACAAGCTGATCCGGGAAGTGA AAGTGATCACCTGAAGTCCAAGCTGGTGTCCGATTTCCGGAAGG ATTTCCAGTTTTACAAAGTGCAGGAGATCAACAACCTACCACCAGC CCACGACGCCTACCTGAACGCCGTCTGGGAACCGCCCTGATCA AAAAGTACCCTAAGCTGGAAGCGAGTTCTGTACGGCGACTACA AGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGGAGGAGAA ATCGGCAAGGCTACCGCAAGTACTTCTTCTACAGCAACATCATG AACTTTTTCAAGACCGAGATTACCCTGGCCAACGGCGAGATCCGG AAGCGGCCTCTGATCGAGACAAACGGCGAAACCGGGGAGATCGT GTGGGATAAGGGCCGGGATTTTGCACCGTGCAGAAAGTGTGA GCATGCCCAAGTGAATATCGTGA AAAAGACCGAGGTGCAGACAG GCGGCTTCAGCAAAGAGTCTATCCTGCCAAGAGGAACAGCGATA AGCTGATCGCCAGAAAGAGGACTGGGACCCTAAGAAGTACGGC GGCTTCGACAGCCCCACCGTGGCCTATTCTGTGCTGGTGGTGGC</p>

No.	Name	Purpose	Generation strategy
			<p>CAAAGTGGAAAAGGGCAAGTCCAAGAACTGAAGAGTGTGAAAGA GCTGCTGGGGATCACCATCATGGAAAGAAGCAGCTTCGAGAAGAA TCCCATCGACTTTCTGGAAGCCAAGGGCTACAAAGAAGTGAAAAA GGACCTGATCATCAAGCTGCCTAAGTACTCCCTGTTCCGAGCTGGA AAACGGCCGGAAGAGAATGCTGGCCTCTGCCGGCGAACTGCAGA AGGGAAACGAACTGGCCCTGCCCTCCAAATATGTGAACTTCCTGT ACCTGGCCAGCCACTATGAGAAGCTGAAGGGCTCCCCCGAGGAT AATGAGCAGAAACAGCTGTTTTGTGGAACAGCACAAAGCACTACCTG GACGAGATCATCGAGCAGATCAGCGAGTTCTCCAAGAGAGTGATC CTGGCCGACGCTAATCTGGACAAAGTGTGTCCGCCTACAACAAG CACCGGGATAAGCCCATCAGAGAGCAGGGCCGAGAATATCATCCA CCTGTTTACCCTGACCAATCTGGGAGCCCTGCCGCCTTCAAGTA CTTTGACACCACCATCGACCGGAAGAGGTACACCAGCACCAGGAA GGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCGGCCTGT ACGAGACACGGATCGACCTGTCTCAGCTGGGAGGCCACAAAAGG CCGGCGGCCACGAAAAAGGCCGGccaggcaaaaaagaaaaaggGATCC TCTGAGACACCAGGCACCTCCGAGTCTGCCACCCCTGAGCGAGG AAGCGGAGGAAGCGGCTCCGTGCAGATCCCACAGAACCCCTGA TCCTGGTGGACGGCAGCTCCTACCTGTATCGGGCCTACCACGCCT TCCCACCTTGACAACTCCGCCGGAGAGCCAACCGGAGGCCATG TATGGCGTGTGAATATGCTGAGGAGCCTGATCATGAGTACAAG CCTACACACGCCGCCGTGGTGTGATGCCAAGGGCAAGACCTC CGCGACGAGCTGTTTGAGCACTACAAGAGCCACAGGCCACCAAT GCCTGACGATCTGAGGGCACAGATCGAGCCACTGCACGCAATGG TGAAGGCCATGGGCTGCCTCTGCTGGCCGTGAGCGGAGTGGAG GCCGACGATGTGATCGGCACACTGGCAAGGGAGGCAGAGAAGGC AGGCCGCCAGTGCTGATCTCCACCGGCCACAAGGATATGGCCC AGCTGGTGACACCAAACATCACCTGATCAACACCATGACAAATA CCATCCTGGGCCCCGAGGAGGTGGTGAATAAGTATGGCGTGCCT CCAGAGCTGATCATCGATTTCTGGCCCTGATGGCGACTTAGC GATAACATCCCTGGCGTGCCAGGAGTGGGAGAAAAAGCCGCACA GGCCCTGCTGCAGGGCCTGGGAGGCCTGGACACCCTGTACGCC GAGCCAGAGAAGATCGCCGGCCTGTCTTTAGGGGCGCCAAGAC AATGGCCGCCAAGCTGGAGCAGAATAAGGAGGTGGCCTACCTGT CTTATCAGCTGGCCACAATCAAGACCGAGCTGGAGCTGGAGCTGA CCTGCGAGCAGCTGGAGGTGACAGCAGCCTGCAGCAGAGGAGCTG CTGGGCCTGTTCAAGAAGTACGAGTTTAAAGAGATGGACAGCCGAT GTGGAGCCGGCAAGTGGCTGCAGGCAAGGGAGGCAGCAAGCCAG CAGCAAAGCCACAGGAGACAAGCGTGGCAGACGAGGCACCAGAG GTGACAGCCACCGTGTCTCCTACGATAACTATGTGACAATCCTG GACGAGGAGACACTGAAGGCCTGGATCGCCAAGCTGGAGAAGGC CCCCGTGTTGCCTTTGATACAGAGACAGACAGCCTGGATAACAT CTCCGCCAATCTGGTGGCCTGTCTTTGCCATCGAGCTGGCCTGGC GGCCGCTATATCCCAGTGGCCACGACTACCTGGATGCCCCCG ACCAGATCAGCAGGGAGAGAGCCCTGGAGCTGCTGAAGCCTCTG CTGGAGGATGAGAAGGCCCTGAAGGTGGCCAGAACCCTGAAGTA TGACAGGGGCACTCCTGGCCAATTACGGCATCGAGCTGAGAGGCA TCGCCTTTGACACCATGCTGGAGTCTTATATCCTGAATAGCGTGG CAGGCCGGCACGACATGGATTCCCTGGCCGAGAGGTGGCTGAAG CACAAGACAATCACCTTCGAGGAGATCGCCGGCAAGGGCAAGAA CCAGCTGACCTCAACCAGATCGCCCTGGAGGAGGCAGGCAGGT ACGACGAGAGGACGCAGATGTGACCCTGCAGCTGCACCTGAAG ATGTGGCCAGATCTGCAGAAGCACAAGGGCCCCCTGAACGTCTTT GAGAATATCGAGATGCCCTGGTGCCTGTGCTGAGCCGGATCGA CGCAACGGCGTGAAGATCGACCCTAAGGTGCTGCACAATCACTC CGAGGAGCTGACCCTGAGACTGGCCGAGCTGGAGAAGAAGGCCCC ACGAGATCGCCGGCAGGAGTTCAACCTGTCTCTACAAAGCAGC TGCAGACCATCCTGTTTGAGAAGCAGGGCATCAAGCCCCTGAAGA AAACCCCTGGAGGAGCACCATCTACCAGCGAGGAGGTGCTGGAG GAGCTGGCCCTGGATTATCCCCTGCCATAAAGTATCCTGGAGTAC CGGGGCTGGCCAAGCTGAAGTCTctagctcgctttctgctccaattctattaa aggttcctttgtccctaagtccaactactaa. Peptide sequence: MDYKDHGDYKDHIDYKDDDDKMAPKKRKRKVIHGVPAADKKYSI GLDIGTNSVGWAVITDEYKVPKFKVLGNTDRHSIKKLNIGALLFDS GETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSSFFHRL ESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADL RLIYLALAHMIKFRGHFLIEGDLNPDNSDVKLFIQLVQTYNQLFEENPI NASGVDAKAILSARLSKSRRLLENLIAQLPGEKKNGLFGLIALSLGLTP</p>

No.	Name	Purpose	Generation strategy
			<p>NFKSNFDLAEDAKLQLSKDYYDDDLNLLAQIGDQYADLFLAAKNLSD AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYK EIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKNLRE DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILTF RIPYYVGLPARGNSRFAMTRKSEETITPWNFEEVVDKGASQAQSFIE RMTNFDKKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFL SGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFN ASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIERLKY AHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSD GFANRNFMLIHDDSLTFKEDIQKAQVSGQGDLSLHEHIANLAGSPAIK KGILQTVKVVDELVKVMGRHKPENIVIAMARENQTTQKGQKNSRERM KRIEEGIKELGSQILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELD INRLSDYDVDHIVPQSFLKDDSIDNKVLRSDKNRKGSDNVPSEEVVK KMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVET RQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFY KVREINNYHHAHDAYLNAVVGALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRLIETNGETG EIVWDKGRDFATVRKVLSPQVNVKKEVQTTGGFSKESILPKRNSD KLIARKKDWDPKKGFFDSPTVAYSVLVAVKVEKGSKLLKSVKELL GITIMERSSEFEKNPIDFLEAKGYKEVKDLIIKPKYSLFELENRGRKML ASAGELQKGNELALPSKYVNFLYLASHYEKLGSPEDNEQKQLFVEQ HKHYLDEIIEQISEFSKRVLADANLKVLSAYNKHDRDKPIREQAENIHL FTLTLNLGAPAAFKYFDTTIDRKRYTSTKEVLDTLIHQSIITGLYETRIDL SQLGGDKRPAATKKAGQAKKKKGSSETPGTSESATPEGGSGGSGS VQIQPNLILVDGSSYLYRAYHAFPPLTNSAGEPTGAMYGLVNLMLRSL IMQYKPTHAAVVFDAGKTFRDELFEHYKSHRPPMPDDLRAENLPLH AMVKAMGLPLLAVSGVEADDVIGTLAREAEKAGRPVLISTGDKDMAQ LVTPNITLINTMTNITLGPPEEVNKYGVPELIIDFLALMGDSSDNIPGV PGVGEKTAQALLQGLGLDLYAEPEKIAGLSFRGAKTMAAKLEQNK EVAYLSYQLATIKTDVELELTCEQLLEVQQPAAEELLGLFKKYEFRWT ADVEAGKWLQAKGAKPAKPKQETSVADEAPEVTATVISYDNYVTILD EETLKAWIAKLEKAPVFAFDTETDSLNDNISANLVGLSFAIEPGVAAYIPV AHDYLDAPDQISRERALELLKPLLEDEKALKVGNLKYDRGILANYGIE LRGIAFDTMLS YILNSVAGRHDMSLAERWLKHKITTFEEIAGKGN QLTFNQIALEEAGRYAAEDADVTLQLHLKMWPDLQKHKGPLNVFENI EMPLVPVLSRIERNVGVKIDPKVLHNSSEELTLRLAELEKKAHEIAGEEF NLSSTKQLQTLFEKQGIKPLKKTGGAPSTSEEVLLEALDYPLPKVIL EYRGLAKLKSARFLAVQFLKVPKPKSNY (Color Key: Cas9-linker- exo)</p>
6	pspCas9-5Exo-tetra-com-hCLCN5-sp-g1	Plasmid for expressing a fusion protein of spCas9 and the 5' exonuclease domain of POLI, and sgRNA targeting human CLCN5 gene.	<p>Cut pspCas9-POLI-tetra-com-hCLCN5-sp-g1 with ClaI+NheI to remove majority of the POLI coding DNA. Then use 5Exo-F (CAGAGCTGATCATCGATTTCCTGG) and 5Exo-R (AagaagcgagctagccCACGGTGGCTGTCACCTCTGG) to amplify a 400 bp band coding for the 5' exonuclease domain from pspCas9-POLI-tetra-com-hCLCN5-sp-g1. This DNA was inserted into the ClaI+NheI linearized vector by infusion reaction.</p> <p>Nucleic acid sequence: ATGGACTATAAGGACCACGACGGAGACTACAAGGATCATGATATT GATTACAAAGACGATGACGATAAGATGGCCCCAAAGAAGAAGCGG AAGGTCGGTATCCACGGAGTCCCAGCAGCCGACAAGAAGTACAG CATCGGCCTGGACATCGGCACCAACTCTGTGGGCTGGGCCGTGA TCACCGACGAGTACAAGGTGCCAGCAAGAAATTCAAGGTGCTGG GCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGAGCC CTGCTGTTGACAGCGGCGAAACAGCCGAGGCCACCCGGCTGAA GAGAACC GCCAGAAGAAGATACACCAGACGGAAGAACCGGATCT GCTATCTGCAAGAGATCTTCAAGCAACGAGATGGCCAAGGTGGACG ACAGCTTCTCCACAGACTGGAAGAGTCTTCTCGTGGGAAGAGG ATAAGAAGCACGAGCGGCACCCCATCTTCGGCAACATCGTGGAC GAGGTGGCCTACCACGAGAAGTACCCACCATCTACCACCTGAGA AAGAACTGGTGACAGCACCGACAAGGCCGACCTGCGGCTGAT CTATCTGGCCCTGGCCACATGATCAAGTTCGGGGCCCACTTCT GATCGAGGGCGACCTGAACCCCGACAACAGCGACCTGGACAAGC TGTTCCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCCGAGGAAA</p>

No.	Name	Purpose	Generation strategy
			<p> ACCCCATCAACGCCAGCGGCGTGGACGCCAAGGCCATCTGTCT GCCAGACTGAGCAAAGCAGACGGCTGGAAAATCTGATCGCCCA GCTGCCCGGCGAGAAGAAGAATGGCCTGTTCCGAAAACCTGATTG CCCTGAGCCTGGGCCTGACCCCAACTTCAAGAGCAACTTCGACC TGGCCGAGGATGCCAAACTGCAGCTGAGCAAGGACACCTACGAC GACGACCTGGACAACTGCTGGCCAGATCGGCGACCACTACGC CGACCTGTTTCTGGCCGCAAGAACCTGTCCGACGCCATCTGTCT GAGCGACATCTGAGAGTGAACACCGAGATCACCAAGGCCCCCC TGAGCGCCTCTATGATCAAGAGATACGACGAGCACCACCAGGACC TGACCCTGCTGAAAGCTCTCGTGCGGCAGCAGCTGCCTGAGAAG TACAAAGAGATTTTCTTCGACCAGAGCAAGAACGGCTACGCCGGC TACATTGACGGCGGAGCCAGCCAGGAAGAGTTTACAAGTTTCATC AAGCCCATCCTGGAAAAGATGGACGGCACCGAGGACCTCCCTGT GAAGCTGAACAGAGAGGACCTGCTGCGGAAGCAGCGGACCTTCG ACAACGGCAGCATCCCCACCAAGATCCACCTGGGAGAGCTGCAC GCCATTCTGCGGCGGCAGGAAGATTTTTACCCATTCTGAAGGAC AACCGGAAAAAGATCGAGAAGATCCTGACCTTCGCTCCCTAC TACGTGGGCCCTCTGGCCAGGGGAAACAGCAGATTCGCTGGAT GACCAGAAAGAGCGAGGAAACCATCACCCCTGGAACTTCGAGG AAGTGGTGGACAAGGGCGCTTCCGCCAGAGCTTCATCGAGCGG ATGACCAACTTCGATAAGAACCTGCCAACGAGAAGGTGCTGCC AAGCACAGCCTGCTGTACGAGTACTTACCGTGTATAACGAGCTG ACCAAAGTGAATACGTGACCGAGGGAATGAGAAAGCCCGCCTTC CTGAGCGGCGAGCAGAAAAAGGCCATCGTGGACCTGCTGTTCAA GACCAACCGGAAAGTGACCGTGAAGCAGCTGAAAGAGGACTACTT CAAGAAAAATCGAGTGCTTCCGACTCCGTGGAAATCTCCGGCTGGA AGATCGGTTCAAGCCTCCCTGGGCACATACCACGATCTGCTGAA AATTATCAAGGACAAGGACTTCTGGACAATGAGGAAAAACGAGGA CATTCTGGAAGATATCGTGCTGACCCTGACACTGTTTGAGGACAG AGAGATGATCGAGGAACGGCTGAAAACCTATGCCACCTGTTTGA CGACAAAGTGATGAAGCAGCTGAAGCGGCGGAGATACACCGGCT GGGGCAGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAA GCAGTCCGGCAAGACAATCCTGGATTTCTGAAGTCCGACGGCTT CGCCAACAGAAAACCTTCATGCAGCTGATCCACGACGACAGCTGAC CTTTAAAGAGGACATCCAGAAAGCCAGGTGTCCGGCCAGGGCG ATAGCCTGCACGACACATTGCCAATCTGGCCGGCAGCCCGCC ATTAAGAAGGGCATCCTGCAGACAGTGAAGGTGGTGGACGAGCT CGTGAAGTGATGGGCGGCGACAAGCCGAGAACATCGTGATCG AAATGGCCAGAGAGAACCAGACCACCCAGAAGGGACAGAAGAAC AGCCGCGAGAGAATGAAGCGGATCGAAGAGGGCATCAAAGAGCT GGGCAGCCAGATCCTGAAAGAACACCCCGTGGAAAAACACCCAGC TGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAATGGGCG GATATGTACGTGGACCAGGAACCTGGACATCAACCGGCTGTCCGAC TACGATGTGGACCATATCGTGCCTCAGAGCTTTCTGAAGGACGAC TCCATCGACAACAAGGTGCTGACCAGAAGCGACAAGAACCAGGGG CAAGAGCGACAACGTGCCCTCCGAAGAGGTCTGAAAGAAGATGA AGAACTACTGGCGGACGCTGCTGAACGCCAAGCTGATTACCCGAA GAAAGTTCGACAATCTGACCAAGGCCGAGAGAGGGCGGCTGAGC GAACTGGATAAGGCCGGCTTTCATCAAGAGACAGCTGGTGGAAAC CCGGCAGATCACAAGACAGTGGCACAGATCCTGGACTCCCGGA TGAACACTAAGTACGACGAGAATGACAAGCTGATCCGGGAAGTGA AAGTGATCACCTGAAGTCCAAGCTGGTGTCCGATTTCCGGAAGG ATTTCCAGTTTTACAAAGTGCAGGAGATCAACAACCTACCACCAGC CCACGACGCCTACCTGAACGCCGTCTGTGGGAACCGCCCTGATCA AAAAGTACCCTAAGCTGGAAAGCGAGTTCGTGTACGGCGACTACA AGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAA ATCGGCAAGGCTACCGCAAGTACTTCTTCTACAGCAACATCATG AACTTTTTCAAGACCGAGATTACCCTGGCCAACGGCGAGATCCGG AAGCGGCTCTGATCGAGACAAACGGCGAAACCGGGGAGATCGT GTGGGATAAGGGCCGGGATTTTTGCCACCGTGCAGGAAAGTGTGA GCATGCCCAAGTGAATATCGTAAAAAGACCGAGGTGCAGACAG GCGGCTTCAGCAAAGAGTCTATCCTGCCAAAGAGGAACAGCGATA AGCTGATCGCCAGAAAGAGGACTGGGACCCCTAAGAAGTACGGC GGCTTCGACAGCCCAACCGTGGCCTATTCTGTGCTGGTGGGCG CAAAGTGGAAAAAGGCAAGTCCAAGAACTGAAGAGTGTGAAAGA GCTGCTGGGGATCACCATCATGGAAAGAGCAGCTTCGAGAAGAA TCCCATCGACTTTCTGGAAGCCAAGGGCTACAAAGAAGTGAAAA GGACCTGATCATCAAGCTGCCTAAGTACTCCCTGTTCCGAGCTGGA </p>

No.	Name	Purpose	Generation strategy
			<p>AAACGGCCGGAAGAGAATGCTGGCCTCTGCCGGCGAACTGCAGA AGGGAAACGAACTGGCCCTGCCCTCCAAATATGTGAACCTCCTGT ACCTGGCCAGCCACTATGAGAAGCTGAAGGGCTCCCCGAGGAT AATGAGCAGAAACAGCTGTTTGTGGAACAGCACAAGCACTACCTG GACGAGATCATCGAGCAGATCAGCGAGTTCTCCAAGAGAGTGATC CTGGCCGACGCTAATCTGGACAAAGTGTGTCCGCCTACAACAAG CACCGGGATAAGCCCATCAGAGAGCAGGCCGAGAATATCATCCA CCTGTTTACCCTGACCAATCTGGGAGCCCCTGCCGCCTTCAAGTA CTTTGACACCACCATCGACCGGAAGAGGTACACCAGCACCAAGA GGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCGGCCTGT ACGAGACACGGATCGACCTGTCTCAGCTGGGAGGCACAAAAGG CCGGCGGCCACGAAAAAGGCCGgccaggcaaaaaagaaaaggGATCC TCTGAGACACCAGGCACCTCCGAGTCTGCCACCCCTGAGGGAGG AAGCGGAGGAAGCGGCTCCGTGCAGATCCCACAGAACCCCTGA TCCTGGTGGACGGCAGCTCCTACCTGTATCGGGCCTACCACGCCT TCCCACCTCTGACAACTCCGCCGGAGAGCCAACCGGAGCCATG TATGGCGTCTGAATATGCTGAGGAGCCTGATCATGACGAAG CCTACACACGCCCGCTGGTGTGGTGTGATGCCAAGGGCAAGACCTC CGCGACGAGCTGTTTGAAGCACTACAAGAGCCACAGGCCACCAAT GCCTGACGATCTGAGGGCACAGATCGAGCCACTGCACGCAATGG TGAAGGCATGGGCCTGCCTCTGCTGGCCGTGAGCGAGTGGAG GCCGACGATGTGATCGGCACACTGGCAAGGGAGGCAGAGAAGGC AGGCCGCCAGTGCTGATCTCCACCGGCCACAAGGATATGGCCC AGCTGGTGACACCAACATCACCCCTGATCAACACCATGACAAATA CCATCCTGGGCCCCGAGGAGGTGGTGAATAAGTATGGCTGCCT CCAGAGCTGATCATCGATTTCTGGCCCTGATGGGCGACTTAGC GATAACATCCCTGGCGTGCCAGGAGTGGGAGAAAAGACCGCACA GGCCCTGCTGCAGGGCCTGGGAGGCCTGGACACCCTGTACGCC GAGCCAGAGAAGATCGCCGGCCTGTCTTTAGGGGCGCCAAGAC AATGGCCGCAAGCTGGAGCAGAATAAGGAGGTGGCCTACCTGT CTTATCAGCTGGCCACAATCAAGACCGACGTGGAGCTGGAGCTGA CCTGCGAGCAGCTGGAGGTGCAGCAGCCTGCAGCAGAGGAGCTG CTGGGCCTGTTCAAGAAGTACGAGTTTAAGAGATGGACAGCCGAT GTGGAGCCGGCAAGTGGCTGCAGGCAAGGGAGCAAAAGCCAG CAGCAAAGCCACAGGAGACAAGCGTGGCAGACGAGGCACCAGAG GTGACAGCCACCGTgtag. Peptide sequence: MDYKDHGDYKDHIDYKDDDDKMAPKKRKRKVIHGVPAADKKYSI GLDIGTNSVGVAVITDEYKVPSSKFKVLGNDRHSIKKNLIGALLFDS GETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRL ESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKLVDSTDKADL RLIYLALAHMIKFRGHFLIEGDLNPDNSVDKLFQQLVQTYNQLFEENPI NASGVDAKAILSARLSKSRLENLIAQLPGEKKNLFGNLIALLSLGTP NFKSNFDLAEDAQLSKDYYDDLDNLLAQIGDQYADLFLAAKNLSD AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYK EIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKNLRE DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPLKDNREKIEKILTF RIPYYVGLPARGNSRFAMTRKSEETITPWNFEVVDKGASAQSFIE RMTNFDKPLPNEKVLPHSLLYEFVTVYNELTKVKYVTRMRKPAFL SGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFN ASLGTYHDLLKIKDKDFLDNEENEDILEDIVLTLTLFEDREMIERLKY AHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKLTILDFLKSD GFANRNFMLIHDDSLTFKEDIQKAQVSGGQDLSHEHIANLAGSPAIK KGILQTVKVVDELVKVMGRHKPENIVIAMARENQTTQKGQKNSRERM KRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELD INRLSDYDVDHIVPQSFLKDDSIDNKVLRSDKNRKGSDNVPSEEVVK KMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRLQVET RQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSXLVDFRKFQFY KVREINNYHHAHDAYLNAVVGTAALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRLIETNGETG EIVWDKGRDFATVRKVLSPQVNVKKEVQTTGGFSKESILPKRNSD KLIARKKDWDPKKGFFSPTVAYSVLVAKVEKKGSKKLKSVKELL GITIMERSSEKPNIDFLEAKGYKEVKKDLIILPKYSLFELENGRKRML ASAGELQKGNELALPSKYVNFLLASHYEKLGSPEDNEQKQLFVEQ HKHYLDEIIEQISEFSKRVLADANLDKVL SAYNKHDRDKPIREQAENIHL FTLNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSTGLYETRIIDL SQLGGDKRPAATKKAGQAKKKKGSSETPGTSESATPEGGSGSGS VQIQNPLILVDGSSYLYRAYHAFPPLTNSAGEPTGAMYGVNLMLRSL IMQYKPTHAAVVFDAKGKTRDELFEHYKSHRPPMPDDLRAQIEPLH</p>

No.	Name	Purpose	Generation strategy
			AMVKAMGLPLLAVSGVEADDVIGTLAREAEKAGRPVLISTGDKDMAQLVTPNITLINTMTNTILGPPEEVNKGVPPELIIDFLALMGDSSDNIPGVPVGGEKTAQALLQGLGGLDTLYAEPEKIAGLSFRGAKTMAAKLEQNKEVAYLSYQLATIKTDVELELTCEQLEVVQPPAAEELLGLFKKYEFKRWTADVEAGKWLQAKGAKPAAKPQETSVADEAPEVTATVG (Color Key: Cas9-linker-5' exo)
7	pspCas9-POLI-ST2-com-53-sp-g2	Vector plasmid for expressing spCas9-POLI fusion protein and sgRNA targeting human DMD exon 53.	The AflIII+Acc65I fragment of pspCas9-3'UTR-ST2-com-52-sp-g2 was used to replace that of pspCas9-POLI-tetra-com-hCLCN5-sp-g1 by restriction enzyme digestion and T4 DNA ligase.
8	pspCas9-Klenow-ST2-com-53-sp-g2	Plasmid for expressing spCas9-Klenow fusion protein and sgRNA targeting human DMD exon 53.	The AflIII+Acc65I fragment of pspCas9-3'UTR-ST2-com-52-sp-g2 was used to replace that of pspCas9-Klenow-tetra-com-hCLCN5-sp-g1 by restriction enzyme digestion and T4 DNA ligase.
9	pspCas9-POLI-ST2-com vector	Vector plasmid for expressing spCas9-POLI fusion protein and sgRNA. There are two copies of HBB 3' UTR in the 3'UTR of spCas9-POLI to enhance expression. The ST2 loop of the sgRNA scaffold was replaced by com-aptamer to enable Cas9 RNP encapsulation into viral capsids via com-COM interaction.	The AflIII and Acc65I fragment of pspCas9-3'UTR-ST2-com vector was used to replace the AflIII and Acc65I fragment of pspCas9-POLI-Tetra-com vector by restriction enzyme digestion and T4 DNA ligase.
10	pspCas9-3'UTR-ST2-com-HBB-W	Plasmid for expressing spCas9 and wild type HBB sgRNA. The ST2 loop of the sgRNA scaffold was replaced by com-aptamer to enable Cas9 RNP encapsulation into viral capsids via com-COM interaction.	The annealed HBB-W oligoes were inserted into the BbsI site of pspCas9-3'UTR-ST2-com-vector by T4 DNA ligase.
11	pspCas9-Klenow-ST2-com-vector	Vector plasmid for expressing spCas9-Klenow fusion protein and sgRNA. There are two copies of HBB 3' UTR in the 3'UTR of	The AflIII-Acc65I fragment containing the U6-sgRNA cassette was cut from pspCas9-poli-ST2-com vector and was inserted into the AflIII-Acc65I Sites of pspCas9-Klenow-ST2-com-53-sp-g2 by T4 DNA ligase.

No.	Name	Purpose	Generation strategy
		spCas9-Klenow to enhance expression. The ST2 loop of the sgRNA scaffold was replaced by com-aptamer to enable Cas9 RNP encapsulation into viral capsids via com-Com interaction.	
12	pspCas9-Klenow-ST2-com-HBB-W	Plasmid for expressing spCas9-Klenow fusion protein and sgRNA targeting human HBB.	The annealed HBB oligonucleotides (HBB-sp-WF and HBB-sp-WR) were inserted into the BbsI site of pspCas9-Klenow-ST2-com-vector by T4 DNA ligase.
13	pspCas9-3'UTR-ST2-com-DMD44	Plasmid for expressing spCas9 and sgRNA targeting human DMD exon 44.	The annealed DMD44 oligonucleotides (DMD44-g1-F and DMD44-g1-R) were inserted into the BbsI site of pspCas9-3'UTR-ST2-com-vector by T4 DNA ligase.
14	pspCas9-Klenow-ST2-com-DMD44	Plasmid for expressing spCas9-Klenow fusion protein and sgRNA targeting human DMD exon 44.	The annealed DMD44 oligonucleotides were inserted into the BbsI site of pspCas9-Klenow-ST2-com-vector by T4 DNA ligase.
15	pspCas9-3'UTR-ST2-com-g5	Plasmid for expressing spCas9 and sgRNA targeting human Intragenic 1 (GRCh38.p13, chromosome 20, 32752960–32752979).	Reported in CRISPR J. 2021 Dec;4(6):914-928. doi: 10.1089/crispr.2020.0106. Epub 2021 Mar 16.
16	pspCas9-Klenow-ST2-com-g5	Plasmid for expressing spCas9-Klenow fusion protein and sgRNA targeting Intragenic 1.	The annealed oligonucleotides ABE-g5-F and ABE-g5-R were inserted into the BbsI site of pspCas9-Klenow-ST2-com-vector by T4 DNA ligase.
17	pspCas9-3'UTR-ST2-com-P53	Plasmid for expressing spCas9 and sgRNA targeting human P53.	Reported in CRISPR J. 2021 Dec;4(6):914-928. doi: 10.1089/crispr.2020.0106. Epub 2021 Mar 16.
18	pspCas9-Klenow-ST2-com-P53	Plasmid for expressing spCas9-Klenow fusion protein and sgRNA targeting human P53.	The annealed P53 oligonucleotides (P53-g1F1 and P53-g1R1) were inserted into the BbsI site of pspCas9-Klenow-ST2-com-vector by T4 DNA ligase.

No.	Name	Purpose	Generation strategy
19	pspCas9-3'UTR-ST2-com-MRE11	Plasmid for expressing spCas9 and sgRNA targeting human MRE11.	The annealed MRE11 oligonucleotides (Mre11-gF and Mre11-gR) were inserted into the BbsI site of pspCas9-3'UTR-ST2-com-vector by T4 DNA ligase.
20	pspCas9-Klenow-ST2-com-MRE11	Plasmid for expressing spCas9-Klenow fusion protein and sgRNA targeting human MRE11.	The annealed MRE11 oligonucleotides were inserted into the BbsI site of pspCas9-Klenow-ST2-com-vector by T4 DNA ligase.
21	pspCas9-3'UTR-ST2-com-CtIP	Plasmid for expressing spCas9 and sgRNA targeting human RBBP8.	The annealed CtIP oligonucleotides (CtIP-gF and CtIP-gR) were inserted into the BbsI site of pspCas9-3'UTR-ST2-com-vector by T4 DNA ligase.
22	pspCas9-Klenow-ST2-com-CtIP	Plasmid for expressing spCas9-Klenow fusion protein and sgRNA targeting human TBBP8.	The annealed CtIP oligonucleotides were inserted into the BbsI site of pspCas9-Klenow-ST2-com-vector by T4 DNA ligase.
23	Ppoli-CLCN5-g1	Plasmid for expressing <i>E. coli</i> polymerase I with nuclear targeting signals and sgRNA for <i>CLCN5</i> .	pspCas9-poli-Tetra-com-clcn5-sp-g1 was cut with PpuMI to delete the 2.6 kb encoding the Cas9 functional domains, and the rest DNA was self-ligated.
24	pspCas9-poli(D705A)-Tetra-com-clcn5-sp-g1	Plasmid for expressing sgRNA for <i>CLCN5</i> and Cas9-pol fusion protein with D705A mutation to inactivate the polymerase activity.	Cut pspCas9-poli-Tetra-com-clcn5-sp-g1 with Accl and NheI, and recover the vector (remove the 1 bk band); Use Pol-F1 (GCCAAGCTGAAGTCTACATATAC) and Pol-MR (CTGAGAGTAGGCGGCGGACACG) as primers and use pspCas9-poli-Tetra-com-clcn5-sp-g1 as the template to amplify a fragment of 225 bp (Fragment 1); Use Pol-MF (CGTGTCCGCGCCTACTCTCAG) and pol-R1 (aagaaagcgagctagcaatgaa) as primers and use pspCas9-poli-Tetra-com-clcn5-sp-g1 as the template to amplify a fragment of 840 bp (Fragment 2). Insert Fragment 1 and 2 into the recovered vector by Infusion reaction.
25	pspCas9-Klenow(D705A)-Tetra-com-clcn5-sp-g1	Plasmid for expressing sgRNA for <i>CLCN5</i> and Cas9-Klenow fusion protein with D705A mutation to inactivate the polymerase activity.	The Accl-Fsel fragment of pspCas9-poli(D705A)-Tetra-com-clcn5-sp-g1 was replaced by the Accl-Fsel fragment of pspCas9-Klenow-Tetra-com-clcn5-sp-g1 to remove the 5' exonuclease coding region by T4 DNA ligase.

Supplementary Table S2. Sequence information for primers and analysis

Primer name	SEQ	Purpose
Reporter-F	tccatttcaggtgtcgtgag	To amplify the DNA in the GFP reporter cassette.
Reporter-R2	TCCAGCTCGACCAGGATG	
HBB-F1	AGCCAGGGCTGGGCATAAAAG	Used to amplify the HBB region for NGS with HBB-R3
HBB-R3	TGGGAAAATAGACCAATAGGCAGAG	Used to amplify the HBB region for NGS with HBB-R or HBB-R
DMD53-F	TCCTGTTGTTTCATCATCCTAGC	To amplify the DMD 53 exon target region for NGS
DMD53-R	TCCAGCCATTGTGTTGAATC	
hCLCN5-F	GTTTAAGGGCCCGCCTTTTG	To amplify the CLCN5 target region for NGS
hCLCN5-R	TGTCTTACCTCTCGGTGCCT	
hCLCN5-F3	GACCCAGGTTTCTTGAGCTG	To amplify the CLCN5 target region for SMRT sequencing
hCLCN5-R3	TTCAGAGCTTCCCTCCAAGC	
OT4-F	CCCTATACCTGGGCTCCGTT	To amplify the Off-target 4 region for NGS
OT4-R	GAAAGGGCCTCTCTTTGTAATG	
OT5-F	AAGCTCTACAAGGGCAGAGAATG	To amplify the Off-target 8 region for NGS
OT5-R	TCAAAGCTCCCAGATTCACGTT	
OT8-F	CTGCTCTTTGCCTGTTGGAG	To amplify the Off-target 8 region for NGS
OT8-R	GCTAAAGCTGGAAGGCTGTG	
HBD-F2	AAAAGGCAGGGCAGAGTCGACTG	To amplify HBD region for NGS
HBD-R2	GGTAGGAAAACAGCCCAAGGGAC	
<i>DMD44-F</i>	CCATCACCTTCAGAACCTGA	To amplify DMD exon 44 region for NGS
<i>DMD44-R</i>	tcagtggttaacagaagctga	
<i>CLCN5 5' Sequence</i>	GTAGGATTCTAATCACTGCCTGCTC	Sequences used to find deletions in analyzing SMRT sequencing data
<i>CLCN5 3' Sequence</i>	CTTTCTGCACCTCCTGATAGCCTTG	
MRE11-onF	GCCAAGTGTGAATGTGCACA	To amplify the on-target of for MRE11 NGS
MRE11-onR	CCTCTTAGGCTATGACCAGGG	
Ctip-onF	TCATTGGGAGGCCGAACATC	To amplify the on-target of RBBP8 for NGS
CtIP-onR	AAGGGCTGAAGGATGATGCA	
P53-onF	CTGGCATTCTGGGAGCTTCA	To amplify the on-target of p53 for NGS
P53-onR	GAGACCTGTGGGAAGCGAAA	
g5-onF	GTCTGAGGTCACACAGTGGG	To amplify the on-target of intragenic 1 for NGS
g5-onR	CTGAGAGCAGGGACCACATC	
ABE-g5-F	ACCGGATGAGATAATGATGAGTCA	To make construct for expressing intragenic 1 sgRNA
ABE-g5-R	aaacTGACTCATCATTATCTCATC	
P53-g1F1	ACCGCCATTGTTCAATATCGTCCG	To make construct for expressing P53 sgRNA
P53-g1R1	AAACCGGACGATATTGAACAATGG	
DMD44-g1-F	CACCGtttagcatgttccaatttc	To make construct for expressing DMD exon 44 sgRNA
DMD44-g1-R	AAACgagaattgggaacatgctaa	
HBB-sp-WF	ACCGGTAACGGCAGACTTCTCCTC	

HBB-sp-WR	AAACGAGGAGAAGTCTGCCGTTAC	To make construct for expressing <i>HBB</i> sgRNA
CtlP-gF	accgTTGCCCAAAGATTCCCCAG	To make construct for expressing <i>RBBP8</i> sgRNA
CtlP-gR	aaacCTGGGGAATCTTTGGGCAA	
Mre11-gF	accgTGACTGAGATCTGAGTGCTC	To make construct for expressing <i>MRE11</i> sgRNA
Mre11-gR	aaacGAGCACTCAGATCTCAGTCA	
53-sp-g2-F	ACCGactggttgccctccggttctga	To make construct for expressing DMD exon 53 sgRNA
53-sp-g2-R	AAACtcagaaccggaggcaacagt	
hCLCN5-sp-g1-F	ACCGGAGGACAAGTCGTACAATGG	To make construct for expressing <i>CLCN5</i> sgRNA
hCLCN5-sp-g1-R	AAACCCATTGTACGACTTGTCTTC	

Supplementary Table S3. Single guide RNA target sequences

Name	Protospacer	PAM
<i>CLCN5</i>	GAGGACAAGTCGTACAATGG	TGG
<i>DMD53</i>	ACTGTTGCCTCCGTTCTGA	AGG
<i>DMD44</i>	TTAGCATGTCCCAATTCTC	AGG
<i>HBB</i>	GTAACGGCAGACTTCTCCTC	AGG
<i>Intragenic site 1</i>	GATGAGATAATGATGAGTCA	GGG
<i>P53</i>	CCATTGTTCAATATCGTCCG	GGG
<i>MRE11</i>	TGACTGAGATCTGAGTGCTC	TGG
<i>RBBP8</i>	TTGCCCAAAGATTCCCCAG	GGG

Supplementary Table S4. Overall INDEL rates of co-targeted genes

	<i>RBBP8/CLCN5</i>	
	<i>RBBP8</i>	<i>CLCN5</i>
Cas9	48.9 ± 1.9	42.4 ± 1.9
Cas9-Klenow	1.7 ± 0.03	1.1 ± 0.17

Replicate number is three for all groups. No difference in INDEL rates was observed for co-transfected genes.