

CLAIMS

1. A guide ribonucleic acid (gRNA) scaffold comprising a sequence having at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to any one of the sequences selected from the group consisting of SEQ ID NOS: 2292, 2291, 2307, 2281-2290, 2293-2306, 2308-2332 and 2353-2398.
2. The gRNA scaffold of claim 1, comprising a sequence having one or more modifications relative to SEQ ID NO: 2238, wherein the one or more modifications comprise one or more nucleotide substitutions, insertions, and/or deletions, wherein the one or more modifications result in an improved characteristic, and wherein the improved characteristic comprises one or more functional properties selected from the group consisting of increased editing activity, increased pseudoknot stem stability, increased triplex region stability, increased scaffold stem stability, extended stem stability, reduced off-target folding intermediates, and increased binding affinity to a Class 2, Type V CRISPR protein.
3. The gRNA scaffold of claim 1, comprising a sequence having one or more modifications relative to SEQ ID NO: 2239, wherein the one or more modifications comprise one or more nucleotide substitutions, insertions, and/or deletions, wherein the one or more modifications result in an improved characteristic, and wherein the improved characteristic comprises one or more functional properties selected from the group consisting of increased editing activity, increased pseudoknot stem stability, increased triplex region stability, increased scaffold stem stability, extended stem stability, reduced off-target folding intermediates, and increased binding affinity to a Class 2, Type V CRISPR protein.
4. The gRNA scaffold of claim 3, comprising one or more modifications relative to the sequence of SEQ ID NO: 2239 selected from the group consisting of C9U, U11C, C17G, U24C, A29C, an insertion of G at position 54, an insertion of C at position 64, A88G, and A95G.
5. The gRNA scaffold of any one of claims 1-4, wherein the gRNA scaffold comprises one or more heterologous RNA sequences in the extended stem, wherein the heterologous RNA is selected from the group consisting of a MS2 hairpin, Q β hairpin, U1 hairpin II, Uvsx hairpin, and a PP7 stem loop, or sequence variants thereof, and wherein the one or more heterologous RNA sequences is capable of binding a protein, a RNA, a DNA, or a small molecule.
6. A gRNA comprising the gRNA scaffold of any one of claims 1-5, and a targeting sequence at the 3' end of the gRNA scaffold, wherein the targeting sequence is

complementary to a target nucleic acid sequence, and wherein the targeting sequence has 15, 16, 17, 18, 19, or 20 nucleotides.

7. The gRNA of any one of claims 1-6, wherein the gRNA is chemically modified.

8. An chimeric Class 2, Type V CRISPR protein comprising:

a. a non-target strand binding (NTSB) domain isolated or derived from *Deltaproteobacteria* comprising a sequence of QPASKKIDQNKLKPEMDEKGNLTTAGFACSQCGQPLFVYKLEQVSEK GKAYTNYFG RCNVAEHEKLILLAQLKPEKDSDEAVTYSLGKFGQ (SEQ ID NO: 2335), or a sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity thereto;

b. a helical I-II domain isolated or derived from *Deltaproteobacteria* comprising a sequence of RALDFYSIHVTKESTHPVKPLAQIAGNRYASGPVGKALSDACMGTIASFLSKYQDIIIE HQKVVKGNQKRLESLRELAGKENLEYPSVTLPQPHTKEGVDA YNEVIARVRMWV NLNLWQKLKLSRDDAKPLLRLKGFP SF (SEQ ID NO: 2336), or a sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity thereto; and

c. a RuvC-I domain isolated or derived from *Planctomycetes* comprising a sequence of SSNIKPMNLIGVDRGENIPAVIALTDPEGCPLSRFKDSLGNP THILRIGESYKEKQRTIQ AKKEVEQRRAGGYSRKYASKAKNLADDMVRNTARDLLYYAVTQDAMLIFENLSRG FGRQGKRTFMAERQYTRMEDWLTAKLAYEGLPSKTYLSKTLAQYTSKTC (SEQ ID NO: 2352), or a sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity thereto.

9. The chimeric Class 2, Type V CRISPR protein of claim 8, comprising a helical I-I domain, a helical II domain, an oligonucleotide binding domain (OBD)-I domain, an OBD-II, a target strand loading (TSL) domain, and a RuvC-II domain.

10. The chimeric Class 2, Type V CRISPR protein of claim 9, wherein the helical I-I domain, helical II domain, OBD-I domain, OBD-II, TSL domain, and RuvC-II domain are each isolated or derived from *Planctomycetes*.

11. The Class 2, Type V CRISPR protein of claim 9 or claim 10, wherein:

a. the helical I-I domain comprises a sequence of PISNTSRANLNKLLTDYTEMKKAILHVYWEEFQKDPVGLMSRVA

(SEQ ID NO: 2343), or a sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto;

- b. the helical II domain comprises a sequence of
PLVERQANEVDWWDMMVCNVKKLINEKKEDGKVFWQNLAGYKRQEA
LRPYLSSEEDRKKGKKFARYQLGDLHLEKKHGEDWGKVYDEAWE
RIDKKVEGLSKHIKLEEERRSEDAQSKAALTDWLRAKASFVIEGLKEA
DKDEFRCRCELKLQKWYGDLRGKPF AIEAE (SEQ ID NO: 2351), or a
sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least
98% or at least 99% identity thereto;
- c. the OBD-I domain comprising a sequence of
QEIKRINKIRRLVKDSNTKKAGKTGPMKTLLVRVMTPDLRERLENLR
KKPENIPQ (SEQ ID NO: 2342), or a sequence having at least 90%, at least
95%, at least 96%, at least 97%, at least 98%, or at least 99% identity thereto;
- d. the OBD-II domain comprises a sequence of
NSILDISGFSKQYNCAFIWQKDGVKLNLYLIINYFKGGKLRFKKIKPE
AFEANRFYTVINKKSGEIVPMEVNFNFDDPNLILPLAFGKRQGREFIW
NDLLSLETGSLKLANGRVIEKTLYNRRTRQDEPALFVALTFERREVLD
(SEQ ID NO: 2347), or a sequence having at least 90%, at least 95%, at least
96%, at least 97%, at least 98%, or at least 99% identity thereto;
- e. the TSL domain comprises a sequence of
SNCGFTITSADYDRVLEKLKKTATGWMTTINGKELKVEGQITYYNYR
KRQNVVKDLSVELDRLSEESVNNDISSWTKGRSGEALSLLKKRFSHRP
VQEKFVCLNCGFETH (SEQ ID NO: 2349), or a sequence having at least
90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%
identity thereto; and
- f. the RuvC-II domain comprises a sequence of
ADEQAALNIARSWLFLRSQEYKKYQTNKTTGNTDKRAFVETWQSFYR
KKLKEVWKPAV (SEQ ID NO: 2350), or a sequence having at least 90%, at
least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity
thereto.

12. The Class 2, Type V CRISPR protein of any one of claims 8-11, comprising the
sequence of SEQ ID NO: 416, or a sequence having at least 90%, at least 95%, at least 96%,
at least 97%, at least 98%, or at least 99% identity thereto, wherein the Class 2, Type V
CRISPR protein comprises a proline amino acid at position 793 relative to a *Planctomycetes*

sequence of SEQ ID NO: 2, and wherein the Class 2, Type V CRISPR protein is a chimeric protein.

13. The Class 2, Type V CRISPR protein of claim 11, wherein the Class 2, Type V CRISPR protein comprises at least one modification in one or more domains, wherein the at least one modification comprises:

a. a modification at one or more amino acid positions in the NTSB domain relative to SEQ ID NO: 2335 selected from the group consisting of P2, S4, Q9, E15, G20, G33, L41, Y51, F55, L68, A70, E75, K88, and G90, an insertion of G at position 2, an insertion of I at position 4, an insertion of L at position 4, Q9P, E15S, G20D, a deletion of S at position 30, G33T, L41A, Y51T, F55V, L68D, L68E, L68K, A70Y, A70S, E75A, E75D, E75P, K88Q, and G90Q relative to SEQ ID NO: 2335;

b. a modification at one or more amino acid positions in the helical I-II domain relative to SEQ ID NO: 2336 selected from the group consisting of I24, A25, Y29 G32, G44, S48, S51, Q54, I56, V63, S73, L74, K97, V100, M112, L116, G137, F138, S140, an insertion of T at position 24, an insertion of C at position 25, Y29F, G32Y, G32N, G32H, G32S, G32T, G32A, G32V, a deletion of G at position 32, G32S, G32T, G44L, G44H, S48H, S48T, S51T, Q54H, I56T, V63T, S73H, L74Y, K97G, K97S, K97D, K97E, V100L, M112T, M112W, M112R, M112K, L116K, G137R, G137K, G137N, an insertion of Q at position 138, and S140Q relative to SEQ ID NO: 2336;

c. a modification at one or more amino acid positions in the helical II domain relative to SEQ ID NO: 2351 selected from the group consisting of L2, V3, E4, R5, Q6, A7, E9, V10, D11, W12, W13, D14, M15, V16, C17, N18, V19, K20, L22, I23, E25, K26, K31, Q35, L37, A38, K41, R42, Q43, E44, L46, K57, Y65, G68, L70, L71, L72, E75, G79, D81, W82, K84, V85, Y86, D87, I93, K95, K96, E98, L100, K102, I104, K105, E109, R110, D114, K118, A120, L121, W124, L125, R126, A127, A129, I133, E134, G135, L136, E138, D140, K141, D142, E143, F144, C145, C147, E148, L149, K150, L151, Q152, K153, L158, E166, A167, an insertion of A at position 2, an insertion of H at position 2, a deletion of L at position 2 and a deletion of V at position 3, V3E, V3Q, V3F, a deletion of V at position 3, an insertion of D at position 3, V3P, E4P, a deletion of E at position 4, E4D, E4L, E4R, R5N, Q6V, an insertion of Q at position 6, an insertion of G at position 7, an insertion of H at position 9, an insertion of A at position 9, VD10, an insertion of T1 at position 0, a deletion of V at position 10, an insertion of F at position 10, an insertion of D at position 11, a deletion of D at position 11, D11S, a deletion of W at position 12, W12T, W12H, an insertion of P at position 12, an insertion of Q at position 13, an insertion of G at position 12, an insertion of R at

position 13, W13P, W13D, an insertion of D at position 13, W13L, an insertion of P at position 14, an insertion of D at position 14, a deletion of D at position 14 and a deletion of M at position 15, a deletion of M at position 15, an insertion of T at position 16, an insertion of P at position 17, N18I, V19N, V19H, K20D, L22D, I23S, E25C, E25P, an insertion of G at position 25, K26T, K27E, K31L, K31Y, Q35D, Q35P, an insertion of S at position 37, a deletion of L at position 37 and a deletion of A at position 38, K41L, an insertion of R at position 42, a deletion of Q at position 43 and a deletion of E at position 44, L46N, K57Q, Y65T, G68M, L70V, L71C, L72D, L72N, L72W, L72Y, E75F, E75L, E75Y, G79P, an insertion of E at position 79, an insertion of T at position 81, an insertion of R at position 81, an insertion of W at position 81, an insertion of Y at position 81, an insertion of W at position 82, an insertion of Y at position 82, W82G, W82R, K84D, K84H, K84P, K84T, V85L, V85A, an insertion of L at position 85, Y86C, D87G, D87M, D87P, I93C, K95T, K96R, E98G, L100A, K102H, I104T, I104S, I104Q, K105D, an insertion of K at position 109, E109L, R110D, a deletion of R at position 110, D114E, an insertion of D at position 114, K118P, A120R, L121T, W124L, L125C, R126D, A127E, A127L, A129T, A129K, I133E, an insertion of C at position 133, an insertion of S at position 134, an insertion of G at position 134, an insertion of R at position 135, G135P, L136K, L136D, L136S, L136H, a deletion of E at position 138, D140R, an insertion of D at position 140, an insertion of P at position 141, an insertion of D at position 142, a deletion of E at position 143+a deletion of F at position 144, an insertion of Q at position 143, F144K, a deletion of F at position 144, a deletion of F at position 144 and a deletion of C at position 145, C145R, an insertion of G at position 145, C145K, C147D, an insertion of V at position 148, E148D, an insertion of H at position 149, L149R, K150R, L151H, Q152C, K153P, L158S, E166L, and an insertion of F at position 167 relative to SEQ ID NO: 2351;

d. a modification at one or more amino acid positions in the RuvC-I domain relative to SEQ ID NO: 2352 selected from the group consisting of I4, K5, P6, M7, N8, L9, V12, G49, K63, K80, N83, R90, M125, L146, an insertion of I at position 4, an insertion of S at position 5, an insertion of T at position 6, an insertion of N at position 6, an insertion of R at position 7, an insertion of K at position 7, an insertion of H at position 8, an insertion of S at position 8, V12L, G49W, G49R, S51R, S51K, K62S, K62T, K62E, V65A, K80E, N83G, R90H, R90G, M125S, M125A, L137Y, an insertion of P at position 137, a deletion of L at position 141, L141R, L141D, an insertion of Q at position 142, an insertion of R at position 143, an insertion of N at position 143, E144N, an insertion of P at position 146, L146F, P147A, K149Q, T150V, an insertion of R at position 152, an insertion of H153, T155Q, an

insertion of H at position 155, an insertion of R at position 155, an insertion of L at position 156, a deletion of L at position 156, an insertion of W at position 156, an insertion of A at position 157, an insertion of F at position 157, A157S, Q158K, a deletion of Y at position 159, T160Y, T160F, an insertion of I at position 161, S161P, T163P, an insertion of N at position 163, C164K, and C164M relative to SEQ ID NO: 2352;

e. a modification at one or more amino acid positions in the OBD-I domain relative to SEQ ID NO: 2342 selected from the group consisting of I3, K4, R5, I6, N7, K8, K15, D16, N18, P27, M28, V33, R34, M36, R41, L47, R48, E52, P55, Q56, an insertion of G at position 3, I3G, I3E, an insertion of G at position 4, K4G, K4P, K4S, K4W, K4W, R5P, an insertion of P at position 5, an insertion of G at position 5, R5S, an insertion of S at position 5, R5A, R5P, R5G, R5L, I6A, I6L, an insertion of G at position 6, N7Q, N7L, N7S, K8G, K15F, D16W, an insertion of F at position 16, an insertion of F18, an insertion of P at position 27, M28P, M28H, V33T, R34P, M36Y, R41P, L47P, an insertion of P at position 48, E52P, an insertion of P at position 55, a deletion of P at position 55 and a deletion of Q at position 56, Q56S, Q56P, an insertion of D at position 56, an insertion of T at position 56, and Q56P relative to SEQ ID NO: 2342;

f. a modification at one or more amino acid positions in the OBD-II domain relative to SEQ ID NO: 2347 selected from the group consisting of S2, I3, L4, K11, V24, K37, R42, A53, T58, K63, M70, I82, Q92, G93, K110, L121, R124, R141, E143, V144, L145, a deletion of S at position 2, I3R, I3K, a deletion of I at position 3 and a deletion of L4, a deletion of L at position 4, K11T, an insertion of P at position 24, K37G, R42E, an insertion of S at position 53, an insertion of R at position 58, a deletion of K at position 63, M70T, I82T, Q92I, Q92F, Q92V, Q92A, an insertion of A at position 93, K110Q, R115Q, L121T, an insertion of A at position 124, an insertion of R at position 141, an insertion of D at position 143, an insertion of A at position 143, an insertion of W at position 144, and an insertion of A at position 145 relative to SEQ ID NO: 2347; or

g. a modification at one or more amino acid positions in the TSL domain relative to SEQ ID NO: 2349 selected from the group consisting of S1, N2, C3, G4, F5, I7, K18, V58, S67, T76, G78, S80, G81, E82, S85, V96, E98, or an insertion of M at position 1, a deletion of N at position 2, an insertion of V at position 2, C3S, an insertion of G at position 4, an insertion of W at position 4, F5P, an insertion of W at position 7, K18G, V58D, an insertion of A at position 67, T76E, T76D, T76N, G78D, a deletion of S at position 80, a deletion of G

at position 81, an insertion of E at position 82, an insertion of N at position 82, S85I, V96C, V96T, and E98D relative to SEQ ID NO: 2349;

wherein the Class 2, Type V CRISPR protein is capable of forming a ribonuclear protein complex (RNP) with a gRNA and wherein the Class 2, Type V CRISPR protein exhibits an improved characteristic relative to SEQ ID NO: 416.

14. The Class 2, Type V CRISPR protein of claim 13, wherein the improved characteristic comprises increased binding affinity to a gRNA, increased binding affinity to the target nucleic acid, improved ability to utilize a greater spectrum of PAM sequences in the editing of the target nucleic acid, improved unwinding of the target nucleic acid, increased editing activity, improved editing efficiency, improved editing specificity for cleavage of the target nucleic acid, decreased off-target editing or cleavage of the target nucleic acid, increased percentage of a eukaryotic genome that can be edited, increased activity of the nuclease, increased target strand loading for double strand cleavage, decreased target strand loading for single strand nicking, increased binding of the non-target strand of DNA, improved protein stability, increased protein:gRNA (RNP) complex stability, improved fusion characteristics, and/or increased cleavage activity at a target nucleic acid sequence comprising an TTC, ATC, GTC, or CTC PAM sequence.

15. The Class 2, Type V CRISPR protein of claim 14, wherein the increased cleavage activity is an enrichment score ($\log_2$) of at least about 1.5, at least about 2.0, at least about 2.5, at least about 3, at least about 3.5, at least about 4, at least about 4.5, at least about 5, at least about 6, at least about 7, at least about 8 or more greater compared to score of the sequence of SEQ ID NO: 416 in an in vitro assay.

16. The Class 2, Type V CRISPR protein of any one of claims 8-13, wherein the Class 2, Type V CRISPR protein has a sequence selected from the group consisting of the sequences of SEQ ID NOS: 417-592 and 1147-1231, as set forth in Table 3, or a sequence having at least about 70%, at least about 80%, at least about 90%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99%, sequence identity thereto.

17. The Class 2, Type V CRISPR protein of any one of claims 8-16, comprising one or more nuclear localization signals (NLS) positioned at or near the N-terminus and/or the C-terminus of the protein.

18. A gene editing pair, characterized by comprising:

a. a guide ribonucleic acid (gRNA) scaffold, comprising a sequence having at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence

identity to any one of the sequences selected from the group consisting of SEQ ID NOS: 2292, 2291, 2307, 2281-2290, 2293-2306, 2308-2332 and 2353-2398; and

b. a Class 2, Type V CRISPR protein of any one of claims 8-17

wherein the gRNA and the Class 2, Type V CRISPR protein are capable of forming a ribonuclear protein complex (RNP), and wherein an RNP of the Class 2, Type V CRISPR protein and the gRNA exhibit at least one or more improved characteristics as compared to an RNP of a reference protein of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3 and a gRNA comprising a sequence of SEQ ID NO: 4 or SEQ ID NO: 5.

19. The gene editing pair of claim 18, wherein the improved characteristic is selected from one or more of the group consisting of increased binding affinity of the Class 2, Type V CRISPR protein to the gRNA; increased binding affinity to a target nucleic acid; increased ability to utilize a greater spectrum of one or more PAM sequences, including ATC, CTC, GTC, or TTC, in the editing of target nucleic acid; increased unwinding of the target nucleic acid; increased editing activity; increased editing efficiency; increased editing specificity of the target nucleic acid; increased nuclease activity; increased target strand loading for double strand cleavage; decreased target strand loading for single strand nicking; decreased off-target cleavage of the target nucleic acid; increased binding of non-target nucleic acid strand; increased protein:gRNA complex (RNP) stability; and increased fusion characteristics.

20. The gene editing pair of claim 19, wherein the improved characteristic is at least about 1.1 to about 100-fold or more increased relative to the RNP of a reference protein of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3 and the gRNA comprising a sequence of SEQ ID NO: 4 or SEQ ID NO: 5 in a comparable in vitro assay system.

21. The gene editing pair of any one of claims 18-20, wherein the Class 2, Type V CRISPR and the gRNA are able to form RNP having at least about a 5%, at least about a 10%, at least about a 15%, or at least about a 20% higher percentage of cleavage-competent conformation compared to an RNP of any one of the reference proteins of SEQ ID NOS: 1-3 and the gRNA of SEQ ID NO: 4 or SEQ ID NO: 5, when assessed in a comparable in vitro assay system.

22. The gene editing pair of any one of claims 18-21, wherein the RNP comprising the Class 2, Type V CRISPR and the gRNA exhibit a cleavage rate for the target nucleic acid in a timed in vitro assay that is at least about 5-fold, at least about 10-fold, or at least about 20-fold higher compared to an RNP of any one of the reference proteins of SEQ ID-NOS: 1-3 and the gRNA of SEQ ID NO: 4 or SEQ ID NO: 5, when assessed in a comparable in vitro assay system.

- 23.** A catalytically-dead Class 2, Type V CRISPR protein, comprising a sequence selected from the group consisting of SEQ NOs: 44-62 and 1232-1235, or a sequence having at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99% thereto, and comprising at least one modification relative to SEQ ID NO: 2, forming a catalytically-dead Class 2, Type V CRISPR protein, wherein an RNP of the catalytically-dead Class 2, Type V CRISPR protein and a gRNA of claim 6, retains the ability to bind target nucleic acid.
- 24.** A nucleic acid, comprising a sequence that encodes or comprises:
- a.** a guide ribonucleic acid (gRNA) scaffold comprising: a sequence having at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to any one of the sequences selected from the group consisting of SEQ ID NOS: 2292, 2291, 2307, 2281-2290, 2293-2306, 2308-2332 and 2353-2398, and a targeting sequence at the 3' end of the gRNA scaffold, wherein the targeting sequence is complementary to a target nucleic acid sequence, and wherein the targeting sequence has 15, 16, 17, 18, 19, or 20 nucleotides;
 - b.** the Class 2, Type V CRISPR protein of any one of claims 8-23; or
 - c.** a combination of (a) and (b).
- 25.** A vector comprising the nucleic acid of claim 24, wherein the vector is selected from the group consisting of a retroviral vector, a lentiviral vector, an adenoviral vector, an adeno-associated viral (AAV) vector, a herpes simplex virus (HSV) vector, a virus like particle, a plasmid, a minicircle, a nanoplasmid, a DNA vector, and an RNA vector.
- 26.** The vector of claim 25, wherein the AAV vector is selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV-Rh74, or AAVRh10.
- 27.** A lipid nanoparticle comprising the nucleic acid of claim 24.
- 28.** A method of modifying a target nucleic acid in a eukaryotic cell, comprising contacting the target nucleic acid with: i) the gene editing pair of any one of claims 18-22; ii) one or more nucleic acids encoding the gene editing pair of (i); iii) a vector comprising the nucleic acid of (ii); iv) a virus like particle comprising the gene editing pair of (i); or v) combinations of two or more of (i) to (iv), wherein the contacting comprises binding the target nucleic acid and introducing one or more double-stranded breaks in the target nucleic acid, wherein the modifying comprises introducing a mutation, an insertion, or a deletion in the target nucleic acid of the cell.
- 29.** A composition comprising the gene editing pair of any one of claims 18-22.

30. A kit comprising a guide ribonucleic acid (gRNA) scaffold comprising: a sequence having at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to any one of the sequences selected from the group consisting of SEQ ID NOS: 2292, 2291, 2307, 2281-2290, 2293-2306, 2308-2332 and 2353-2398, and a targeting sequence at the 3' end of the gRNA scaffold, wherein the targeting sequence is complementary to a target nucleic acid sequence, and wherein the targeting sequence has 15, 16, 17, 18, 19, or 20 nucleotides; the Class 2, Type V CRISPR protein of any one of claims 8-17; and a container.

31. A composition comprising: the gene editing pair of any one of claims 18-22, for use as a medicament for the treatment of a subject having a disease.