Thus, there remains a need for developing assays, such as DSF, that can be used to evaluate the quality of Cas9 molecule/gRNA molecule complexes, e.g., to quantitate productive complex formation, a prerequisite for RNP mediated gene editing.

SUMMARY

In one aspect, the present disclosure refers to a method of screening for a Cas9 molecule/guide RNA (gRNA) molecule complex, the method comprising: (a) generating a plurality of samples, each sample comprising a Cas9 molecule/gRNA molecule complex generated by combining a Cas9 molecule and one of a plurality of gRNA molecules; (b) detecting a melting temperature (T_m) value of the Cas9 molecule/gRNA molecule complex in each of the plurality of samples; and (c) selecting at least one sample from the plurality of samples based on one or more of (i) a comparison of the T_m values in the plurality of samples to a T_m value of a reference Cas9 molecule/gRNA molecule complex or a pre-determined threshold T_m value, wherein the Cas9 molecule/gRNA molecule complex is selected if the Tm of the Cas9 molecule/gRNA molecule complex is at least 8°C greater than the Tm value of the reference Cas9 molecule/gRNA molecule complex or the pre-determined threshold Tm value. In another aspect, the present disclosure refers to the Cas9 molecule/gRNA molecule complex identified by the method as disclosed herein for use in therapy, wherein the Cas9 molecule/gRNA molecule complex is to be administered to a subject. Methods are disclosed herein for screening for a Cas9 molecule/gRNA molecule complex for administration to a subject that includes (a) generating a plurality of samples, each sample comprising a Cas9 molecule/gRNA molecule complex generated by combining a Cas9 molecule and one of a plurality of gRNA molecules; (b) detecting a melting temperature (T_m) value of the Cas9 molecule/gRNA molecule complex in each of the plurality of samples; and (c) selecting at least one sample from the plurality of samples based on one or more of (i) a comparison of the T_m values in the plurality of samples to a T_m value of a reference Cas9 molecule/gRNA molecule complex or a pre-determined threshold T_m value, or (ii) a relative ordering of the T_m values of the plurality of samples. In certain examples, the step of detecting the T_m value of the Cas9 molecule/gRNA molecule complex in each sample of the plurality of samples may include assessing each sample in the plurality of samples by differential scanning fluorimetry (DSF). In certain examples, the gRNA may be a chimeric gRNA. In certain examples, the gRNA may be a modular gRNA. In certain examples, the sample may comprise a component comprising an additive, a small molecule, a stabilizing reagent, buffer, pH, salt concentration, glycerol concentration, or other buffer component. In certain examples, a sample comprising a Cas9 molecule/gRNA molecule complex having a T_m of at least 8° C greater than a T_m value of the Cas9 molecule absent the gRNA molecule may be selected.

Disclosed herein in certain examplesare isolated complexes of a Cas9 molecule and a gRNA molecule having a T_m at least 8° C greater than a T_m value of the Cas9 molecule absent the gRNA molecule selected according to the methods provided herein.

What is claimed is:

1. A method of screening for a Cas9 molecule/guide RNA (gRNA) molecule complex, the method comprising:

(a) generating a plurality of samples, each sample comprising a Cas9 molecule/gRNA molecule complex generated by combining a Cas9 molecule and one of a plurality of gRNA molecules;

(b) detecting a melting temperature (T_m) value of the Cas9 molecule/gRNA molecule complex in each of the plurality of samples; and

(c) selecting at least one sample from the plurality of samples based on one or more of a comparison of the Tm values in the plurality of samples to a T_m value of a reference Cas9 molecule/gRNA molecule complex or a pre-determined threshold T_m value, wherein the Cas9 molecule/gRNA molecule complex is selected if the T_m of the Cas9 molecule/gRNA molecule complex is at least 8°C greater than the T_m value of the reference Cas9 molecule/gRNA molecule complex or the pre-determined threshold T_m value.

2. The method according to claim 1, wherein the step of detecting the T_m value of the Cas9 molecule/gRNA molecule complex in each sample of the plurality of samples includes assessing each sample in the plurality of samples by differential scanning fluorimetry (DSF).

3. The method according to claim 1, wherein the gRNA is a chimeric gRNA.

4. The method according to claim 1, wherein the gRNA is a modular gRNA.

5. The method of any of claims 1-4, wherein the T_m value is detected using a thermal shift assay.

6. The method of claim 5, wherein the thermal shift assay is selected from differential scanning fluorimetry (DSF), differential scanning calorimetry (DSC), or isothermal titration calorimetry (ITC).

7. The method of any of claims 1-6, wherein the Cas9 molecule is a chimeric Cas9 molecule, or a synthetic or engineered Cas9 molecule, e.g., a Cas9 molecule with a portion or portions deleted.

8. The method of any of claims 1-7, wherein the Cas9 molecule comprises a *S. pyogenes* or a *S. aureus* Cas9 molecule.

9. The method of any of claims 1-8, wherein the reference Cas9 molecule/gRNA molecule complex is a reference Cas9 molecule complexed with a second gRNA molecule.

10. The method of claim 9, wherein the reference Cas9 molecule is the same as the Cas9 molecule of the complex being evaluated.

11. The method of claim 9, wherein the reference Cas9 molecule is different from the Cas9 molecule of the complex being evaluated.

12. The method of claim 9, wherein the reference Cas9 molecule differs in primary sequence from the Cas9 molecule of the complex being evaluated.

13. The method of any of claims 1 and 9-12, wherein the gRNA molecule of the reference Cas9 molecule/gRNA molecule complex is the same as the gRNA molecule of the complex being evaluated.

14. The method of any of claims 1 and 9-12, wherein the gRNA molecule of the reference Cas9 molecule/gRNA molecule complex is different from the gRNA molecule of the complex being evaluated.

15. The method of claim 14, wherein the gRNA molecule of the reference Cas9 molecule/gRNA molecule complex differs in sequence or differs by a modification from the gRNA molecule of the complex being evaluated.

16. The method of claim 7, wherein the Cas9 molecule is a Cas9 molecule with a portion or portions deleted

17. The Cas9 molecule/gRNA molecule complex identified by the method of any of claims 1-15 for use in therapy, wherein the Cas9 molecule/gRNA molecule complex is to be administered to a subject.

18. The Cas9 molecule/gRNA molecule complex of claim 17, wherein the subject is a human.