time consuming processes. Accordingly, there is a need for new systems and methods for the high throughput delivery of nucleic acid manipulation reagents.

SUMMARY

[0004] Provided herein are compositions, systems and methods for the delivery of reagents into individual cells.

[0005] Provided herein is a method of delivering reagents into individual cells. Such a method includes without limitation the steps of: (a) providing a plurality of capsules, wherein the capsules comprise reagents for altering expression of at least one gene product in a cell; (b) delivering the capsules into discrete partitions, wherein the discrete partitions further comprise individual cells; and (c) causing the capsules to release their contents into the discrete partitions under conditions enabling uptake of the reagents by the individual cells, thereby delivering the reagents into the individual cells. In a first aspect, there is provided a method of delivering reagents into individual cells, the method comprising: (a) providing a plurality of capsules, wherein the capsules comprise reagents for altering expression of at least one gene product in a cell; (b) delivering the capsules into discrete partitions, wherein the discrete partitions further comprise individual cells; (c) causing the capsules to release their contents into the discrete partitions under conditions enabling uptake of the reagents by the individual cells, thereby delivering the reagents into the individual cells.

[0006] In some embodiments and in accordance with the above, the reagents for altering expression of at least one gene product includes (i) a first regulatory element operably linked to at least one nucleotide sequence encoding a CRISPR system guide RNA that hybridizes with a target sequence in a DNA molecule within the individual cells, and (ii) a second regulatory element operably linked to a nucleotide sequence encoding a RNA-guided nuclease or an RNA-guided nuclease fusion protein. In some embodiments, the components (i) and (ii) are located on same or different vectors, and the RNA-guided nuclease and the guide RNA do not naturally occur together.

[0007] In an exemplary embodiment, the second regulatory element is operably linked to a nucleotide sequence encoding a RNA-guided nuclease. In such an embodiment, the guide RNA targets the target sequence and the RNA-guided nuclease cleaves the DNA molecule, whereby expression of the at least one gene product is altered. In some embodiments, the reagents for altering gene expression further includes a donor nucleic acid that is inserted into the DNA molecule following cleavage of the DNA molecule by the RNA-guided nuclease.

[0008] In another exemplary embodiment, the second regulatory element is operably linked to a nucleotide sequence encoding a deactivated RNA-guided nuclease,

embodiments, different individual cells receive different nucleic acid barcode sequences.

[0015] In some embodiments, the reagents for altering expression of at least one gene product described above further comprise a pair of Cas9 nickases or Cas9 fusion proteins that improve specificity of the CRISPR system as compared to when RNA-guided nucleases are used.

[0016] In certain embodiments and in accordance with any of the above, the target sequence has few or no close relatives within the cellular genome.

[0017] In further embodiments and in accordance with any of the above, the reagents for altering expression of at least one gene product further include agents that increase the frequency of homologous recombination in the cell by repressing genes involved in non-homologous end-joining (NHEJ) pathway. In some embodiments, these agents comprise a Cas9 nuclease or a nuclease-null Cas9 protein encoded with the at least one nucleotide sequence encoding a CRISPR system guide RNA.

[0018] In yet further embodiments and in accordance with any of the above, the guide RNA further comprises a spacer that is identical to a targeted protospacer sequence within the cell's genome.

[0019] In another exemplary embodiment and in accordance with any of the above, one or both of the first and second regulatory elements is an inducible promoter. In some embodiments, the inducible promoter is selected from the group consisting of a light-inducible, a heat-inducible and a chemical inducible promoter.

[0020] Provided herein is a method for delivering a reagent to a cell. This method includes without limitation the steps of (a) providing the reagent releasably coupled to a microcapsule; (b) separating the microcapsule into a discrete partition, wherein the discrete partition further comprises an individual cell; and (c) releasing the reagent under conditions that enable uptake of the reagent into the cell. In a second aspect, there is provided a method for delivering a reagent to a cell, the method comprising: (a) providing the reagent releasably coupled to a microcapsule; (b) separating the microcapsule into a discrete partition, wherein the discrete partition further comprises an individual cell; and (c) releasing the reagent under conditions that enable uptake of the reagent into the cell.

[0021] In some embodiments of this second aspect, the reagent includes a vector encoding at least one of a RNA-guided nuclease, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs), or CRISPR guide RNA capable of hybridizing with one or more target sequences in a DNA molecule of the cell, and one or more condition-inducible promoters. In certain embodiments, the reagent includes (i) a first regulatory

some embodiments, the vector or vectors are capable of stable integration into the cell's genome.

[0026] In further embodiments, the releasing step includes applying a stimulus to the microcapsule to release the reagent. In some embodiments, the stimulus is selected from a chemical stimulus, an electrical stimulus, a thermal stimulus, a magnetic stimulus, a change in pH, a change in ion concentration, reduction of disulfide bonds, a photostimulus, and combinations thereof.

[0027] In some embodiments, the uptake of the reagent into the cell is facilitated by electroporation.

[0028] In further embodiments, the microcapsule further comprises one or more additives to improve compatibility of the reagent for uptake into the cell. In some embodiments, the one or more additives includes a transfection agent.

[0029] In some embodiments, the microcapsule includes a member selected from a droplet in an emulsion and a crosslinked polymer.

[0030] In further embodiments, the microcapsule includes a bead. In certain embodiments, the bead is a gel bead.

[0031] In yet further embodiments, the microcapsule further includes a population of nucleic acid barcode sequences releasably coupled thereto, where the barcode sequences substantially all include the same barcode sequence. In some embodiments, the barcode sequences further include a hairpin sequence.

[0032] In still further embodiments, the reagent further includes a pair of Cas9 nickases or Cas9 fusion proteins that improve specificity of the CRISPR system as compared to when RNA-guided nucleases are used.

[0033] Provided herein is a method for altering gene expression in a plurality of cells. The method includes without limitation the steps of (a) providing a plurality of capsules, where capsules include reagents for altering expression of at least one gene product, the reagents include an engineered, non-naturally occurring Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system; (b) delivering the capsules into discrete partitions containing individual cells; (c) providing a stimulus to cause the capsules to release their contents under conditions such that reagents are delivered into the individual cells. In some embodiments, the CRISPR

system includes one or more vectors that include (i) a first regulatory element operably linked to at least one nucleotide sequence encoding a CRISPR-Cas system guide RNA capable of hybridizing with a target sequence in a DNA molecule of the cell, and (ii) a second regulatory element operably linked to a nucleotide sequence encoding a RNA-guided nuclease, wherein components (i) and (ii) are located on same or different vectors of the system. In a third aspect, there is provided a method for altering gene expression in a plurality of cells, the method comprising: (a) providing a plurality of capsules, wherein capsules comprise reagents for altering expression of at least one gene product, the reagents comprising an engineered, non-naturally occurring Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system comprising one or more vectors comprising: (i) a first regulatory element operably linked to at least one nucleotide sequence encoding a CRISPR-Cas system guide RNA capable of hybridizing with a target sequence in the DNA molecule of the cell, and (ii) a second regulatory element operably linked to a nucleotide sequence encoding a RNA-guided nuclease protein, wherein components (i) and (ii) are located on same or different vectors of the system, (b) delivering the capsules into discrete partitions containing individual cells; (c) providing a stimulus to cause the capsules to release their contents under conditions such that reagents are delivered into the individual cells, wherein subsequent to application of the stimulus, the guide RNA hybridizes to the target sequence and the RNA-guided nuclease protein cleaves the DNA molecule containing the target sequence, whereby expression of the at least one gene product is altered. In this aspect, subsequent to application of the stimulus, the guide RNA hybridizes to the target sequence and the RNA-guided nuclease cleaves the DNA molecule containing the target sequence, whereby expression of the at least one gene product is altered. In some embodiments of this aspect, the reagents for altering gene expression further include a donor nucleic acid that is inserted into the DNA molecule following cleavage of the DNA molecule by the RNA-guided nuclease.

[0034] Provided herein is a method for altering gene expression in a plurality of cells. The method includes without limitation the steps of (a) providing a plurality of capsules, where capsules include reagents for altering expression of at least one gene product, the reagents include an engineered, non-naturally occurring Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system; (b) delivering the capsules into discrete partitions containing individual cells; (c) providing a stimulus to cause the capsules to release their contents under conditions such that reagents are delivered into the individual cells. In some embodiments, the CRISPR system includes one or more vectors that include (i) a first regulatory element operably linked to at least one nucleotide sequence encoding a

CRISPR-Cas system guide RNA capable of hybridizing with a target sequence in a DNA molecule of the cell, and (ii) a second regulatory element operably linked to a nucleotide sequence encoding a deactivated RNA-guided nuclease, wherein components (i) and (ii) are located on same or different vectors of the system. In this embodiment, subsequent to application of the stimulus, the guide RNA hybridizes to the target sequence and the deactivated RNA-guided nuclease interferes with the transcription of a nucleic acid encoding the at least one gene product, whereby expression of the at least one gene product is altered. In a fourth aspect, there is provided a method for altering gene expression in a plurality of cells, the method comprising: (a) providing a plurality of capsules, wherein capsules comprise reagents for altering expression of at least one gene product, the reagents comprising an engineered, non-naturally occurring Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system comprising one or more vectors comprising: (i) a first regulatory element operably linked to at least one nucleotide sequence encoding a CRISPR-Cas system guide RNA capable of hybridizing with a target sequence in the DNA molecule of the cell, and (ii) a second regulatory element operably linked to a nucleotide sequence encoding a deactivated RNA-guided nuclease, wherein components (i) and (ii) are located on same or different vectors of the system, (b) delivering the capsules into discrete partitions containing individual cells; (c) providing a stimulus to cause the capsules to release their contents under conditions such that reagents are delivered into the individual cells, wherein subsequent to application of the stimulus, the guide RNA hybridizes to the target sequence and the deactivated RNA-guided nuclease interferes with the transcription of a nucleic acid encoding the at least one gene product, whereby expression of the at least one gene product is altered.

[0035] Provided herein is a method for altering gene expression in a plurality of cells. The method includes without limitation the steps of (a) providing a

plurality of capsules, where capsules include reagents for altering expression of at least one gene product, the reagents include an engineered, non-naturally occurring Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system; (b) delivering the capsules into discrete partitions containing individual cells; (c) providing a stimulus to cause the capsules to release their contents under conditions such that reagents are delivered into the individual cells. In some embodiments, the CRISPR system includes one or more vectors that include (i) a first regulatory element operably linked to at least one nucleotide sequence encoding a CRISPR-Cas system guide RNA capable of hybridizing with a target sequence in a DNA molecule of the cell, and (ii) a second regulatory element operably linked to a nucleotide sequence encoding a a RNA-guided nuclease fusion protein, wherein components (i) and (ii) are located on same or different vectors of the system. In this embodiment, subsequent to application of the stimulus, the guide RNA hybridizes to the target sequence and the RNA-guided nuclease fusion protein interferes with the expression of the at least one gene product, whereby expression of the at least one gene product is altered. In a fifth aspect, there is provided a method for altering gene expression in a plurality of cells, the method comprising: (a) providing a plurality of capsules, wherein capsules comprise reagents for altering expression of at least one gene product, the reagents comprising an engineered, non-naturally occurring Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system comprising one or more vectors comprising: (i) a first regulatory element operably linked to at least one nucleotide sequence encoding a CRISPR-Cas system guide RNA capable of hybridizing with a target sequence in the DNA molecule of the cell, and (ii) a second regulatory element operably linked to a nucleotide sequence encoding a RNA-guided nuclease fusion protein, wherein components (i) and (ii) are located on same or different vectors of the system, (b) delivering the capsules into discrete partitions containing individual cells; (c) providing a stimulus to cause the capsules to release their contents under conditions such that reagents are delivered into the individual cells, wherein subsequent to application of the stimulus, the guide RNA hybridizes to the target sequence and the RNA-guided nuclease fusion protein interferes with the expression of the at least one gene product, whereby expression of the at least one gene product is altered. In some embodiments, the RNA-guided nuclease fusion protein includes a deactivated RNA-guided nuclease and a transcription activator or a transcription repressor. In certain embodiments, the nuclease fusion protein includes a deactivated RNA-guided nuclease and an epigenetic modifier.

[0036] In some embodiments of the third, fourth and fifth aspects, the RNA-guided nuclease is a Cas9 protein or a Cpf1 protein.

[0037] In some embodiments of the third, fourth and fifth aspects, the different capsules include guide RNAs that are capable of hybridizing to different target sequences within the individual cells, such that expression of different gene products is altered in different cells.

[0038] In further embodiments, the plurality of capsules includes about 500 to about 100,000 capsules. In some embodiments, the plurality of capsules includes about 10,000 to about 50,000 capsules. In other embodiments, the plurality of capsules comprises about 15,000 to about 30,000 capsules, where only a single capsule is delivered into each discrete partition.

5. The method of claim 2, wherein the second regulatory element is operably linked to a nucleotide sequence encoding a deactivated RNA-guided nuclease, whereby the guide RNA targets the target sequence and the deactivated RNA-guided nuclease interferes with the transcription of a nucleic acid encoding the at least one gene product, whereby expression of the at least one gene product is altered.

6. The method of claim 2, wherein the second regulatory element is operably linked to a nucleotide sequence encoding a RNA-guided nuclease fusion protein, whereby the guide RNA targets the target sequence and the RNA-guided nuclease fusion protein interferes with the expression of the at least one gene product, whereby expression of the at least one gene product is altered.

7. The method of claim 6, wherein the RNA-guided nuclease fusion protein comprises a deactivated RNA-guided nuclease and a transcription activator or a transcription repressor.

8. The method of claim 6, wherein the nuclease fusion protein comprises a deactivated RNA-guided nuclease and an epigenetic modifier.

9. The method of claim 1, wherein the RNA-guided nuclease is a Cas9 protein or a Cpf1 protein.

10. The method of claim 1, wherein the capsules are configured to release their contents upon the application of a stimulus.

11. The method of claim 10, wherein the stimulus is selected from a chemical stimulus, an electrical stimulus, a thermal stimulus, a magnetic stimulus, a change in pH, a change in ion concentration, reduction of disulfide bonds, a photostimulus, and combinations thereof.

12. The method of claim 1, wherein the plurality of capsules comprises about 100-100,000 different reagents for altering expression of at least one gene product, such that different individual cells receive different reagents.

13. The method of claim 1, wherein the capsules further comprise one or more additives for compatibility of the capsules or their contents with the individual cells.

14. The method of claim 13, wherein the one or more additives comprises a transfection agent.

15. The method of claim 2, wherein the reagents for altering expression of at least one gene product further comprise oligonucleotides that comprise a nucleic acid barcode sequence, and wherein different individual cells receive different nucleic acid barcode sequences.

16. The method of claim 2, wherein the reagents for altering expression of at least one gene product further comprise a pair of Cas9 nickases or Cas9 fusion proteins that improve specificity of the CRISPR system as compared to when RNA-guided nucleases are used.

17. The method of claim 2, wherein the reagents for altering expression of at least one gene product further comprise agents that increase frequency of homologous recombination in the cell by repressing genes involved in non-homologous end-joining (NHEJ) pathway.

18. The method of claim 17, wherein the agents comprise a Cas9 nuclease or a nuclease-null Cas9 protein encoded with the at least one nucleotide sequence encoding a CRISPR system guide RNA.

19. A method for delivering a reagent to a cell, the method comprising:

(a) providing the reagent releasably coupled to a microcapsule;

(b) separating the microcapsule into a discrete partition, wherein the discrete partition further comprises an individual cell; and

(c) releasing the reagent under conditions that enable uptake of the reagent into the cell.

20. The method of claim 19, wherein the reagent comprises a vector encoding at least one of a RNA-guided nuclease, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs), or CRISPR guide RNA capable of hybridizing with one or more target sequences in a DNA molecule of the cell, and one or more condition-inducible promoters.

21. A method for altering gene expression in a plurality of cells, the method comprising:

(a) providing a plurality of capsules, wherein capsules comprise reagents for altering expression of at least one gene product, the reagents comprising an engineered, non-naturally occurring Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system comprising one or more vectors comprising:

(i) a first regulatory element operably linked to at least one nucleotide sequence encoding a CRISPR-Cas system guide RNA capable of hybridizing with a target sequence in the DNA molecule of the cell, and

(ii) a second regulatory element operably linked to a nucleotide sequence encoding a RNA-guided nuclease protein, wherein components (i) and (ii) are located on same or different vectors of the system,

(b) delivering the capsules into discrete partitions containing individual cells;

(c) providing a stimulus to cause the capsules to release their contents under conditions such that reagents are delivered into the individual cells,

wherein subsequent to application of the stimulus, the guide RNA hybridizes to the target sequence and the RNA-guided nuclease protein cleaves the DNA

molecule containing the target sequence, whereby expression of the at least one gene product is altered.

22. A method for altering gene expression in a plurality of cells, the method comprising:

(a) providing a plurality of capsules, wherein capsules comprise reagents for altering expression of at least one gene product, the reagents comprising an engineered, non-naturally occurring Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system comprising one or more vectors comprising:

(i) a first regulatory element operably linked to at least one nucleotide sequence encoding a CRISPR-Cas system guide RNA capable of hybridizing with a target sequence in the DNA molecule of the cell, and

(ii) a second regulatory element operably linked to a nucleotide sequence encoding a deactivated RNA-guided nuclease, wherein components (i) and (ii) are located on same or different vectors of the system,

(b) delivering the capsules into discrete partitions containing individual cells;

(c) providing a stimulus to cause the capsules to release their contents under conditions such that reagents are delivered into the individual cells,

wherein subsequent to application of the stimulus, the guide RNA hybridizes to the target sequence and the deactivated RNA-guided nuclease interferes with the transcription of a nucleic acid encoding the at least one gene product, whereby expression of the at least one gene product is altered.

23. A method for altering gene expression in a plurality of cells, the method comprising:

(a) providing a plurality of capsules, wherein capsules comprise reagents for altering expression of at least one gene product, the reagents comprising an engineered, non-naturally occurring Clustered Regularly Interspaced Short

Palindromic Repeats (CRISPR) system comprising one or more vectors comprising:

(i) a first regulatory element operably linked to at least one nucleotide sequence encoding a CRISPR-Cas system guide RNA capable of hybridizing with a target sequence in the DNA molecule of the cell, and

(ii) a second regulatory element operably linked to a nucleotide sequence encoding a RNA-guided nuclease fusion protein, wherein components (i) and (ii) are located on same or different vectors of the system,

(b) delivering the capsules into discrete partitions containing individual cells;

(c) providing a stimulus to cause the capsules to release their contents under conditions such that reagents are delivered into the individual cells,

wherein subsequent to application of the stimulus, the guide RNA hybridizes to the target sequence and the RNA-guided nuclease fusion protein interferes with the expression of the at least one gene product, whereby expression of the at least one gene product is altered.