CLAIMS

What is claimed:

1. A method of delivering reagents into individual cells, the method comprising:

   (a) providing a plurality of beads, wherein the beads are attached to reagents for altering expression of at least one gene product in a cell, wherein the reagents comprise:

   (i) a first regulatory element operably linked to at least one nucleotide sequence encoding a CRISPR system guide RNA, and

   (ii) a second regulatory element operably linked to a nucleotide sequence encoding an RNA-guided nuclease or an RNA-guided nuclease fusion protein;

   (b) delivering the beads into discrete partitions comprising a single cell, wherein each of the discrete partitions is a droplet or a microwell;

   (c) causing the beads to release the reagents into the discrete partitions under conditions enabling uptake of the reagents by the individual cells,

   thereby delivering the reagents into the individual cells.

2. The method of claim 1, wherein the second regulatory element is operably linked to a nucleotide sequence encoding a RNA-guided nuclease, whereby the guide RNA targets a target sequence in a DNA molecule of the individual cells and the RNA-guided nuclease cleaves the DNA molecule, whereby expression of the at least one gene product is altered.

3. The method of claim 2, wherein the reagents for altering gene expression further comprise a donor nucleic acid that is inserted into the DNA molecule following cleavage of the DNA molecule by the RNA-guided nuclease.

4. The method of claim 1, wherein the second regulatory element is operably linked to a nucleotide sequence encoding a deactivated RNA-guided nuclease, whereby the guide RNA targets a target sequence in a DNA molecule of the individual cells 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.

5. The method of claim 1, wherein the second regulatory element is operably linked to a nucleotide sequence encoding a RNA-guided nuclease fusion protein, whereby the guide RNA targets a target sequence in a DNA molecule of the individual cells 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.

6. The method of claim 5, wherein the RNA-guided nuclease fusion protein comprises a deactivated RNA-guided nuclease and a transcription activator or a transcription repressor.

7. The method of claim 5, wherein the nuclease fusion protein comprises a deactivated RNA-guided nuclease and an epigenetic modifier.

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

9. The method of claim 1, wherein the beads are configured to release the reagents upon the application of a stimulus.

10. The method of claim 9, 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.

11. The method of claim 1, wherein the plurality of beads comprises 100-100,000 different guide RNAs for altering expression of at least one gene product, such that different cells receive different guide RNAs.

12. The method of claim 1, 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 cells receive different nucleic acid barcode sequences.

13. The method of claim 1, 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.

14. The method of claim 1, 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.

15. The method of claim 14, 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.

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

(a) providing the reagent releasably coupled to a bead,

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;

(b) separating the bead into a discrete partition, wherein the discrete partition is a microwell or droplet that comprises a single cell; and

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

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

(a) providing a plurality of beads, wherein each bead is attached to 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 a 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 beads into discrete partitions, wherein the partitions are droplets or microwells that contain individual cells;

(c) providing a stimulus to cause the beads to release the reagents 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.

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

(a) providing a plurality of beads, wherein each of the beads is attached to 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 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,

(b) delivering the beads into discrete partitions, wherein each of the partitions are microwells or droplets that contain a single cell of the plurality of cells;

(c) providing a stimulus to cause the beads to release the reagents 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.

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

(a) providing a plurality of beads, wherein each of the beads is attached to 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 a 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 beads into discrete partitions, wherein each of the partitions are microwells or droplets that contain a single cell of the plurality of cells;

(c) providing a stimulus to cause the beads to release the reagents 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.