

CLAIMS

What is claimed is:

1. A method of expressing a therapeutic sequence in a target airway cell, the method
5 comprising:
 - (i) exposing the target airway cell to a synthetic circular DNA vector comprising the therapeutic sequence, wherein the target airway cell is in electrical communication with an electrode; and
 - (ii) transmitting one or more pulses of electrical energy through the electrode at
10 conditions suitable for electrotransfer of the synthetic circular DNA vector into the target airway cell, thereby expressing the therapeutic sequence.
2. The method of claim 1, wherein the synthetic circular DNA vector comprises a therapeutic sequence having a 5' end and a 3' end, wherein the 5' end is connected to the 3'
15 end by a non-bacterial sequence.
3. The method of claim 2, wherein the non-bacterial sequence corresponds to a restriction enzyme cut site overhang.
- 20 4. The method of claim 1, wherein the synthetic circular DNA vector is a naked synthetic circular DNA vector.
5. The method of claim 1, wherein the synthetic circular DNA vector is encapsulated in, or associated with, a particulate structure.
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6. The method of claim 5, wherein the particulate structure is a liposome.
7. The method of claim 1, wherein the therapeutic sequence comprises a therapeutic protein-encoding sequence.
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8. The method of claim 7, wherein the therapeutic protein is cystic fibrosis transmembrane receptor (CFTR).
9. The method of claim 1, wherein the target airway cell is an airway epithelial cell.

10. The method of claim 1, wherein the conditions suitable for electrotransfer of the therapeutic agent into the target airway cell comprise a voltage at the target lung cell of less than 2,400 V.
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11. The method of any one of claims 1-10, wherein 1-6 pulses of electrical energy are transmitted.
12. The method of any one of claims 1-11, wherein the total number of pulses of electrical energy are transmitted within 1-20 seconds.
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13. The method of claim 11, wherein a single pulse of electrical energy is transmitted.
14. The method of any one of claims 1-13, wherein the one or more pulses of electrical energy have an amplitude from 100 V to 5,000 V.
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15. The method of any one of claims 1-14, wherein each of the pulses of electrical energy is from 0.01 to 200 milliseconds in duration.
16. The method of claim 15, wherein each of the pulses of electrical energy is from 10 to 50 milliseconds in duration.
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17. The method of any one of claims 1-16, wherein the expression level of the therapeutic sequence from the synthetic circular DNA vector is greater than expression level of a therapeutic sequence from a reference plasmid DNA vector under analogous conditions.
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18. The method of any one of claims 1-17, wherein the target airway cell has a mutation in a gene correctable by the therapeutic sequence.
19. The method of any one of claims 1-18, wherein step (ii) is performed *in situ* in an individual by inserting a catheter comprising the electrode into an airway lumen of the individual.
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20. The method of claim 19, wherein the airway lumen is trachea, bronchi, or bronchiole.

21. The method of claim 19 or 20, wherein the individual has been diagnosed with a disease or disorder.
- 5 22. The method of claim 21, wherein the disease or disorder is a monogenic respiratory disease.
23. The method of claim 22, wherein the monogenic respiratory disease is cystic fibrosis.
- 10 24. The method of any one of claims 1-23, wherein the synthetic circular DNA vector has been administered to the individual locally.
25. The method of claim 24, wherein the local administration was intranasal administration or intramuscular administration.
- 15 26. The method of claim 24, wherein the local administration was by flood, spray, or aerosolization of the synthetic circular DNA vector.
27. The method of any one of claims 1-23, wherein the synthetic circular DNA vector
- 20 has been administered to the individual systemically.
28. The method of any one of claims 1-27, wherein the method further comprises administering the synthetic circular DNA vector.
- 25 29. The method of any one of claims 1-28, wherein partial airway epithelial ablation therapy has preceded the administration of the synthetic circular DNA vector.
- 30 30. The method of claim 29, wherein the partial airway epithelial ablation was administered using a pulsed electric field.
31. The method of any one of claims 1-30, wherein the method further comprises administering the partial airway epithelial ablation therapy.
32. The method of any one of claims 1-31, wherein the target airway cell is a human cell.

33. A method of delivering a therapeutic agent to a target airway cell in an individual, the method comprising:

- 5 (i) inserting a catheter comprising a monopolar electrode into an airway lumen of the individual;
- (ii) positioning the electrode within a target region of the airway lumen comprising the target airway cell and the therapeutic agent;
- 10 (iii) while the electrode is within the target region, transmitting one or more pulses of electrical energy through the electrode at conditions suitable for electrotransfer of the therapeutic agent into the target airway cell.

34. The method of claim 33, wherein the individual has been treated with a partial airway epithelial ablation therapy.