WHAT IS CLAIMED:

1. A method of recombinantly engineering a *Leishmania* cell comprising
(a) introducing a first DNA fragment and a second DNA fragment into the *Leishmania* cell, and
(b) incubating the *Leishmania* cell to allow homologous recombination of the first and the second DNA fragments,
wherein the first DNA fragment comprises a 5' homologous region and/or a 3' homologous region; and
wherein the first DNA fragment comprises a 5' homologous region and/or a 3' homologous region; and
wherein the 5' homologous region of the first DNA fragment is homologous to the 3' homologous region of the second DNA fragment, or the 3' homologous region of the first DNA fragment is homologous to the 5' homologous region of the second DNA fragment; and
wherein: (i) the nucleotide sequence of the first DNA fragment outside the 5' homologous region and/or the 3' homologous region is not homologous to the nucleotide sequence of the second DNA fragment outside the 5' homologous region and/or the 3' homologous region; (ii) the nucleotide sequence of the first DNA fragment outside the 5' homologous region and/or the 3' homologous region, and the nucleotide sequence of the second DNA fragment outside the 5' homologous region and/or the 3' homologous region, are not homologous to a sequence in the *Leishmania* cell's genome; and/or (iii) the nucleotide sequence of the first DNA fragment outside the 5' homologous region and/or the 3' homologous region has no homologies within the first DNA fragment; and/or (iv) the nucleotide sequence of the second DNA fragment outside the 5' homologous region and/or the 3' homologous region has no homologies within the second DNA fragment.

2. The method of claim 1, wherein each of the first and the second DNA fragments comprises a 5' homologous region and/or a 3' homologous region; wherein the 5' homologous region of each of the first and the second DNA fragments is homologous to a 3' homologous region of another one of the two or more DNA fragments or the 3' homologous region of the each of the two or more DNA fragments is homologous to a 5' homologous region of another one of the two or more DNA fragments; and wherein the nucleotide sequences outside the homologous regions in each DNA fragment are not homologous to each other; are not homologous to a sequence in the *Leishmania* cell's genome; and/or have no homologies within the respective DNA fragment.

3. The method of claim 1 or claim 2, wherein the first and the second DNA fragments are suitable for integration into a chromosome of the *Leishmania* cell.

4. The method of claim 3, wherein the first and the second DNA fragments are integrated into the chromosome of the *Leishmania* cell.

5. The method of claim 4, wherein the first and the second DNA fragments are integrated into the chromosome of the Leishmania cell after the first and the second DNA fragments are recombined with each other.

6. The method of claim 4 or claim 5, wherein the first and the second DNA fragments are integrated in tandem into the paraflagellar rod protein (Pfr) locus.

7. The method of claim 4 or claim 5, wherein the first and the second DNA fragments are integrated at the start of the 18S coding region (Ssu-PolI).

8. The method of claim 1 or claim 2, wherein the first and the second DNA fragments are not integrated in a chromosome of the *Leishmania* cell.

9. The method of claim 8, wherein the homologous recombination of the first and the second DNA fragments results in a circular plasmid.

10. The method of any one of claims 1 to 9, comprising:
in part (a), introducing three or more DNA fragments into the *Leishmania* cell, and
in part (b) incubating the *Leishmania* cell to allow homologous recombination of the three or more DNA fragments,
wherein each of the three or more DNA fragments comprises a 5' homologous region and/or a 3' homologous region.

11. The method of any one of claims 1 to 10, wherein the nucleotide sequence of (i) the first DNA fragment outside the homologous region, (ii) the second DNA fragment outside the homologous region, (iii) both of the first and the second DNA fragments outside the homologous region, and/or (iv) all of the three or more DNA fragments outside the homologous region, is at least 10 nucleotides, at least 20 nucleotides, at least 30 nucleotides, at least 40 nucleotides, at least 50

nucleotides, at least 100 nucleotides, at least 200 nucleotides, at least 300 nucleotides, at least 400 nucleotides, at least 500 nucleotides, at least 600 nucleotides, at least 700 nucleotides, at least 800 nucleotides, at least 900 nucleotides, at least 1000 nucleotides, at least 2000 nucleotides, at least 5000 nucleotides, at least 10000 nucleotides, at least 15000 nucleotides, at least 20000 nucleotides, at least 25000 nucleotides, at least 30000 nucleotides, at least 35000 nucleotides, at least 40000 nucleotides, at least 45000 nucleotides, or at least 50000 nucleotides in length.

12. The method of any one of claims 1 to 11, wherein the nucleotide sequence of (i) the first DNA fragment outside the homologous region, (ii) the second DNA fragment outside the homologous region, (iii) both of the first and the second DNA fragments outside the homologous region, and/or (iv) all of the three or more DNA fragments outside the homologous region, is between 10 and 50,000 nucleotides in length.

13. The method of any one of claims 1 to 12, wherein the nucleotide sequences of (i) both of the first and the second DNA fragments outside the homologous region and/or (ii) all of the three or more DNA fragments outside the homologous region, is between 50 and 10,000 nucleotides in length.

14. The method of any one of claims 1 to 13, wherein (i) the nucleotide sequences of both of the first and the second DNA fragments outside the homologous region and/or (ii) all of the three or more DNA fragments outside the homologous region, is between 150 and 2,500 nucleotides in length.

15. The method of any one of claims 1 to 14, wherein (i) the nucleotide sequences of both of the first and the second DNA fragments outside the homologous region and/or (ii) all of the three or more DNA fragments outside the homologous region, is between 250 and 2,000 nucleotides in length.

16. The method of any one of claims 1 to 15, wherein the homologous recombination of the DNA fragments results in a nucleotide sequence that is 50 nucleotides to 100 nucleotides, 100 nucleotides to 500 nucleotides, 500 nucleotides to 1000 nucleotides, 1000 nucleotides to 5000 nucleotides, 5000 nucleotides to 10000 nucleotides, 10000 nucleotides to 15000 nucleotides, 15000 nucleotides to 20000 nucleotides, 20000 nucleotides to 25000 nucleotides, 25000

nucleotides to 30000 nucleotides, 30000 nucleotides to 35000 nucleotides, 35000 nucleotides to 40000 nucleotides, 40000 nucleotides to 45000 nucleotides, 45000 nucleotides to 50000 nucleotides, 50000 nucleotides to 55000 nucleotides, 55000 nucleotides to 60000 nucleotides, 60000 nucleotides to 65000 nucleotides, 65000 nucleotides to 70000 nucleotides, 70000 nucleotides to 75000 nucleotides, or 75000 nucleotides to 80000 nucleotides in length.

17. The method of any one of claims 1 to 16, wherein the homologous recombination of the DNA fragments results in a nucleotide sequence that is 100 nucleotides to 500 nucleotides in length.

18. The method of any one of claims 1 to 16, wherein the homologous recombination of the DNA fragments results in a nucleotide sequence that is 500 nucleotides to 1000 nucleotides in length.

19. The method of any one of claims 1 to 16, wherein the homologous recombination of the DNA fragments results in a nucleotide sequence that is 1000 nucleotides to 5000 nucleotides in length.

20. The method of any one of claims 1 to 16, wherein the homologous recombination of the DNA fragments results in a nucleotide sequence that is 5000 nucleotides to 10000 nucleotides in length.

21. The method of any one of claims 1 to 20, wherein the 5' homologous region and/or the 3' homologous region of (i) the first DNA fragment, (ii) the second DNA fragment, and/or (iii) all of the three or more DNA fragments, is at least 10 nucleotides, at least 20 nucleotides, at least 30 nucleotides, at least 40 nucleotides, at least 50 nucleotides, at least 100 nucleotides, at least 150 nucleotides, at least 200 nucleotides, at least 250 nucleotides, at least 300 nucleotides, at least 350 nucleotides, at least 400 nucleotides, at least 450 nucleotides, or at least 500 nucleotides in length.

22. The method of any one of claims 1 to 21, wherein the 5' homologous region and/or the 3' homologous region of (i) the first DNA fragment, (ii) the second DNA fragment, and/or (iii) all of the three or more DNA fragments, is at least 90 nucleotides in length.

23. The method of any one of claims 1 to 22, wherein the 5' homologous region and/or the 3' homologous region of (i) the first DNA fragment, (ii) the second DNA fragment, and/or (iii) all of the three or more DNA fragments, is at most 500 nucleotides, at most 550 nucleotides, at most 600 nucleotides, at most 650 nucleotides, at most 700 nucleotides, at most 750 nucleotides, at most 800 nucleotides, at most 850 nucleotides, at most 900 nucleotides, at most 950 nucleotides, at most

1000 nucleotides, at most 1200 nucleotides, at most 1400 nucleotides, at most 1600 nucleotides, at most 1800 nucleotides, at most 2000 nucleotides, at most 2200 nucleotides, at most 2400 nucleotides, at most 2600 nucleotides, at most 2800 nucleotides, at most 3000 nucleotides, at most 3200 nucleotides, at most 3400 nucleotides, at most 3600 nucleotides, at most 3800 nucleotides, at most 4000 nucleotides, at most 4200 nucleotides, at most 4400 nucleotides, at most 4600 nucleotides, at most 4800 nucleotides, or at most 5000 nucleotides in length.

24. The method of any one of claims 1 to 23, wherein the 5' homologous region of the first DNA fragment and the 3' homologous region of the second DNA fragment have at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% sequence identity.

25. The method of any one of claims 1 to 24, wherein the 3' homologous region of the first DNA fragment and the 5' homologous region of the second DNA fragment have at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% sequence identity.

26. The method of any one of claims 1 to 23, wherein the 5' homologous region of the first DNA fragment and the 3' homologous region of the second DNA fragment have at least 95%, or 100% sequence identity, or wherein the 3' homologous region of the first DNA fragment and the 5' homologous region of the second DNA fragment have at least 95%, or 100% sequence identity.

27. The method of any one of claims 1 to 26, wherein the first and the second DNA fragments, and/or each of the three or more DNA fragments, are introduced by transfection.

28. The method of any one of claims 1 to 26, wherein the two or more DNA fragments are introduced concurrently.

29. The method of any one of claims 1 to 28, wherein the number of DNA fragments introduced in part (a), and incubated in part (b), is at least 4.

30. The method of any one of claims 1 to 29, wherein the number of DNA fragments introduced

in part (a), and incubated in part (b), is 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50.

31. The method of any one of claims 1 to 30, wherein the nucleotide sequences of the first and the second DNA fragments, and/or each of the three or more DNA fragments, outside the homologous region are selected from a group consisting of intergenic regions (IRs), untranslated regions (UTRs), and open reading frames (ORFs) encoding polypeptides.

32. The method of claim 31, wherein the IRs, UTRs and ORFs are devoid of homologous sequences within itself, and/or homologous sequences to one another.

33. The method of any one of claims 1 to 32, wherein the nucleotide sequences of the first and the second DNA fragments, and/or each of the three or more DNA fragments, outside the homologous region encode the same polypeptide.

34. The method of claim 33, wherein the *Leishmania* cell is capable of expressing multiple copies of the same polypeptide.

35. The method of claim 33 or claim 34, wherein the method increases the expression level of the polypeptide.

36. The method of any one of claims 1 to 35, wherein the homologous recombination of the DNA fragments results in a nucleotide sequence comprising at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100% of genetic information encoded by the first and the second DNA fragments, and/or the three or more DNA fragments.

37. The method of any one of claims 1 to 36, wherein the homologous recombination of the DNA fragments results in a nucleotide sequence comprising at least 90% or 100% of genetic information encoded by the first and the second DNA fragments, and/or the three or more DNA fragments.

38. The method of any one of claims 1 to 37, wherein the *Leishmania* cell is *Leishmania tarentolae*.

39. A method of making a polypeptide comprising (a) recombinantly engineering a *Leishmania*

cell according to the method of any one of claims 1 to 38; (b) culturing the *Leishmania* cell of under suitable conditions for polypeptide production, wherein the nucleotide sequences of the first and the second DNA fragments, and/or the three or more DNA fragments, outside the homologous region comprise open reading frames (ORFs) encoding the polypeptide; and (c) isolating the polypeptide.