

CLAIMS

What is claimed is:

1. A method for controlling the transcription of a polynucleotide of interest in a cell, wherein the method comprises
 - I. maintaining a cell in a medium without an effective amount of a ligand of both an activator and a repressor, wherein the cell comprises:
 - (A) a promoter operably linked to a polynucleotide of interest and controlled by a first operator operably linked and positioned 5' with respect to the promoter;
 - (B) a polynucleotide encoding the activator;
 - (C) a second operator; and
 - (D) a polynucleotide encoding the repressor,wherein transcription of the polynucleotide of interest is inhibited in the absence of the ligand of both the activator and the repressor; and
 - II. controlling the cell to transcribe the polynucleotide of interest by maintaining the cell in a medium with an effective amount of the ligand of both the activator and the repressor.
2. The method according to claim 1, wherein the second operator is operably linked and positioned 3' with respect to the promoter and 5' with respect to the polynucleotide of interest.
3. The method according to claim 1, wherein the activator binds to the first operator in the presence of the ligand to permit transcription of the polynucleotide of interest.

4. A method for controlling the transcription of a polynucleotide of interest in a cell, wherein the method comprises

I. maintaining a cell in a medium without an effective amount of a ligand of an activator (activator ligand) and with an effective amount of ligand of a repressor (repressor ligand), wherein the cell comprises:

(A) a promoter operably linked to a polynucleotide of interest and controlled by a first operator operably linked and positioned 5' with respect to the promoter;

(B) a polynucleotide encoding the activator;

(C) a second operator; and

(D) a polynucleotide encoding the repressor,

wherein transcription of the polynucleotide of interest is inhibited in the absence of the activator ligand and the presence of the repressor ligand; and

II. controlling the cell to transcribe the polynucleotide of interest by maintaining the cell in a medium with an effective amount of the activator ligand and without an effective amount of the repressor ligand.

5. The method according to claim 4, wherein the second operator is operably linked and positioned 3' with respect to the promoter and 5' with respect to the polynucleotide of interest.

6. A method for controlling the transcription of a polynucleotide of interest in a cell, wherein the method comprises maintaining a cell in a medium without an effective amount of a first ligand of a first regulatory fusion protein (RFP) and with an effective amount of second ligand of a second RFP, wherein the cell comprises

(A) a promoter;

(B) an Arc operator; and

(C) a polynucleotide encoding a reverse tetracycline transactivator fusion protein (rtTA), wherein (A), (B) and (C) are operably linked, and wherein transcription of the rtTA polynucleotide is controlled by a fusion protein comprising an Arc repressor binding domain and an estrogen receptor ligand binding domain (ArcEr); wherein rtTA can control the transcription of a polynucleotide of interest.

7. The method according to claim 6, wherein the first ligand is selected from the group consisting of tetracycline and doxycycline.

8. The method according to claim 6, wherein the second ligand is selected from the group consisting of estrogen, estradiol (E2), tamoxifen, and 4-hydroxytamoxifen (OHT).

9. A cell capable of controlled transcription of at least one polynucleotide of interest, wherein the cell comprises:

(A) a promoter operably linked to a polynucleotide of interest and controlled by a first operator operably linked and positioned 5' with respect to the promoter;

(B) a polynucleotide encoding an activator;

(C) a second operator; and

(D) a polynucleotide encoding a repressor,

wherein transcription of the polynucleotide of interest is inhibited in the absence of a ligand of both the activator and the repressor, and is permitted in the presence of the ligand of both the activator and the repressor.

10. A cell capable of controlled transcription of a polynucleotide of interest, wherein the cell comprises

(A) a promoter operably linked to a polynucleotide of interest and controlled by a first operator operably linked and positioned 5' with respect to the promoter;

(B) a polynucleotide encoding an activator;

(C) a second operator; and

(D) a polynucleotide encoding a repressor;

wherein transcription of the polynucleotide of interest is inhibited in the absence of an effective amount of an activator ligand and the presence of an effective amount of a repressor ligand; and permitted in the presence of an effective amount of the activator ligand and the absence of an effective amount of the repressor ligand.

11. The cell according to claim 10, wherein the second operator is operably linked and positioned 3' with respect to the promoter and 5' with respect to the polynucleotide of interest.

12. The cell according to claim 10, wherein the activator binds to the first operator in the presence of the activator ligand to permit transcription of the polynucleotide of interest.

13. A cell capable of controlled transcription of a polynucleotide of interest, wherein the cell comprises:

(A) a promoter operably linked to a polynucleotide of interest and controlled by a first operator operably linked and positioned 5' with respect to the promoter;

(B) a polynucleotide encoding a first regulatory fusion protein (RFP), where the first RFP comprises:

- (1) a transcription activating domain fused to a first DNA binding domain; and
- (2) a ligand-binding domain; wherein the first ligand is capable of binding to the ligand-binding domain of the first RFP, and wherein the DNA binding domain of the first RFP is capable of binding to the operator positioned 5' when in the presence of the first ligand;

(C) a second operator; and

(D) a polynucleotide encoding the second RFP that differs from the first RFP, wherein the second RFP comprises:

- (1) a DNA-binding domain; and
- (2) a ligand-binding domain; wherein the second ligand is capable of binding to the ligand-binding domain of the second RFP, and wherein the second RFP is capable of binding to the second operator in the presence of the second ligand; wherein transcription of the polynucleotide is inhibited in the absence of an effective amount of the first ligand and in the presence of an effective amount of the second ligand and is permitted in the presence of an effective amount of the first ligand and absence of an effective amount of the second ligand.

14. The cell according to claim 13, wherein the second operator is operably linked and positioned 3' with respect to the promoter and 5' with respect to the polynucleotide sequence encoding the protein of interest.

15. The cell according to claim 13, wherein the second operator is operably linked to the polynucleotide sequence encoding the first RFP, and the second RFP can control transcription of the polynucleotide encoding the first RFP.

16. A cell capable of controlled transcription of a polynucleotide of interest, wherein the cell comprises

(A) a promoter operably linked to a polynucleotide of interest and controlled by a first operator operably linked and positioned 5' with respect to the promoter;

(B) a polynucleotide encoding a regulatory fusion protein (RFP), wherein the RFP comprises:

(1) a transcription activating domain fused to a DNA binding domain; and

(2) a ligand-binding domain; wherein the ligand is capable of binding to the ligand-binding domain of the RFP, and wherein the DNA binding domain of the RFP is capable of binding to the first operator when in the presence of the ligand; and

(C) a second operator; and

(D) a polynucleotide encoding a repressor protein, wherein the repressor protein can bind to the second operator only in the absence of the ligand,

wherein transcription of the polynucleotide of interest is inhibited in the absence of an effective amount of the ligand of both the activator and the repressor,

and is permitted in the presence of an effective amount of the ligand of both the activator and the repressor.

17. The cell according to claim 16, wherein the second operator is operably linked and positioned 3' with respect to the promoter and 5' with respect to the polynucleotide of interest.

18. A cell capable of controlled transcription of a polynucleotide of interest, wherein the cell comprises

(A) a promoter;

(B) an Arc operator; and

(C) a polynucleotide encoding a reverse tetracycline transactivator fusion protein (rtTA), wherein (A), (B) and (C) are operably linked, wherein transcription of the rtTA polynucleotide is controlled by a fusion protein comprising an Arc repressor binding domain and an estrogen receptor ligand binding domain (ArcEr), and wherein rtTA can control the transcription of a polynucleotide of interest.

19. A bioreactor comprising (i) a cell according to claim 9 and (ii) media.

20. A bioreactor comprising (i) a cell according to claim 10 and (ii) media.

21. A bioreactor comprising (i) a cell according to claim 13 and (ii) media.

22. A bioreactor comprising (i) a cell according to claim 16 and (ii) media.

23. A bioreactor comprising (i) a cell according to claim 18 and (ii) media.