CLAIMS

What is claimed is:

1. A method of gene cloning, comprising:

contacting each of a vector and a set of inserts with a pair of first terminal primers and a pair of second terminal primers, , wherein:

the set of insets including at least a first insert comprising a first coding region and a second insert comprising a second coding region,

each of the first terminal primers includes a first region complementary to a region of the vector and a second region complementary to a region of the first insert,

each of the second terminal primers includes a first region complementary to a region of the vector and a second region complementary to a region of an insert different from the first insert, and

wherein each primer includes at least two phosphorothioate internucleotide linkages more than one base apart;

amplifying the vector and at least two inserts to produce a vector amplification product and at least two insert amplification products, each including the at least two phosphorothioate internucleotide linkages;

non-enzymatically cleaving the vector amplification product and the at least two insert amplification products at the at least two phosphorothioate internucleotide linkages to produce complementary single-stranded overhangs;

annealing the vector amplification product and the at least two insert amplification products in the presence of a cation and thereby non-enzymatically assembling a transforming product; and

introducing the transforming product into a host cell.

2. The method of Claim 1, wherein the transforming product is a univariant expression vector comprising:

at least the first coding region and the second coding region, wherein:

the first coding region encoding at least a first gene product, the first coding region being operably linked to a first inducible promoter, the first inducible promoter being of a first strength and being responsive to an inducer; and

the second coding region encoding at least a second gene product, the second coding region being operably linked to a second inducible promoter, the second inducible promoter being of a second strength, different from the first strength, and being responsive to the inducer,

each inducible promoter present in the expression vector differs from each other only within the melting/initialization region but comprise the same polymerase binding region.

3. The method of Claim 1, wherein the set of inserts includes at least one additional insert comprising at least one additional coding region, further including:

contacting the at least one additional insert with a pair of linking primers, wherein each of the linking primers includes a first region complementary to an insert in the set of inserts and a second region complementary to a different insert in the set of inserts;

amplifying the at least one additional insert to produce at least one additional insert amplification product;

non-enzymatically cleaving the at least one additional insert amplification product at the at least two phosphorothioate internucleotide linkages to produce complementary single-stranded overhangs;

annealing the vector amplification product, the at least two insert amplification products, and the at least one additional insert amplification product in the presence of a cation to non-enzymatically assemble the transforming product.

4. The method of Claim 3, wherein:

the complementary single-stranded overhangs are at least 14 base pairs long; and

the at least two phosphorothioate internucleotide linkages are repeated every two or more nucleotides, and

annealing the vector amplification product and the at least two gene amplification products is performed in at least about 0.5 mM of a cation comprising Mg^{2+}, Ca^{2+}, Co^{2+}, Cu^{2+}, or a combination thereof.

5. The method of Claim 2, wherein the expression vector further includes a third coding region encoding at least a third gene product, the third coding region being operably linked to a third inducible promoter, the third inducible promoter being of a third strength, different from the first strength and the second strength, and being responsive to the inducer.

6. The method of Claim 2, wherein:

the first coding region encodes at least a first enzyme, the first enzyme catalyzing a first reaction in a multi-step enzymatic pathway; and

the second coding region encodes at least a second enzyme, the second enzyme catalyzing a second reaction in the multi-step enzymatic pathway.

7. The method of Claim 6, wherein the multi-step enzymatic pathway is the lycopene synthetic pathway or the amorphadiene synthetic pathway.

8. The method of Claim 2, wherein the first and the second inducible promoters originate in a single RNA polymerase promoter.

9. The method of Claim 8, wherein the first inducible promoter comprises a mutation in a melting region or an initiation region.

10. The method of Claim 9, wherein the RNA polymerase promoter is a T7 RNA polymerase promoter, a T5 RNA polymerase promoter, a T3 RNA polymerase promoter, or an SP6 RNA polymerase promoter.

11. A method of expressing at least a first coding region and a second coding region in a cell, the method comprising:

constructing a univariant expression vector using the method of Claim 1, the univariant expression vector comprising at least the first coding region and the second coding region wherein:

the first coding region is operably linked to a first inducible promoter, the first inducible promoter being of a first strength and being responsive to an inducer,

the second coding region is operably linked to a second inducible promoter, the second inducible promoter being of a second strength, different from the first strength, and being responsive to the inducer;

providing a cell comprising the univariant expression vector and

contacting the cell with the inducer, thereby expressing the first coding region and the second coding region.

12. The method of Claim 11, wherein

the first coding region encodes at least a first enzyme, the first enzyme catalyzing a first reaction in a multi-step enzymatic pathway; and

the second coding region encodes at least a second enzyme, the second enzyme catalyzing a second reaction in the multi-step enzymatic pathway.

13. The method of Claim 12, wherein the univariant expression vector further comprises a third coding region, the third coding region being operably linked to a third inducible promoter, the third inducible promoter being of a third strength, different from the first strength and the second strength, and being responsive to the inducer.

14. A method of expressing at least a first coding region and a second coding region in a cell, the method comprising:

constructing a first and a second univariant expression vector using the method of Claim 2,

providing a cell comprising at least the first univariant expression vector comprising at least the first coding region encoding a first gene product, and at least the second univariant expression vector comprising at least the second coding region encoding a second gene product, wherein:

the first coding region is operably linked to a first inducible promoter, the first inducible promoter being of a first strength and being responsive to an inducer,

the second coding region is operably linked to a second inducible promoter, the second inducible promoter being of a second strength, different from the first strength, and being responsive to the inducer; and

contacting the cell with the inducer, thereby expressing the first coding region and the second coding region.

15. The method of claim 6, wherein a ratio of a first strength of the first inducible promoter to a second strength of the second inducible promoter is a ratio for optimally expressing the first and the second enzymes of the multi-step enzymatic pathway in a host cell.

16. The method of Claim 15, further including contacting the host cell with the inducer to induce expression of the first and the second enzymes.

17. The method of Claim 15, wherein the expression vector further comprises a third coding region operably linked to a third inducible promoter, wherein the third coding region encodes at least a third enzyme catalyzing a third reaction in the multi-step enzymatic pathway; the third inducible promoter being of a third strength, different from a first strength and a second strength, and being responsive to an inducer; and wherein a ratio of the first, the second, and the third strengths is a ratio for optimally expressing the first, the

second, and the third enzymes of the multi-step enzymatic pathway in a host cell.

18. The method of Claim 1, wherein the set of inserts includes at least one additional insert comprising at least one additional coding region, further including:

contacting the at least one additional insert with a pair of linking primers, wherein each of the linking primers includes a first region complementary to an insert in the set of inserts and a second region complementary to a different insert in the set of inserts;

amplifying the at least one additional insert to produce at least one additional insert amplification product;

non-enzymatically cleaving the at least one additional insert amplification product at the at least two phosphorothioate internucleotide linkages to produce complementary single-stranded overhangs;

annealing the vector amplification product, the at least two insert amplification products, and the at least one additional insert amplification product in the presence of a cation to non-enzymatically assemble the transforming product.