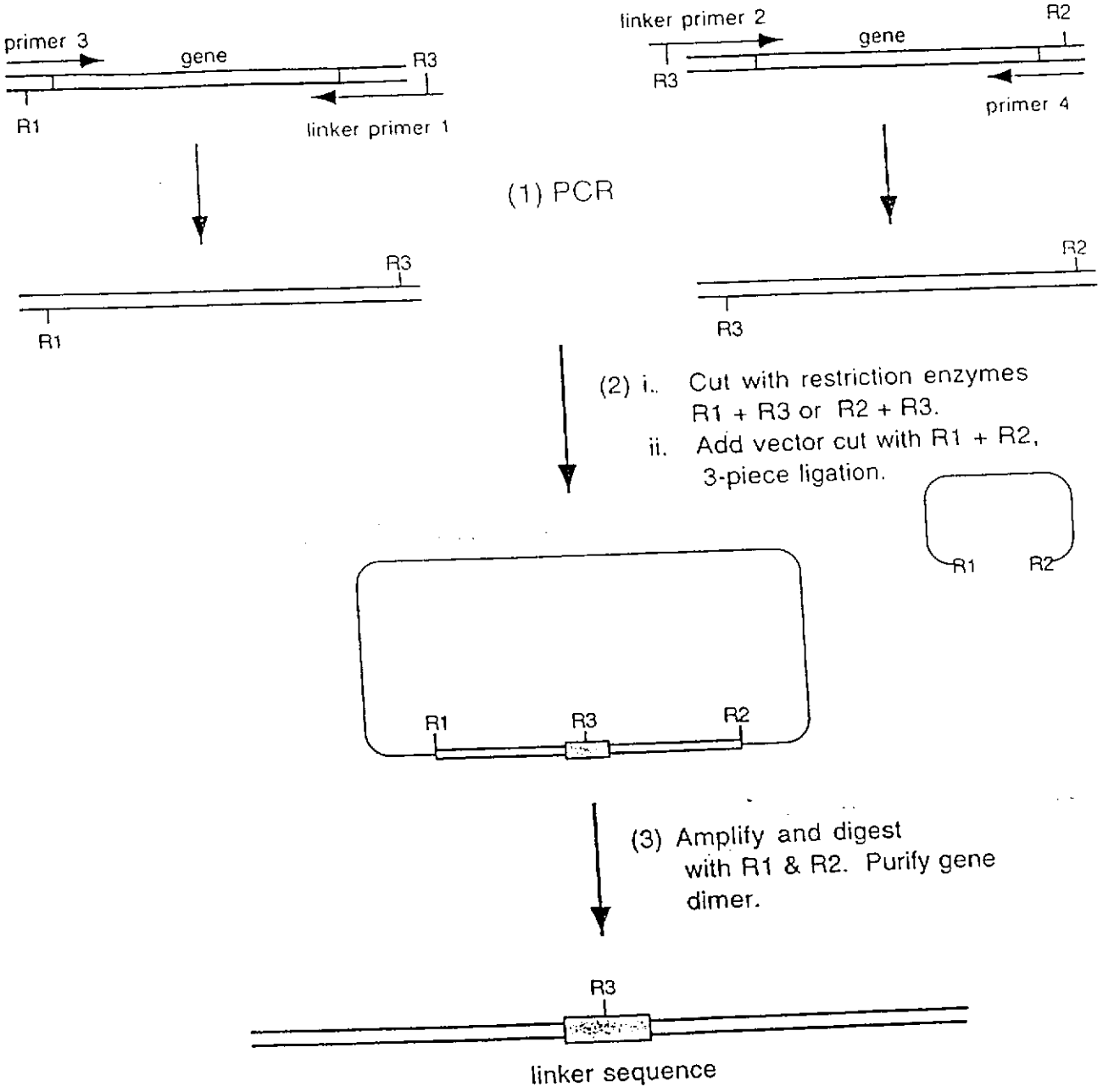


Figure 1A. Construction of a gene dimer. Dimer can be homodimer or heterodimer.

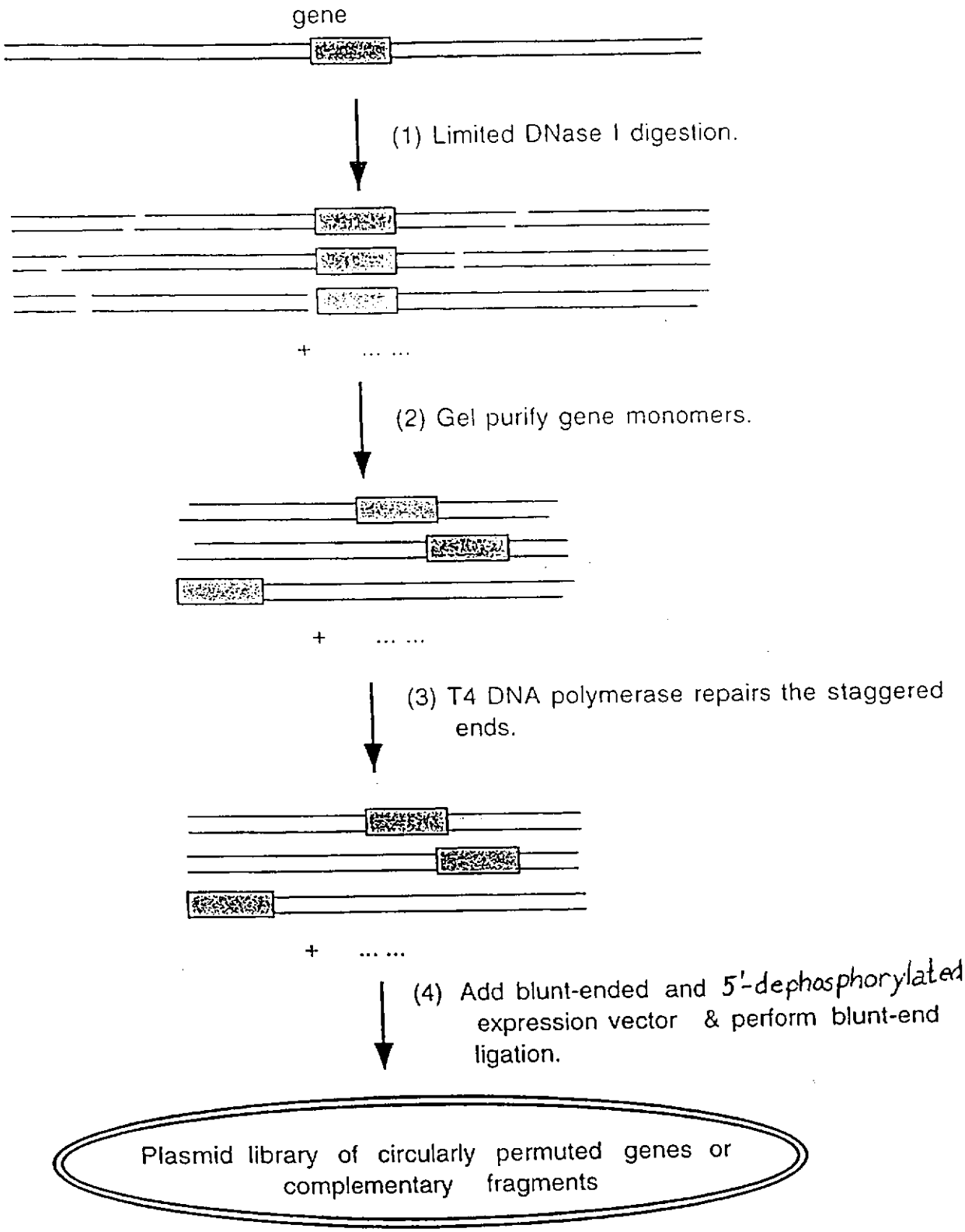
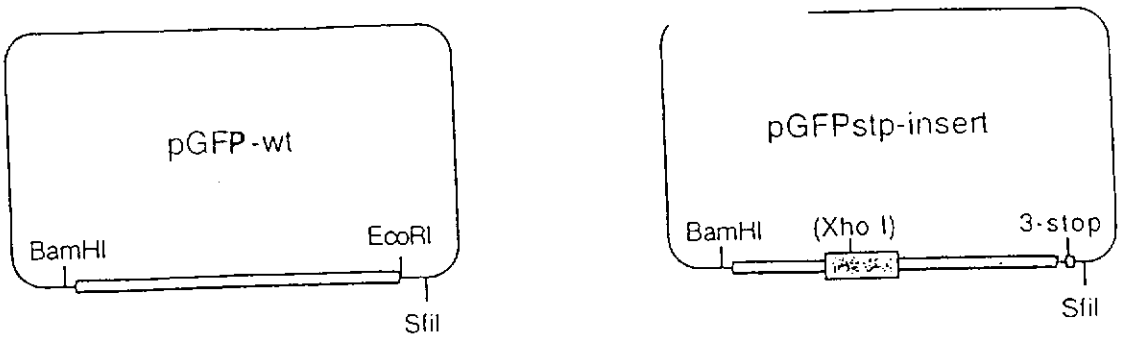


Figure 1B. Strategy for constructing a library of gene fragments corresponding in size to the size of the genes of the original protein(s). Starting gene dimer can be a homo- or heterodimer.



insert length	insert type	number of occurrences
800-850 bp	a.	8
	b.	6
	c.	-
approx. 720 bp	d.	22
	e.	-
500-650 bp	f.	-

Figure 2. Results of screening plasmids from active GFP clones by restriction digestion. A. shows the restriction sites for digestion. B. is a summary of the double-enzyme digestion results, BamHI + EcoRI, BamHI + XhoI and BamHI + SfiI. Insert types: a, intact GFP gene with an extra fragment upstream; b, intact GFP with an extra fragment downstream; c, two overlapped fragments; d, recovered wild-type-like genes; e, complementary fragments; f, truncated genes.

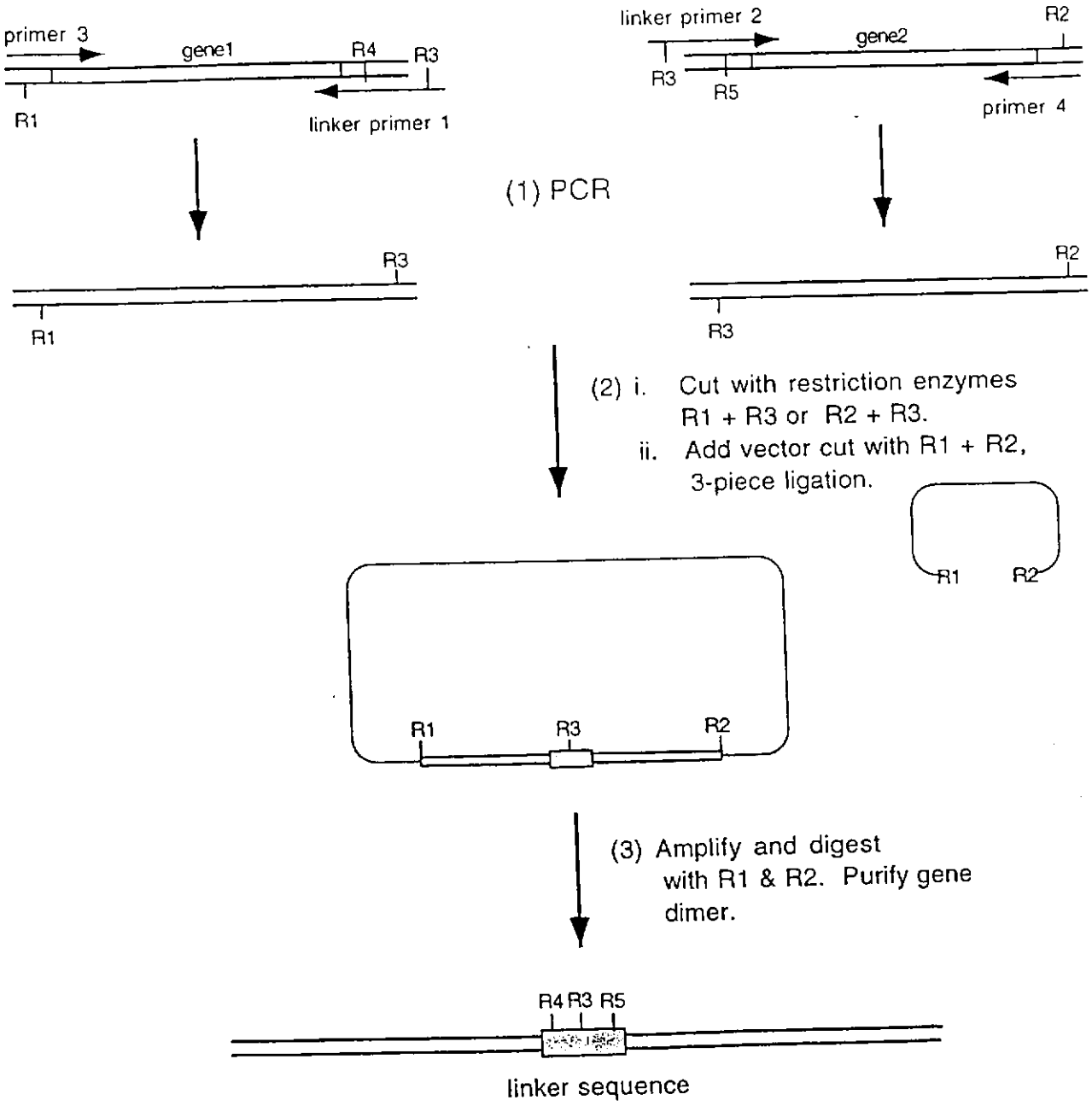


Figure 3. Construction of a gene heterodimer.

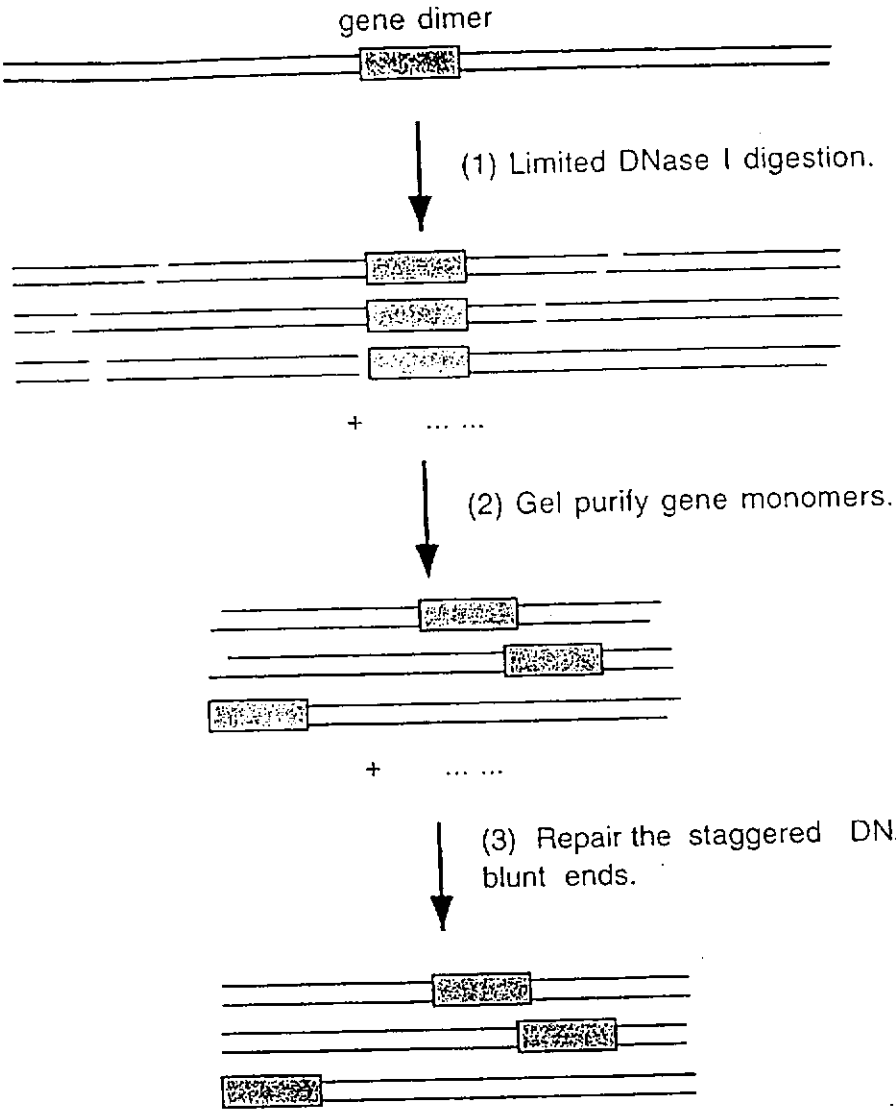


Figure 4A; Strategy for constructing a library of gene fragments corresponding in size to the size of the genes of the original proteins.

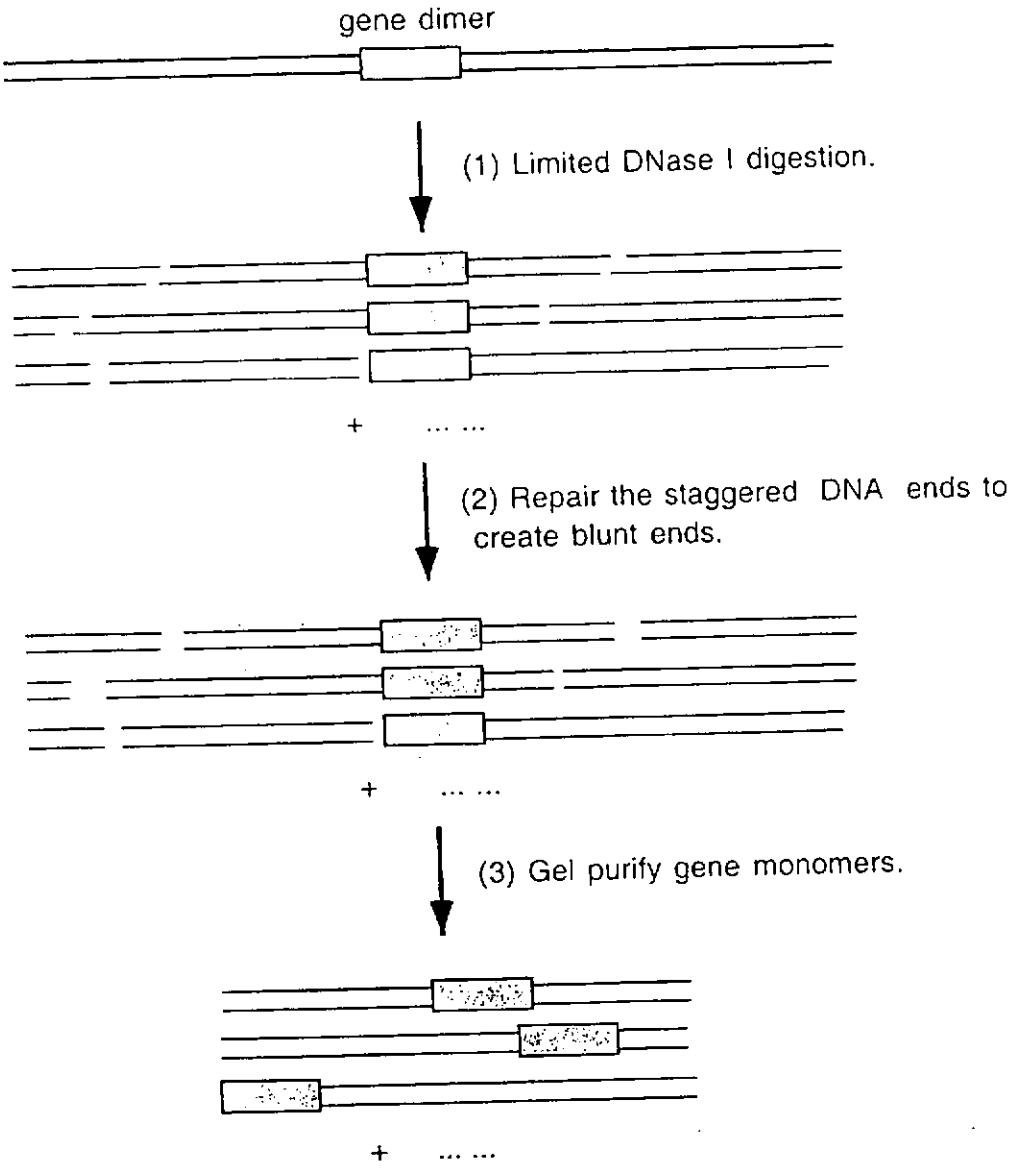


Figure 4B. Strategy for constructing a library of gene fragments corresponding in size to the size of the genes of the original proteins.

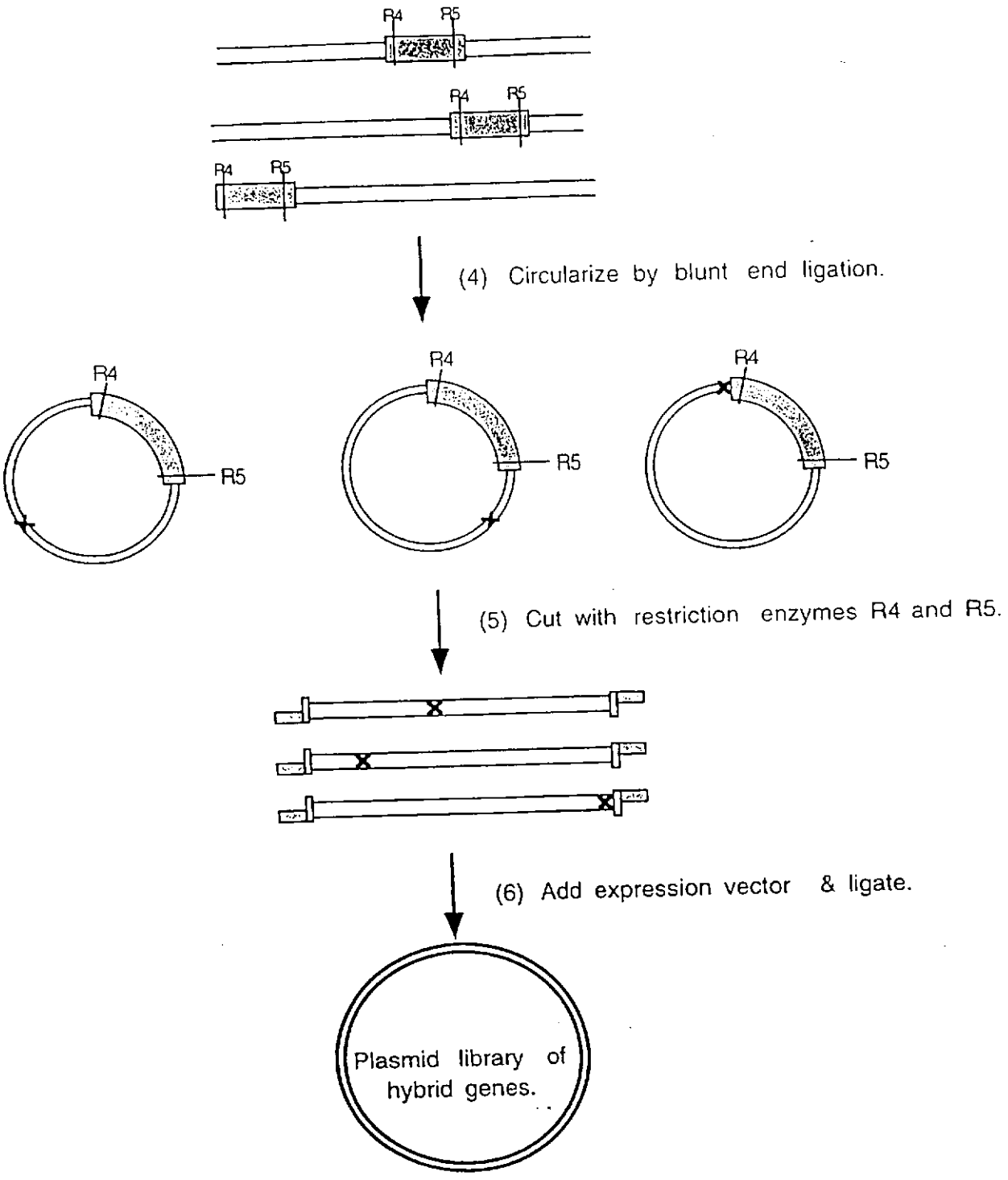


Figure 5. Strategy for constructing a library of hybrid proteins with one single crossover between the two parent proteins or of a library with small interior sequence deletions or duplications. x ... designates the position of the cross over between the two proteins. It is created by the blunt end ligation.

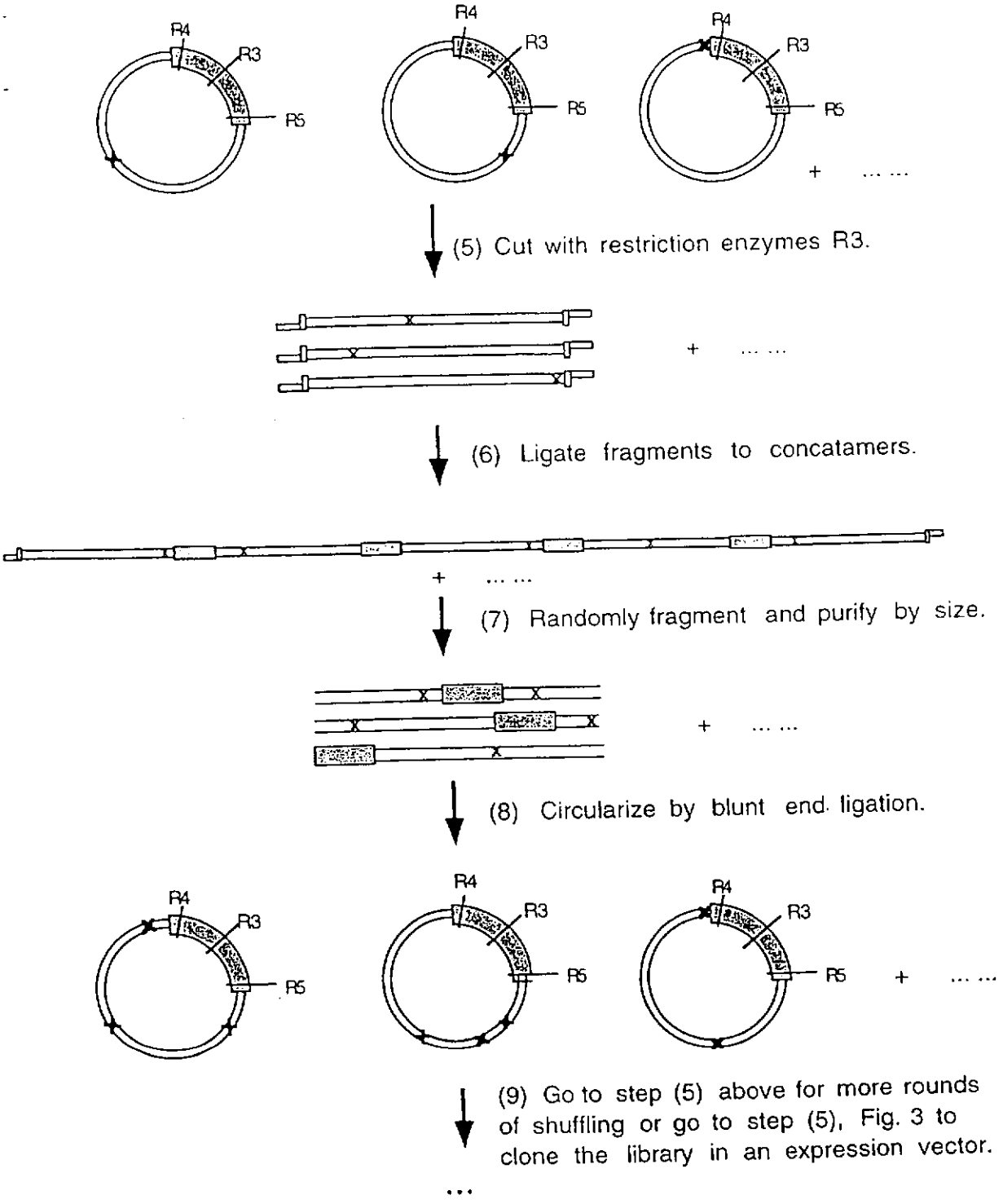


Figure 6. Strategy for constructing a library of hybrid proteins with several crossover points between two or more parent proteins.
x ... designates the position of the cross over between the different proteins.

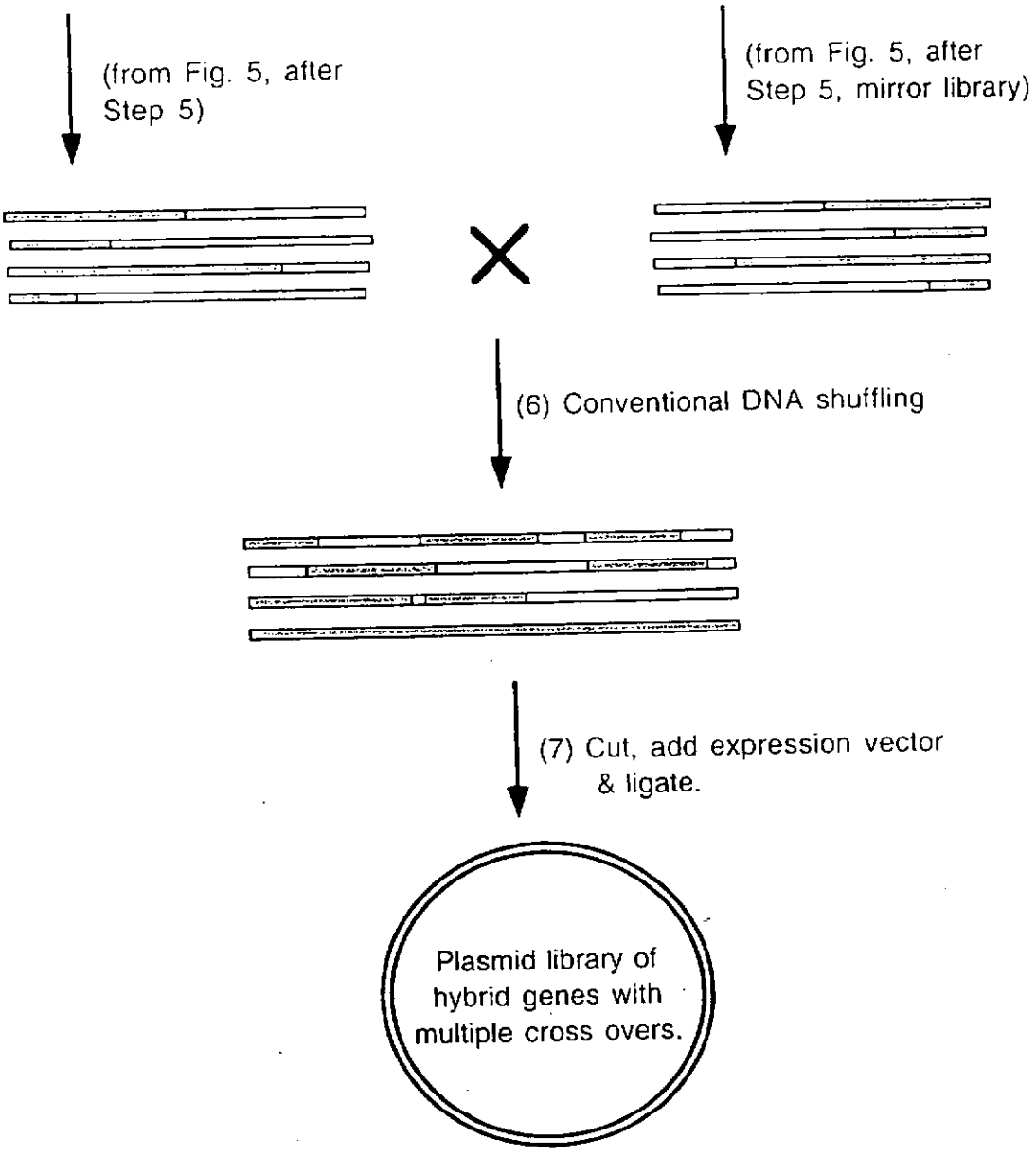


Figure 7. Another strategy for constructing a library of hybrid proteins with several crossover points between two parent proteins.

```
RC1
nt#.......10........20........30........40.46..50........60...
..ATGACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGGTGCTCAAGGGTTTGAGG
..M..T..I..K..E..M..P..Q..P..K..T..F..G..E..V..L..K..G..L..R..
AA#...........5.............10.............16..........20.....

RC2
nt#.......10........20........30........40...75...80........90
..ATGACAATTAAAGAAATGCCTCAGCCAAAACGTTTGGAGAGCCCCAAAGGCCTGAAAAGT
..M..T..I..K..E..M..P..Q..P..K..R..L..E..S..P..K..G..L..K..S..
AA#...........5.............10.............25.............30..
```

Figure 8. N-terminal nucleotide and amino acid sequences of two hybrid proteins.

```
atggctctgt tattagcagt ttttctgttc tgcctggtat tctgggtgct caagggtttg   60
aggcctcggg tcccaaagg cctgaaaagt ccaccagagc catggggctg gcccttgctc   120
gggcatgtgc tgaccctggg gaagaacccg cacctggcac tgtcaaggat gagccagcgc   180
tacgggacg tcctgcagat ccgcattggc tccacgcccg tgctggtgct gagccgcctg   240
gacaccatcc ggcaggccct ggtgcggcag ggcgacgatt tcaaggccg gcctgacctc   300
tacacctcca ccctcatcac tgatggccag agcttgacct tcagcacaga ctctggaccg   360
gtgtgggctg cccgccggcg cctggcccag aatgccctca acaccttctc catcgcctct   420
gacccagctt cctcatcctc ctgctacctg gaggagcatg tgagcaagga ggctaaggcc   480
ctgatcagca ggttgcagga gctgatggca gggcctgggc acttcgaccc ttacaatcag   540
gtgtgggtgt cagtggccaa cgtcattggt gccatgtgct tcggacagca cttccctgag   600
agtagcgatg agatgctcag cctcgtgaag aacactcatg agttcgtgga gactgcctcc   660
tccgggaacc ccctggactt cttccccatc cttcgctacc tgcctaaccc tgccctgcag   720
aggttcaagg ccttcaacca gaggttcctg tggttcctgc agaaaacagt ccaggagcac   780
tatcaggact ttgacaagaa cagtgtccgg gacatcacgg gtgccctgtt caagcacagc   840
aagaagggcc ctagagccag cggcaacctc atcccacagg agaagattgt caaccttgtc   900
aatgacatct ttggagcagg atttgacaca gtcaccacag ccatctcctg gagcctcatg   960
taccttgtga ccaagcctga gatacagaga aggatccaga aggagctgga cactgtgatt   1020
ggcagggagc ggcggccccg gctctctgac agaccccagc tgccctactt ggaggccttc   1080
atcctggaga ccttccgaca ctcctccttc ttgccccttca ccatccccca cagcacaaca   1140
agggacacaa cgctgaatgg cttctacatc cccaagaaat gctgtgtctt cgtaaaccag   1200
tggcaggtca accatgaccc agagctgtgg gaggacccct ctgagttccg gcctgagcgg   1260
ttcctcaccg ccgatggcac tgccattaac aagccccttga gtgagaagat gatgctgttt   1320
ggcatgggca agcgccggtg tatcgggggaa gtcctggcca agtgggagat cttcctcttc   1380
ctgggccatcc tgctacagca actggagttc agcgtgccgc cgggcgtgaa agtcgacctg   1440
acccccatct acgggctgac catgaagcac gcccgctgtg aacatgtcca ggcgcggctg   1500
cgcttctcca tcaat   1515
```

Figure 9. Human cytochrome P450 1A2 with a modified N-terminal.

```
atgacaatta aagaaatgcc tcagccaaaa acgtttggag agcttaaaaa tttaccgtta   60
ttaaacacag ataaaccggt tcaagctttg atgaaaattg cggatgaatt aggagaaatc  120
tttaaattcg aggcgcctgg tcgtgtaacg cgctacttat caagtcagcg tctaattaaa  180
gaagcatgcg atgaatcacg cttttgataa aacttaagtc aagcgcttaa attgtacgt   240
gatttgcag gagacgggtt ggccacaagc tggacgcatg aaaaaaattg gaaaaaagcg  300
cataatatct tacttccaag cttcagtcag caggcaatga aaggctatca tgcgatgatg  360
gtcgatatcg ccgtgcagct tgttcaaaag tgggagcgtc taaatgcaga tgagcatatt  420
gaagtaccgg aagacatgac acgtttaacg cttgatacga ttggtctttg cggctttaac  480
tatcgcttta acagctttta ccgagatcag cctcatccat ttattacaag tatggtccgt  540
gcactggatg aagcaatgaa caagctgcag cgagcaaatc cagacgaccc agcttatgat  600
gaaaacaagc gccagtttca agaagatatc aaggtgatga acgacctagt agataaaatt  660
attgcagatc gcaaagcaag cggtgaacaa agcgatgatt tattaacgca catgctaaac  720
ggaaaagatc cagaaacggg tgagccgctt gatgacgaga acattcgcta tcaaattatt  780
acattcttaa ttgcgggaca cgaaacaaca agtggtcttt tatcatttgc gctgtatttc  840
ttagtgaaaa atccacatgt attacaaaaa gcagcagaag aagcagcacg agttctagta  900
gatcctgttc caagctacaa acaagtcaaa cagcttaaat atgtcggcat ggtcttaaac  960
gaagcgctgc gcttatggcc aactgctcct gcgtttttccc tatatgcaaa agaagatacg 1020
gtgcttggag gagaatatcc tttagaaaaa ggcgacgaac taatggttct gattcctcag 1080
cttcaccgtg ataaaacaat ttggggagac gatgtggaag agttccgtcc agagcgtttt 1140
gaaaatccaa gtgcgattcc gcagcatgcg ttaaaccgt ttggaaacgg tcagcgtgcg 1200
tgtatcggtc agcagttcgc tcttcatgaa gcaacgctgg tacttggtat gatgctaaaa 1260
cactttgact ttgaagatca tacaaactac gagctggata ttaaagaaac tttaacgtta 1320
aaacctgaag gctttgtggt aaaagcaaaa tcgaaaaaaa ttccgcttgg cggtattcct 1380
tcacctagca ct                                                     1392
```

Figure 10. Heme domain of mutant bacterial cytochrome P450 BM3.

```
atgacaatta aagaaatgcc tcagccaaaa acgtttggag aggtgctcaa gggtttgagg   60
cctcgggtcc ccaaaggcct gaaaagtcca ccagagccat ggggctggcc cttgctcggg  120
catgtgctga ccctggggaa gaacccgcac ctggcactgt caaggatgag ccagcgctac  180
ggggacgtcc tgcagatccg cattggctcc acgcccgtgc tggtgctgag ccgcctggac  240
accatccggc aggccctggt gcggcagggc gacgatttca agggccggcc tgacctctac  300
acctccaccc tcatcactga tggccagagc ttgaccttca gcacagactc tggaccggtg  360
tgggctgccc gccggcgcct ggcccagaat gccctcaaca ccttctccat cgcctctgac  420
ccagcttcct catcctcctg ctacctggag gagcatgtga gcaaggaggc taaggccctg  480
atcagcaggt tgcaggaggt gatggcaggg cctgggcact tcgaccctta caatcaggtg  540
gtggtgtcag tggccaacgt cattggtgcc atgtgcttcg gacagcactt ccctgagagt  600
agcgatgaga tgctcagcct cgtgaagaac actcatgagt tcgtggagac tgcctcctcc  660
gggaacccct tggacttctt cccatcctct cgctacctgc ctaaccctgc cctgcagagg  720
ttcaaggcct tcaaccagag gttcctgtgg ttcctgcaga aaacagtcca ggagcactat  780
caggactttg acaagaacag tgtccgggac atcacgggtg ccctgttcaa gcacagcaag  840
aaggggccta gagccagcgg caacctcatc ccacaggaga agattgtcaa ccttgtcaat  900
gacatctttg gagcaggatt tgacacagtc accacagcca tctcctggag cctcatgtac  960
cttgtgacca agcctgagat acagaggaag atccagaagg agctggacac tgtgattggc 1020
agggagcggc ggccccggct ctctgacaga ccccagctgc cctacttgga ggccttcatc 1080
ctggagacct tccgacactc ctccttcttg cccttcacca tccccacag cacaacaagg 1140
gacacaacgc tgaatggctt ctacatcccc aagaaatgct gtgtcttcgt aaaccagtgg 1200
caggtcaacc atgacccaga gctgtgggag gaccctctg agttccggcc tgaggcgttc 1260
ctcaccgccg atggcactgc cattaacaag cccttgagtg agaagatgat gctgtttggc 1320
atgggcaagc gccggtgtat cgggaagtc ctggccaagt gggagatctt cctcttccctg 1380
gccatcctgc tacagcaact ggagttcagc gtgccgcccgg gcgtgaaagt cgacctgacc 1440
cccatctacg ggctgaccat gaagcacgcc cgctgtgaac atgtccaggc gcggctgcgc 1500
ttctccatca at                                                     1512
```

Figure 11. Hybrid polynucleotide according to the invention (RC1).

```
atgacaatta aagaaatgcc tcagccaaaa cgtttggaga gccccaaagg cctgaaaagt   60
ccaccagagc catgggctg gcccttgctc gggcatgtgc tgaccctggg gaagaacccg  120
cacctggcac tgtcaaggat gagccagcgc tacgggggacg tcctgcagat ccgcattggc 180
tccacgcccg tgctggtgct gagccgcctg gacaccatcc ggcaggccct ggtgcggcag  240
ggcgacgatt tcaaggccg gcctgacctc tacacctcca ccctcatcac tgatggccag  300
agcttgacct tcagcacaga ctctggaccg gtgtgggctg cccgccggcg cctggcccag 360
aatgccctca acacttctc catcgcctct gacccagctt cctcatcctc ctgctacctg  420
gaggagcatg tgagcaaggc ggctaaggcc ctgatcagca ggttgcagga gctgatggca 480
gggcctgggc acttcgaccc ttacaatcag gtggtggtgt cagtggccaa cgtcattggt  540
gccatgtgct tcggacagca ctttccctgag agtagcgatg agatgctcag cctcgtgaag 600
aacactcatg agttcgtgga gactgcctcc tccgggaacc ccctggactt cttccccatc  660
cttcgctacc tgcctaaccc tgccctgcag aggttcaagg ccttcaacca gaggttcctg  720
tggttcctgc agaaaacagt ccaggagcac tatcaggact ttgacaagaa cagtgtccgg 780
gacatcacgg gtgccctgtt caagcacagc aagaagggggc ctagagccag cggcaacctc 840
atcccacagg agaagattgt caaccttgtc aatgacatct ttggagcagg atttgacaca  900
gtcaccacag ccatctcctg gagcctcatg taccttgtga ccaagcctga gatacagagg  960
aagatccaga aggagctgga cactgtgatt ggcagggagc ggcggccccg gctctctgac 1020
agaccccagc tgccctactt ggaggccttc atcctggaga ccttccgaca ctcctccttc 1080
ttgcccttca ccatccccca cagcacaaca agggacacaa cgctgaatgg cttctacatc 1140
ccaagaaat gctgtgtctt cgtaaaccag tggcaggtca accatgaccc agagctgtgg 1200
gaggaccccct ctgagttccg gcctgagccg ttcctcaccg ccgatggcac tgccattaac 1260
aagcccttga gtgagaagat gatgctgttt ggcatgggca agcgccggtg tatcgggggaa 1320
gtcctggcca agtgggagat cttcctcttc ctggccatcc tgctacagca actggagttc 1380
agcgtgccgc cgggcgtgaa agtcgacctg accccatct acggggctgac catgaagcac 1440
gcccgctgtg aacatgtcca ggcgcggctg cgcttctcca tcaat                  1485
```

Figure 12. Hybrid polynucleotide according to the invention (RC2).

```
atgacaatta aagaaatgcc tcagccaaaa acgtttggag agcttaaaaa tttaccgtta   60
ttaaacacag ataaaccggt tcaagctttg atgaaaattg cggatgaatt aggagaaatc  120
tttaaattcg aggcgcctgg tcgtgtaacg cgctacttat caagtcagcg tctaattaaa  180
gaagcatgcg atgaatcacg cttgataaa aacttaagtc aagcgcttaa atttgtacgt   240
gatttgcag gagacgggtt ggccacaagc tggacgcatg aaaaaaattg gaaaaaagcg   300
cataatatct tacttccaag cttcagtcag caggcaatga aaggctatca tgcgatgatg  360
gtcgatatcg ccgtgcagct tgttcaaaag tgggagcgtc taaatgcaga tgagcatatt  420
gaagtaccgg aagacatgac acgtttaacg cttgatacga ttggtctttg cggctttaac  480
tatcgcttta acagctttta ccgagatcag cctcatccat ttattacaag tatggtccgt  540
gcactggatg aagcaatgaa caagctgcag cgagcaaatc cagacgaccc agcttatgat  600
gaaaacaagc gccagtttca agaagatatc aaggtgatga acgacctagt agataaaatt  660
attgcagatc gcaaagcaag cggtgaacaa agcgatgatt tattaacgca catgctaaac  720
ggaaaagatc cagaaacggg tgagccgctt gatgacgaga acattcgcta tcaaattatt  780
acattcttaa ttgcgggaca cgaaacaaca agtggtcttt tatcatttgc gctgtatttc  840
ttagtgaaaa atccacatgt attacaaaaa gcagcagaag aagcagcacg agttctagta  900
gatcctgttc caagctacaa acaagtcaaa cagcttaaat atgtcggcat ggtcttaaac  960
gaagcgctgc gcttatggcc aactgctcct gcgtttttccc tatatgtcaa agaagatacg 1020
gtgcttggag gagaatatcc tttagaaaaa ggcgacgaac taatggttct gattcctcag 1080
cttcaccgtg ataaaacaat ttgggggagac gatgtggaag agttccgtcc agagcgtttt 1140
gaaaatccaa gtgcgattcc gcagcatgcg tttaaaccgt ttggacccct ctgagttccg 1200
gcctgagcgg ttcctcaccg ccgatggcac tgccattaac aagcccttga gtgagaagat 1260
gatgctgttt ggcatgggca agcgccggtg tatcgggggaa gtcctgggca agtgggagat 1320
cttcctcttc ctggccatcc tgctacagca actggagttc agcgtgccgc cgggcgtgaa 1380
agtcgacctg accccatct acgggctgac catgaagcac gcccgctgtg aacatgtcca 1440
ggcgcggctg cgcttctcca tcaat                                      1465
```

Figure 13. Hybrid polynucleotide according to the invention (RC3).

```
atgacaatta aagaaatgcc tcagccaaaa acgtttggag agcttaaaaa tttaccgtta   60
ttaaacacag ataaaccggt tcaagctttg atgaaaattg cggatgaatt aggagaaatc  120
tttaaattcg aggcgcctgg tcgtgtaacg cgctacttat caagtcagcg tctaattaaa  180
gaagcatgcg atgaatcacg ctttgataaa aacttaagtc aagcgcttaa atttgtacgt  240
gatttgcag gagacgggtt ggccacaagc tggacgcatg aaaaaaattg gaaaaaagcg  300
cataatatct tacttccaag cttcagtcag caggcaatga aaggcccgcc ggcgcctggc  360
ccaggatgcc ctcaacacct tctccatcgc ctccatgacc gcttcctcat cctcctgcta  420
cctggaggag catgtgagca aggaggctaa ggccctgatc agcaggttgc aggagctgat  480
ggcagggcct gggcacttcg accttacaa tcaggtggtg gtgtcagtgg ccaacgtcat  540
tggtgccatg tgcttcggac agcacttccc tgagagtagc gatgagatgc tcagcctcgt  600
gaagaacact catgagttcg tggagactgc ctcctccggg aacccctgg acttcttccc  660
catccttcgc tacctgccta accctgccct gcagaggttc aaggccttca accagaggtt  720
cctgtggttc ctgcagaaaa cagtccagga gcactatcag gactttgaca agaacagtgt  780
ccgggacatc acgggtgccc tgttcaagca cagcaagaag gggcctagag ccagcggcaa  840
cctcatccca caggagaaga ttgtcaacct tgtcaatgac atctttggag caggatttga  900
cacagtcacc acagccatct cctggagcct catgtaccta gtgaccaagc ctgagataca  960
gaggaagatc cagaaggagc tggacactgt gattggcagg gagcggcggc cccggctctc 1020
tgacagaccc cagctgccct acttggaggc cttcatcctg gagacgttcc gacactcctc 1080
cttcttgccc ttcaccatcc cccacagcac aacaagggac acaacgctga atggcttcta 1140
catccccaag aaatgctgtg tcttcgtaaa ccagtggcag gtcaaccatg acccagagct 1200
gtgggaggac ccctctgagt tccggccctga gcggttcctc accgccgatg gcactgccat 1260
taacaagccc ttgagtgaga agatgatgct gtttggcatg ggcaagcgcc ggtgtatcgg 1320
ggaagtcctg gccaagtggg agatcttcct ctcctgctgc atcctgctac agcaactgga 1380
gttcagcgtg ccgcgggcg tgaaagtcga cctgacccccc atctacgggc tgaccatgaa 1440
gcacgccccgc tgtgaacatg tccaggcgcg gctgcgcttc tccatcaat             1489
```

Figure 14. Hybrid polynucleotide according to the invention (RC4).

```
atgacaatta aagaaatgcc tcagccaaaa acgtttggag agcttaaaaa ttaccgtta    60
atgacaatta aagaaatgcc tcagccaaaa acgtttggag agcttaaaaa ttaccgtta   120
ttaaacacag ataaaccggt tcaagctttg atgaaaattg cggatgaatt aggagaaatc  180
ttaaaattcg aggcgccctgg tcgtgtaacg cgctacttat caagtcagcg tctaattaaa 240
gaagcatgcg atgaatcacg ctttgataaa aacttaagtc aagcgcttaa atttgtacgt  300
gatttgcag gagacgggtt ggccacaagc tggacgcatg aaaaaaattg gaaaaaagcg   360
cataatatct tacttccaag cttcagtcag caggcaatga aaggctatca tgcgatgatg  420
gtcgatatcg ccgtgcagct tgttccaagg gccggcctga cctctacacc tccaccctca  480
tcactgatgg ccagagcttg acctcagca cagactctgg accggtgtgg gctgcccgcc   540
ggcgcctggc ccagaatgcc ctcaacacct tctccatcgc ctctgaccca gcttcctcat  600
cctcctgcta cctggaggag catgtgagca aggaggctaa ggccctgatc agcaggttgc  660
aggagctgat ggcagggcct gggcacttcg accttacaa tcaggtggtg gtgtcagtgg   720
ccaacgtcat tggtgccatg tgcttcggac agcacttccc tgagagtagc gatgagatgc  780
tcagcctcgt gaagaacact catgagttcg tggagactgc ctcctccggg aacccctgg   840
acttcttccc catcctcgc tacctgccta accctgccct gcagaggttc aaggccttca  900
accagaggtt cctgtggttc ctgcagaaaa cagtccagga gcactatcag gactttgaca  960
agaacagtgt ccgggacatc acgggtgccc tgttcaagca cagcaagaag gggcctagag 1020
ccagcggcaa cctcatccca caggagaaga ttgtcaacct tgtcaatgac atctttggag 1080
caggatttga cacagtcacc acagccatct cctggagcct catgtacctt gtgaccaagc 1140
ctgagataca gaggaagatc cagaaggagc tggacactgt gattggcagg gagcggcggc 1200
cccggctctc tgacagaccc cagctgccct acttggaggc cttcatcctg gagaccttcc 1260
gacactcctc cttcttgccc ttcaccatcc cccacagcac aacaagggac acaacgctga 1320
atggcttcta catccccaag aaatgctgtg tcttcgtaaa ccagtggcag gtcaaccatg 1380
acccagagct gtgggaggac ccctctgagt tccggcctga gcggttcctc accgccgatg 1440
gcactgccat taacaagccc ttgagtgaga agatgatgct gtttggcatg ggcaagcgcc 1500
ggtgtatcgg ggaagtccctg gccaagtggg agatcttcct cttcctggcc atcctgctac 1560
agcaactgga gttcagcgtg ccgccgggcg tgaaagtcga cctgacccccc atctacgggc 1620
tgaccatgaa gcacgcccgc tgtgaacatg tccaggcgcg gctgcgcttc tccatcaat   1679
```

Figure 15. Hybrid polynucleotide according to the invention (RC5).