A Step 1: Synthesize Building Blocks (BBs) from oligonucleotides

70 nt

20 nt

PCR →

Building Block
~750 bp

Ligate →

Transform →

B Step 2: Assemble 2-4 kb minichunks

RE 1

2

3

4 RE

Shuttle vector

Cotransform →

RE

40 bp

1 2 3 4 RE
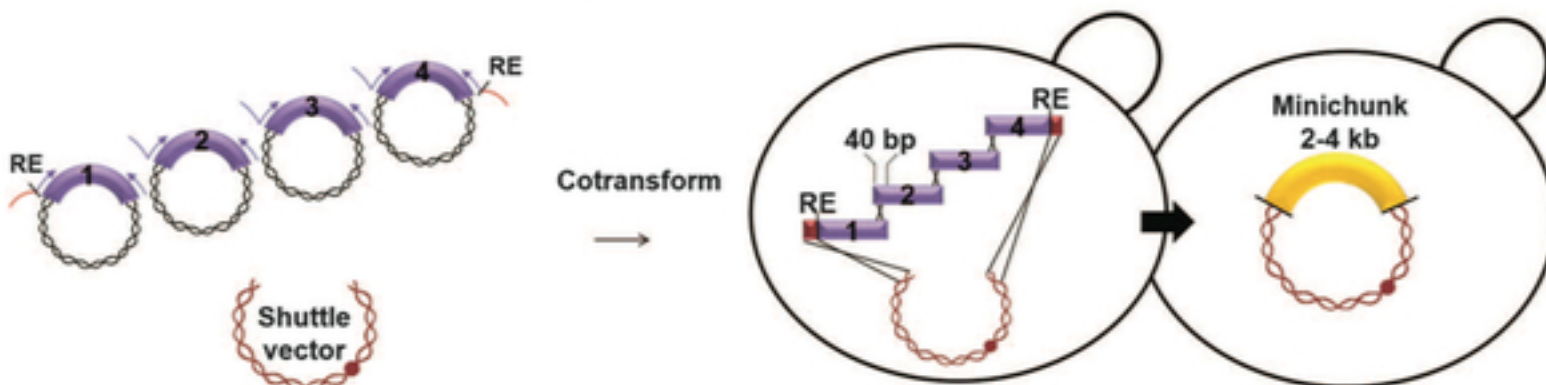
Minichunk
2-4 kb

C Step 3: Replace native *III* with minichunks

Yeast transformation 1

KAN^R

[G418^R, Leu^−, Ura^−]

III

UTC-left

1 ~750 bp

2

2-4 kb

3

4 LEU2   Linker L1

↓

1 2 3 4 LEU2   III

[G418^S, Leu^+, Ura^−]

Yeast transformation 2

[G418^S, Leu^+, Ura^−]

1 2 3 4 LEU2   III

4

5

6

7

8

9

10

11

12

13 URA3   Linker L2

↓

1 2 3 4 5 6 7 8 9 10 11 12 13 URA3   III

[G418^S, Leu^−, Ura^+]