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Direct cloning and refactoring of a silent lipopeptide biosynthetic gene cluster yields the antibiotic taromycin A

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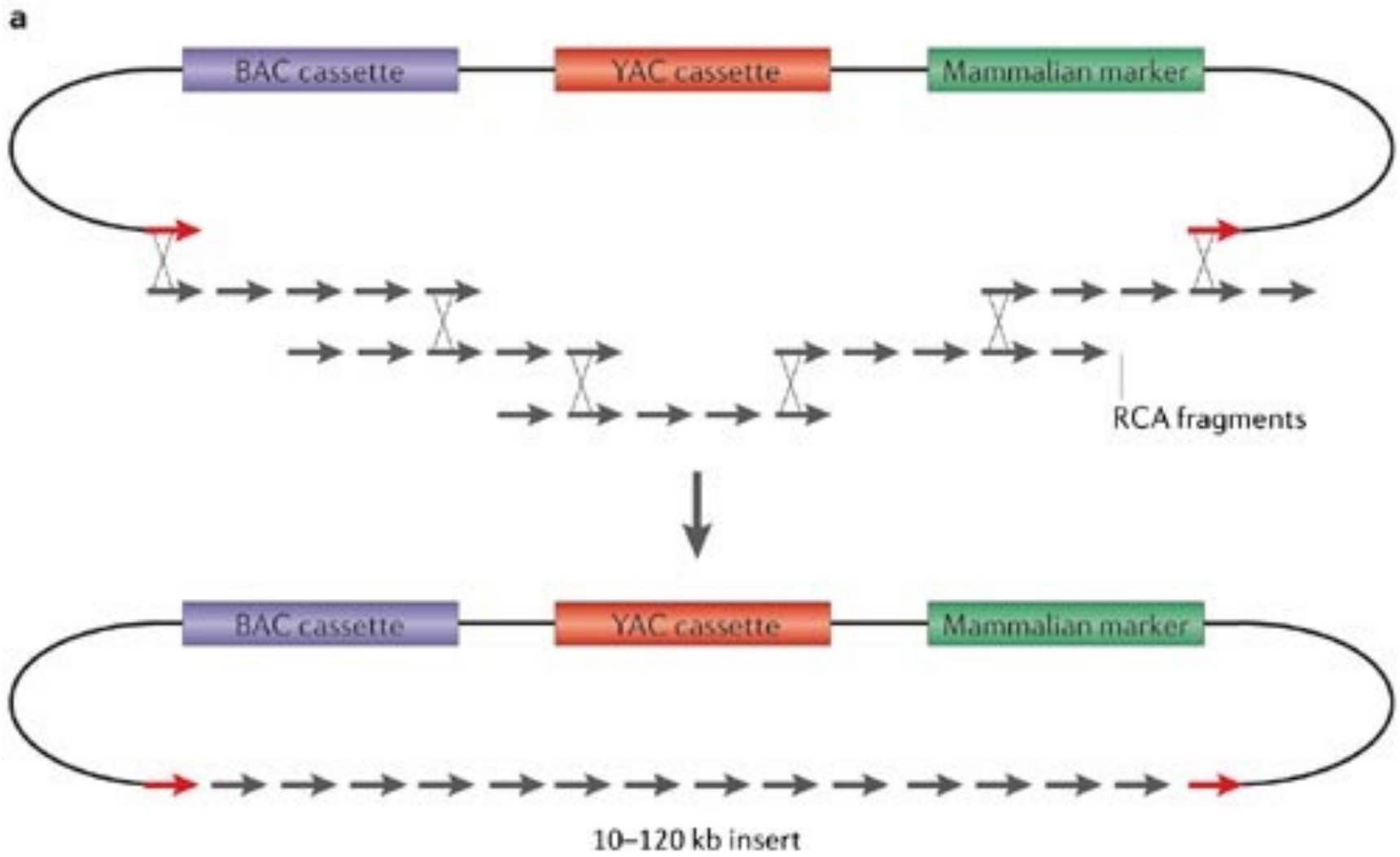
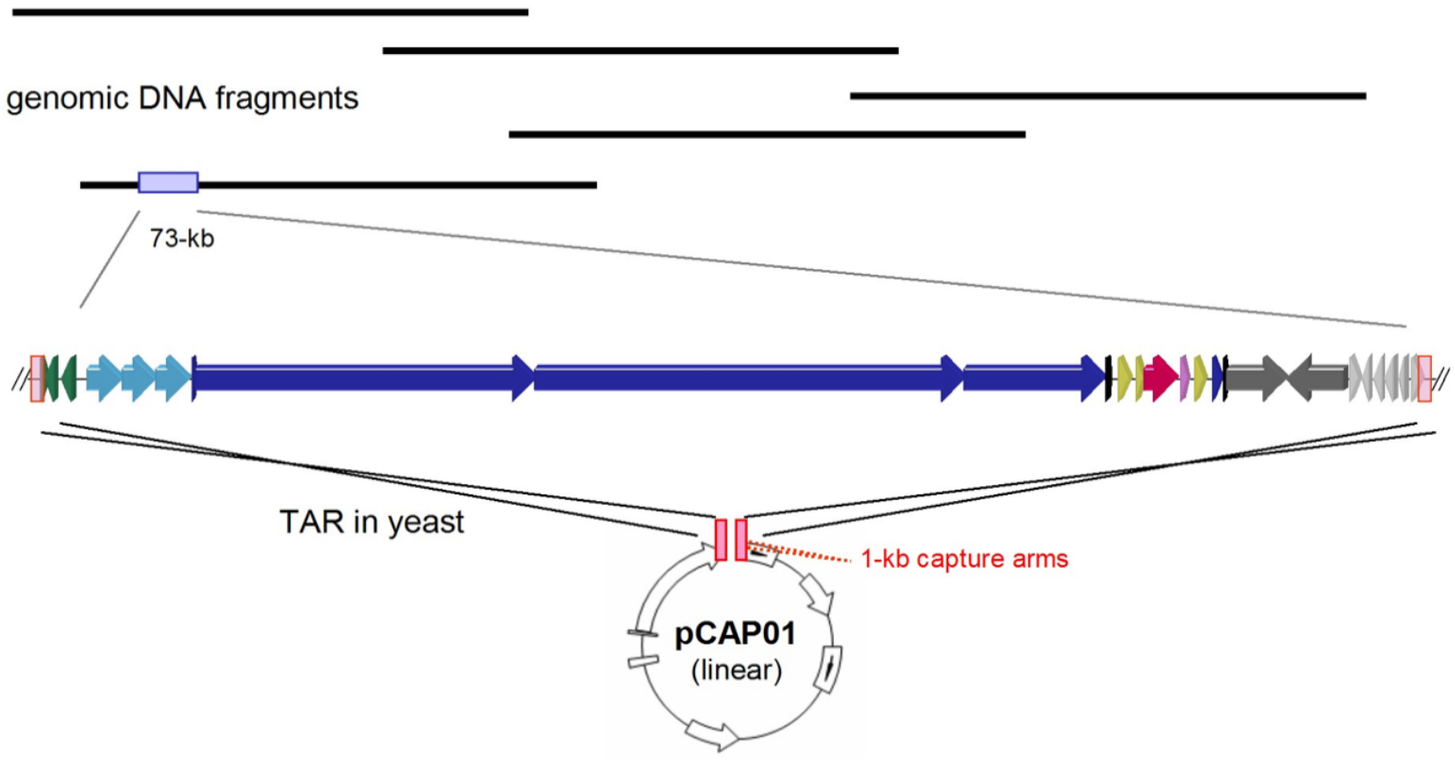
Recent developments in next-generation sequencing technologies have brought recognition of microbial genomes as a rich resource for novel natural product discovery. However, owing to the scarcity of efficient procedures to connect genes to molecules, only a small fraction of secondary metabolomes have been investigated to date. Transformation-associated recombination (TAR) cloning takes advantage of the natural in vivo homologous recombination of *Saccharomyces cerevisiae* to directly capture large genomic loci. Here we report a TAR-based genetic platform that allows us to directly clone, refactor, and heterologously express a silent biosynthetic pathway to yield a new antibiotic. With this method, which involves regulatory gene remodeling, we successfully expressed a 67-kb nonribosomal peptide synthetase biosynthetic gene cluster from the marine actinomycete *Saccharomonospora* sp. CNQ-490 and produced the dichlorinated lipopeptide antibiotic taromycin A in the model expression host *Streptomyces coelicolor*. The taromycin gene duster (*tar*) is highly similar to the clinically approved antibiotic daptomycin from *Streptomyces roseosporus*, but has notable structural differences in three amino acid residues and the lipid side chain. With the activation of the *tar* gene cluster and production of taromycin A, this study highlights a unique

genes (9, 10). Synthetic biology approaches also can be used to refactor orphan gene clusters by optimizing promoters, transcriptional regulation, ribosome-binding sites, and even codon use (11). Examples of successful regulatory gene manipulation to elicit the production of new natural products from silent biosynthetic gene clusters in WT strains have been reported in bacteria and fungi (12, 13); however, this approach requires the de novo development of genetic protocols optimized for each native producer strain. In contrast, the heterologous expression of cryptic pathways in well-investigated biosynthetic host strains has some clear advantages, owing to the wide variety of available genetic tools to manipulate regulatory genes. In fact, many biosynthetic studies of actively expressed secondary metabolite pathways have been explored through mutagenesis and/or heterologous expression of cosmid/fosmid clones selected from genomic libraries (14). Next-generation sequencing technology does not require large insert clonal libraries (3), which have served as a resource for previous heterologous biosynthetic studies. Nonetheless, time-consuming cosmid/fosmid library construction and screening is still routinely practiced in the biosynthetic community.

A further complication with the cosmid/fosmid approach involves its size-selective bias of genomic fragments in the range

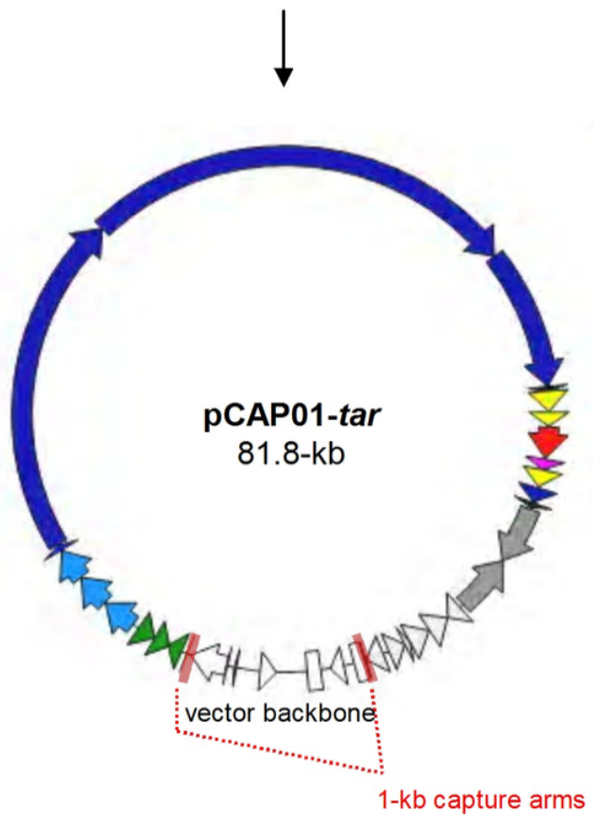
Transformation-Associated Recombination (TAR)-Cloning





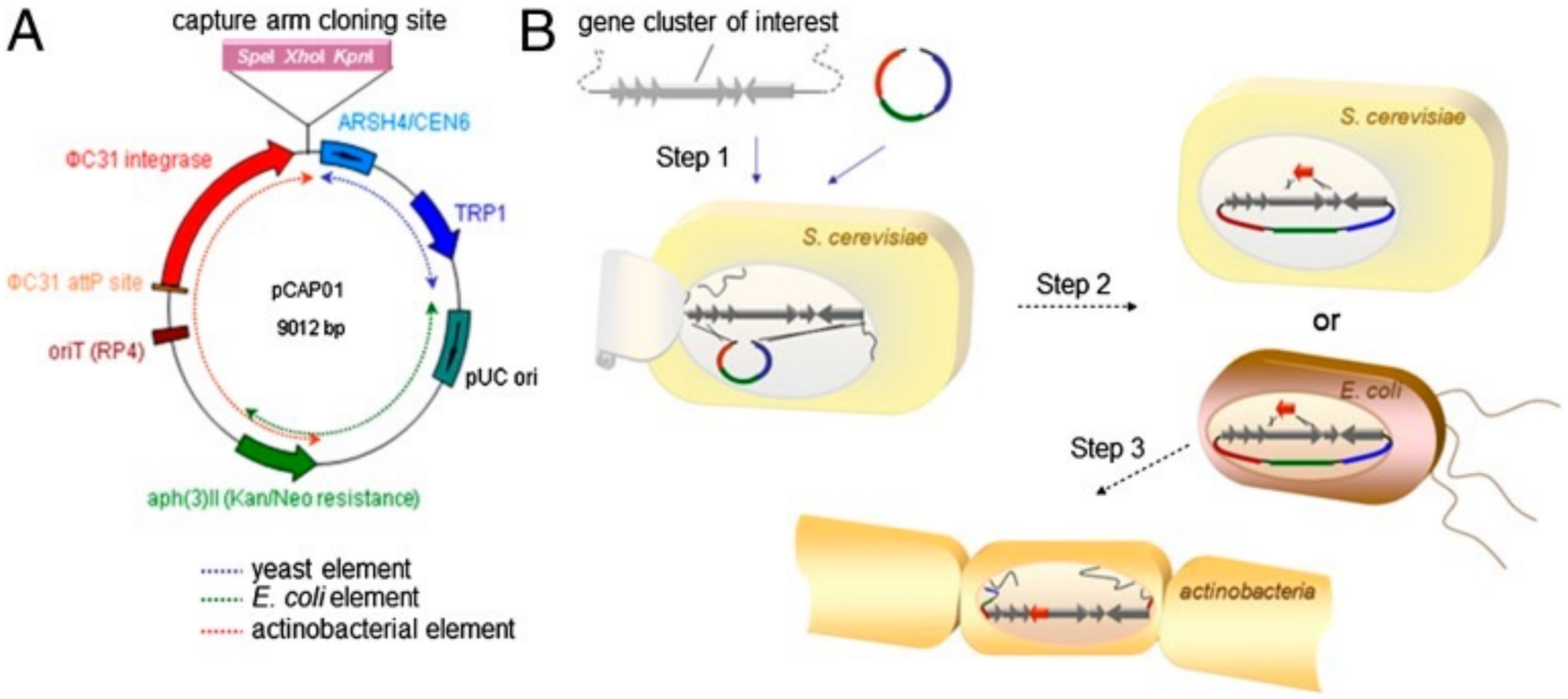
genomic DNA of species of interest is digested.

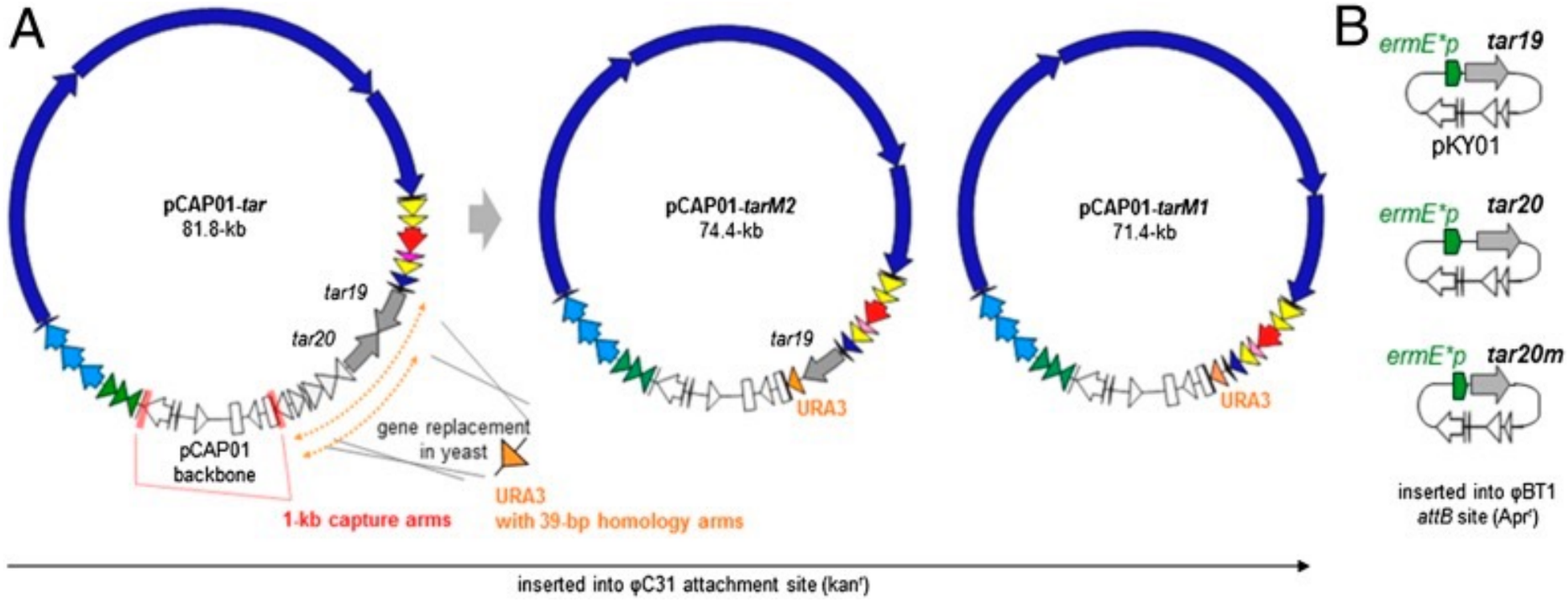
Capture arms on vector match the ends of the gene cluster of interest

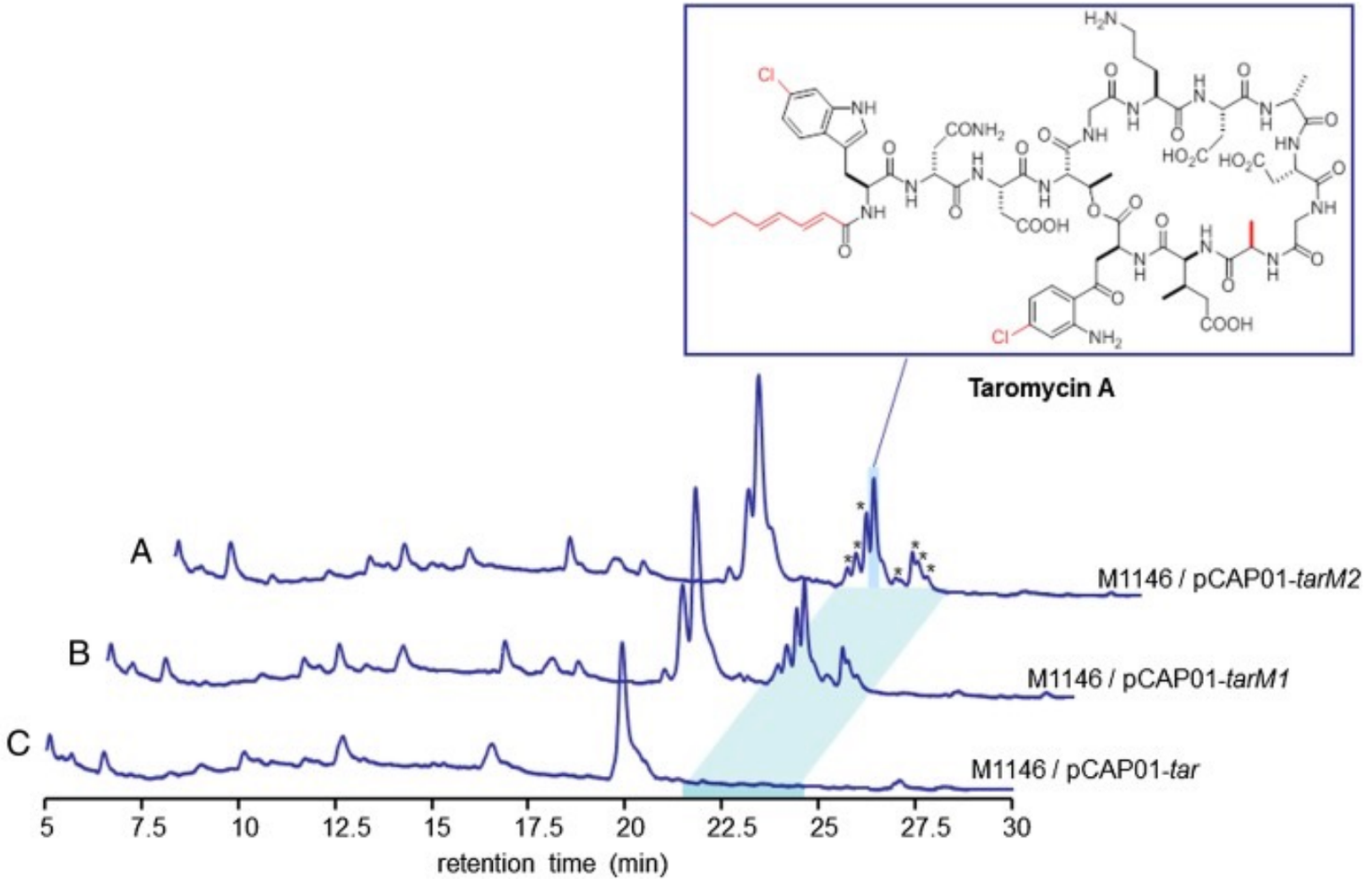


genomic DNA and linear vector are transformed into yeast where they recombine

Capture cluster of interest




A
pCAP01-tar
81.8-kb
tar19
tar20
pCAP01
backbone
gene replacement
in yeast
URA3
with 39-bp homology arms
1-kb capture arms
pCAP01-tarM2
74.4-kb
tar19
URA3
pCAP01-tarM1
71.4-kb
URA3
inserted into φC31 attachment site (kanr)
B
ermE*p tar19
pKY01
ermE*p tar20
ermE*p tar20m
inserted into φBT1
attB site (Aprr)


Taromycin A
A
M1146 / pCAP01-tarM2
B
M1146 / pCAP01-tarM1
C
M1146 / pCAP01-tar
5
7.5
10
12.5
15
17.5
20
22.5
25
27.5
30
retention time (min)