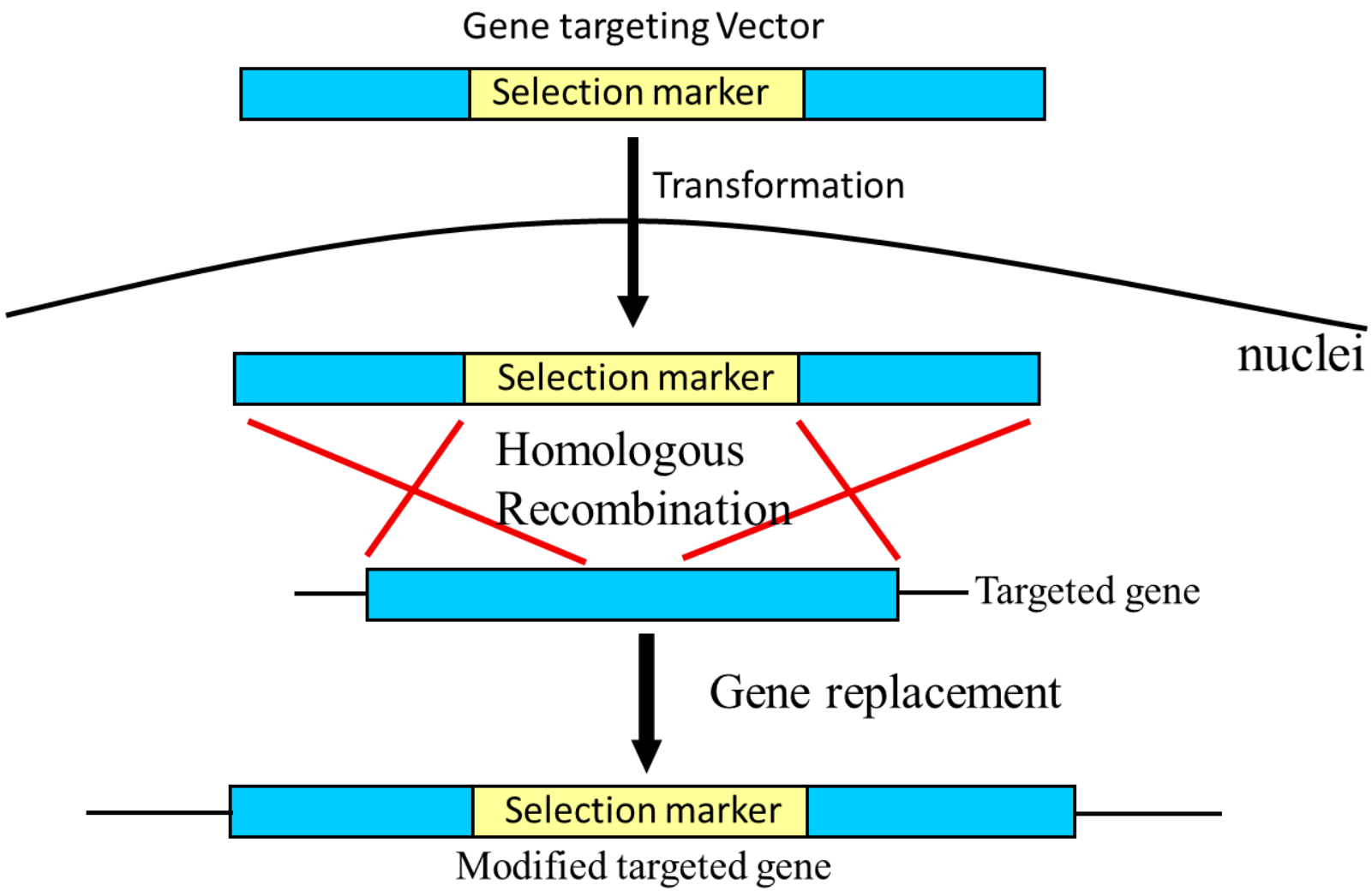
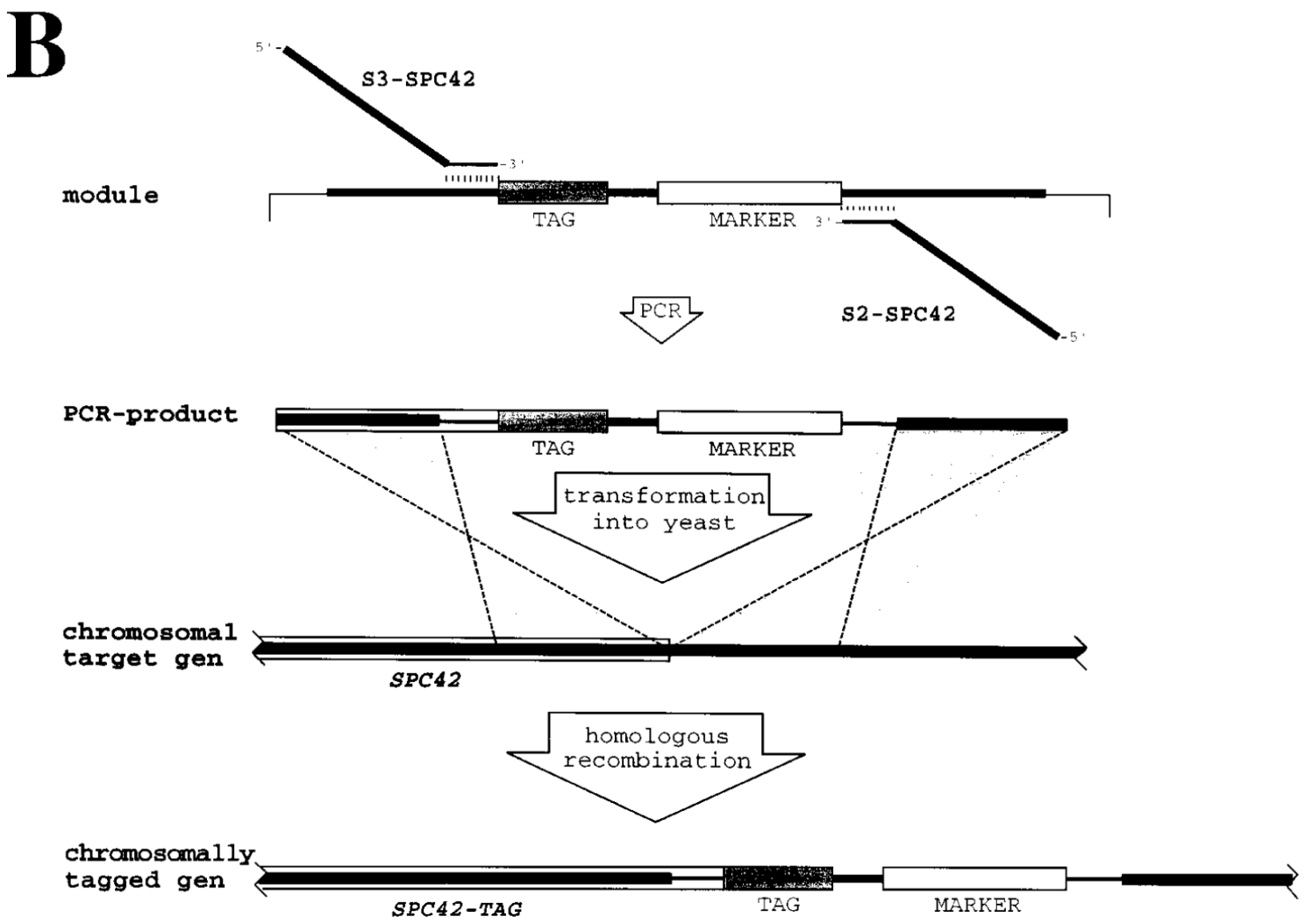
PCR-based TAP tagging strategy via pBS1479

Double cross-over homologous recombinant



PCR-based TAP tagging strategy



Primer design

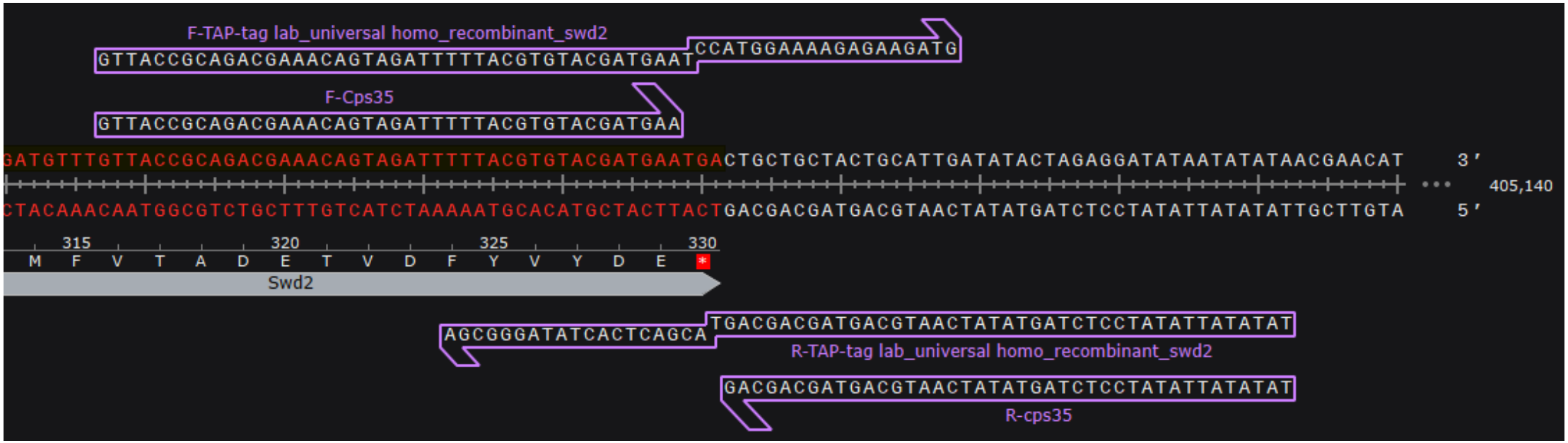
puig *et al.*,2001에서 plasmid DNA에 직접 결합하는 primer 길이를 20mer로 맞추기 위해 3mer를 더 늘린 버전(**볼드체**)을 이용한다.

Forword primer – GTTACCGCAGACGAAACAGTAGATTTTTACGTGTACGATGA **ATCCATGGAAAAGAGAAGATG** (Tm=53˚C, SnapGene)

Reverse primer – TATATATTATATCCTCTAGTATATCAATGCAGTAGCAGCAG **TACGACTCACTATAGGGCGA** (Tm=54˚C, SnapGene)

1.6k 길이의 amplicon을 얻을 수 있다.

Primer design



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볼드체로 표시한 부분의 sequence가 Swd2의 유전자 말단을 뭉개고 들어가도록 homologous recombination을 일으키면서 뒤에 있는 TAP-tag까지 translation되게 한다.

TAP purification