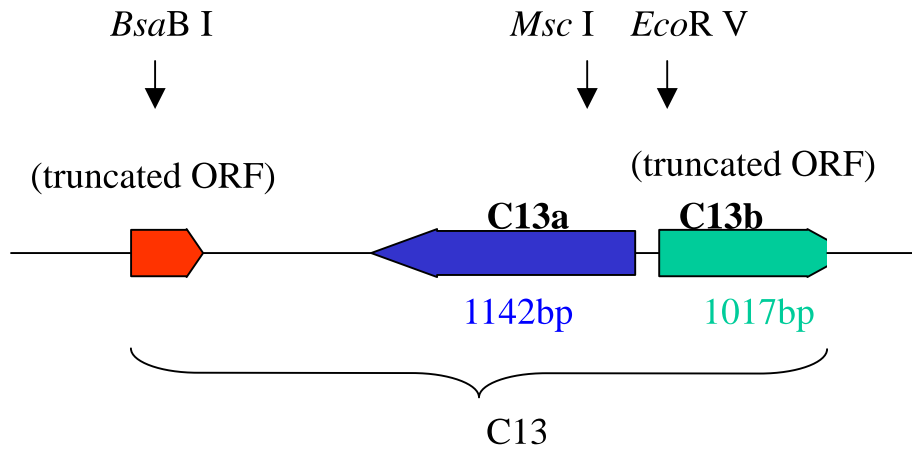
- **C13**



- **C92**

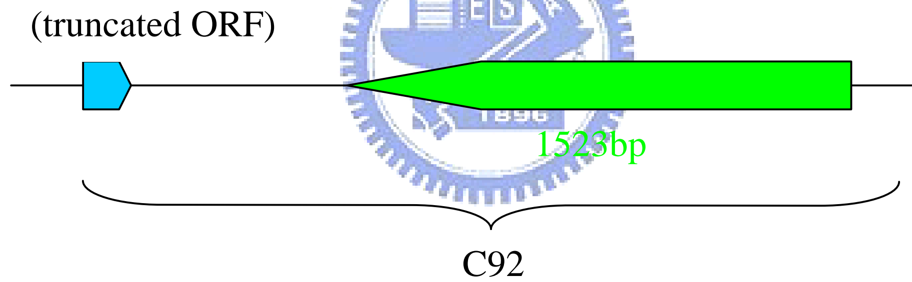


Figure 8. Open reading frames in the insert sequences of C13 and C92. The direction of the arrow means the direction from transcription initiation site to the termination site. The arrows indicated are the recognition sites of restriction enzymes.

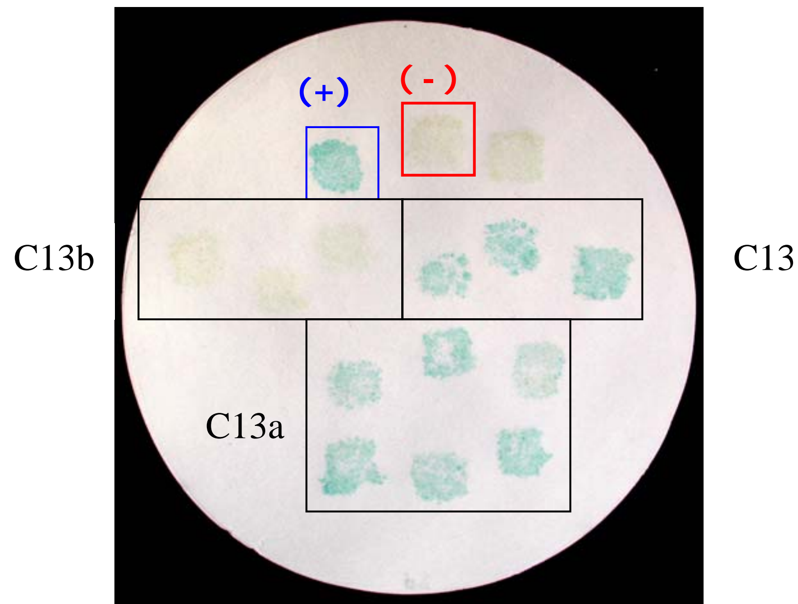


Figure 9. β-galactosidase activity of *S. cerevisiae* cells containing *CDR1*p-*lacZ* fusion and a plasmid carrying one of the open reading frames by filter assay. *S. cerevisiae* cells containing *CDR1*p-*lacZ* fusion and a plasmid carrying one of the open reading frames, C13, C13a, or C13b are as indicated.

(+), Positive control, *S. cerevesiae* cells containing *REP2* insert plasmid (pRS426 based) in 2B/348*CDR1*p-*lacZ*

(-), Negative control, *S. cerevesiae* cells containing vector alone plasmid (pRS426) in 2B/348*CDR1*p-*lacZ*

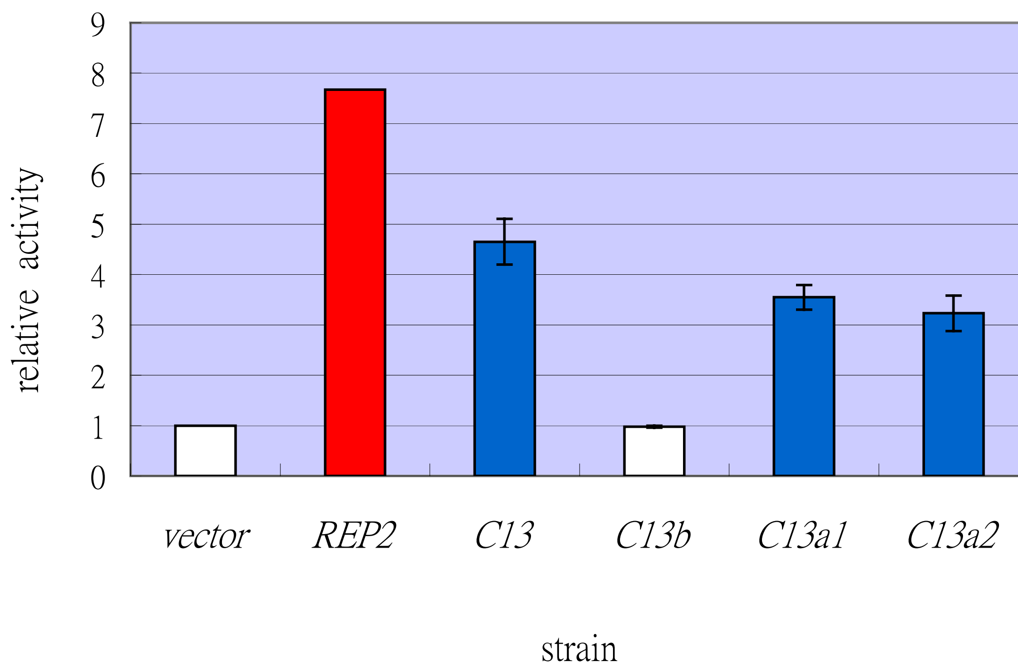


Figure 10. β-galactosidase activity of *S. cerevisiae* cells containing SC5314 *CDR1*p-*lacZ* fusion and carrying one of the candidate plasmids by liquid assay. *S. cerevisiae* cells containing SC5314 *CDR1*promoter-*lacZ* fusion which was integrated into the chromosome and also carrying one of the candidate plasmids, C13a (including C13a1, C13a2), C13b, and C13 are as indicated. Vector, the cells containing plasmid (pRS426) without any insert; *REP2*, the cells containing plasmid (pRS426) with *REP2* insert

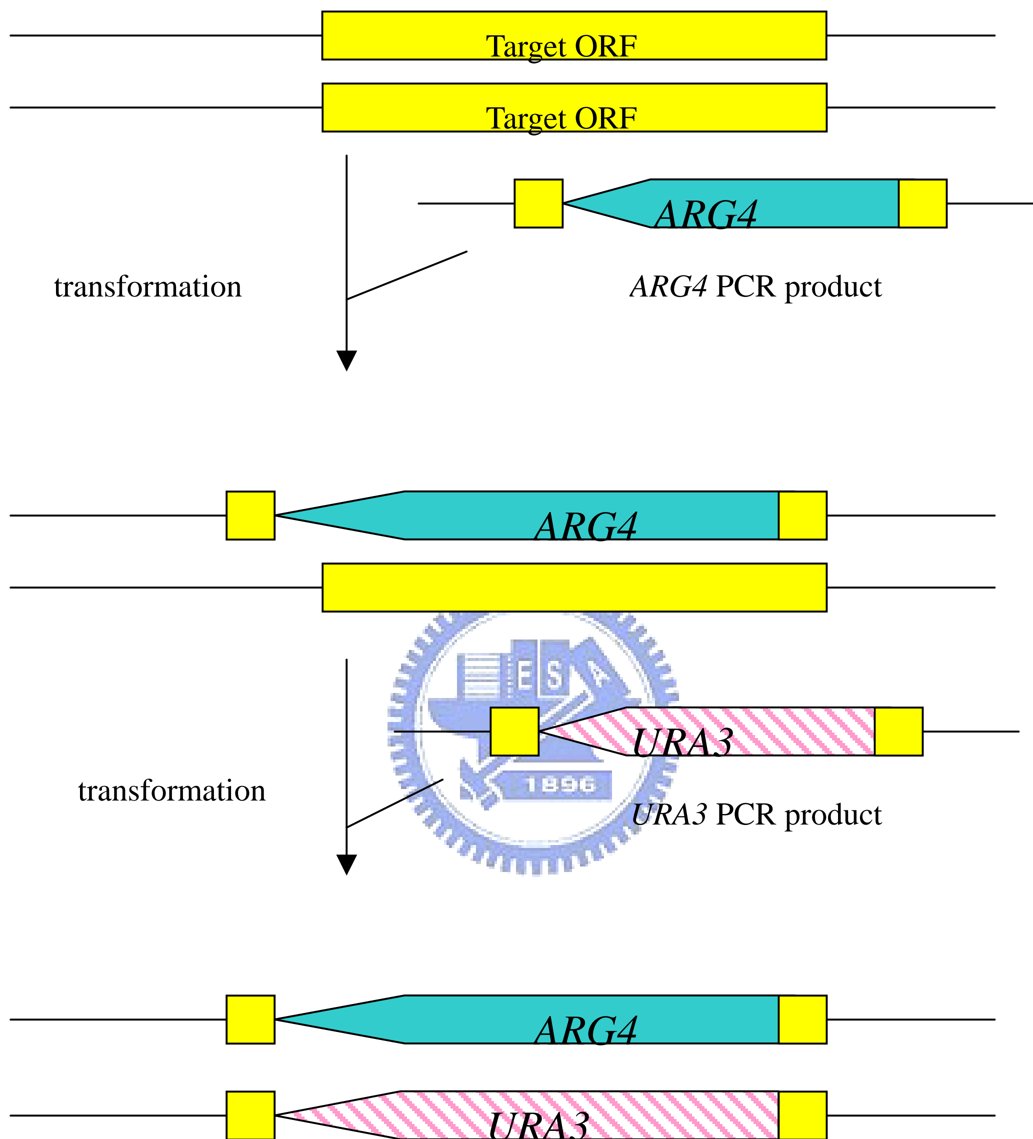
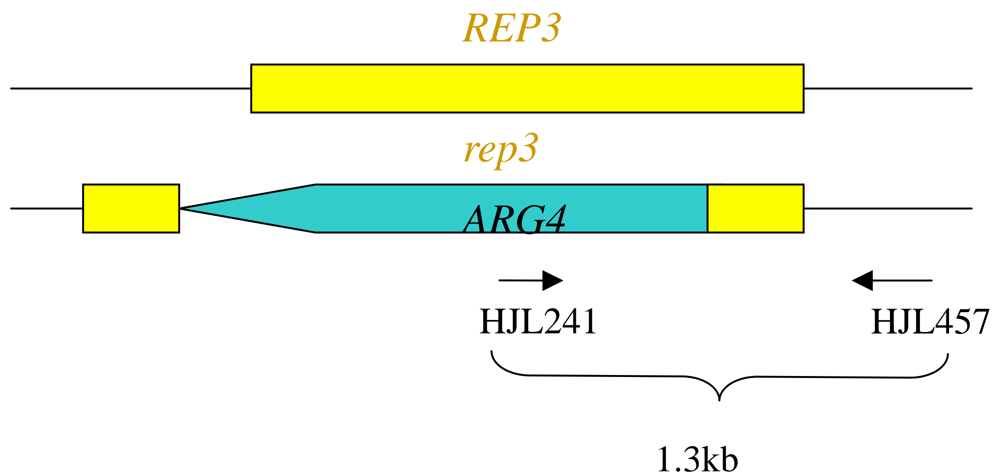


Figure 11. Schematic diagram of *REP* gene disruption.
Target ORF, *REP3* or *REP6* ORF; *ARG4* and *URA3* are gene disruption cassettes carrying the marker genes as indicated. Yellow region is the homology sequence used for homologous recombination.

(a).



(b).

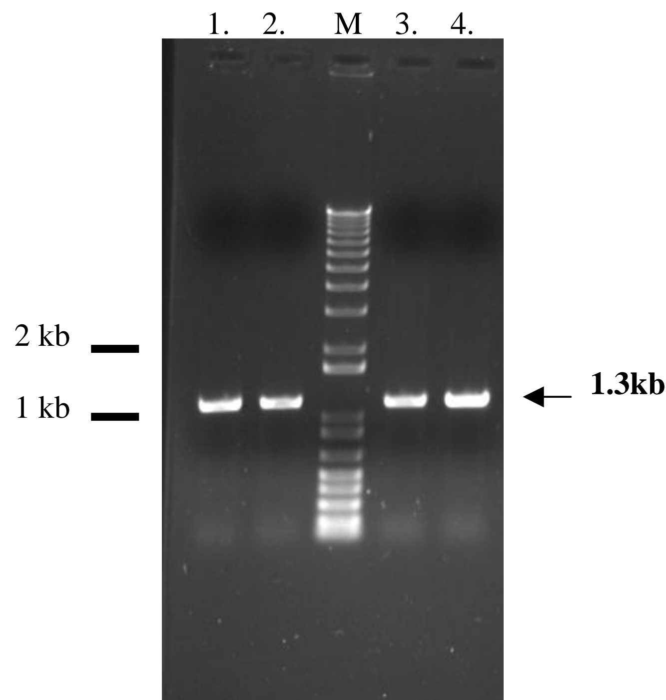


Figure 12. Assessing the of *REP3/rep3* heterozygous mutant strain by PCR. (a). Schematic drawing of assessing *REP3/rep3* hetreozyous mutant strain using primers HJL 241 and HJL 457 **(b).** Result of the PCR. M, 1kb plus DNA ladder; 1.and 2., BWP17 *REP3/rep3* heterozygous mutant strain; 3. and 4., BWP17 tetR-His *REP3/rep3* heterozygous mutant strain. Arrows indicated the positions of the primers on the genome.

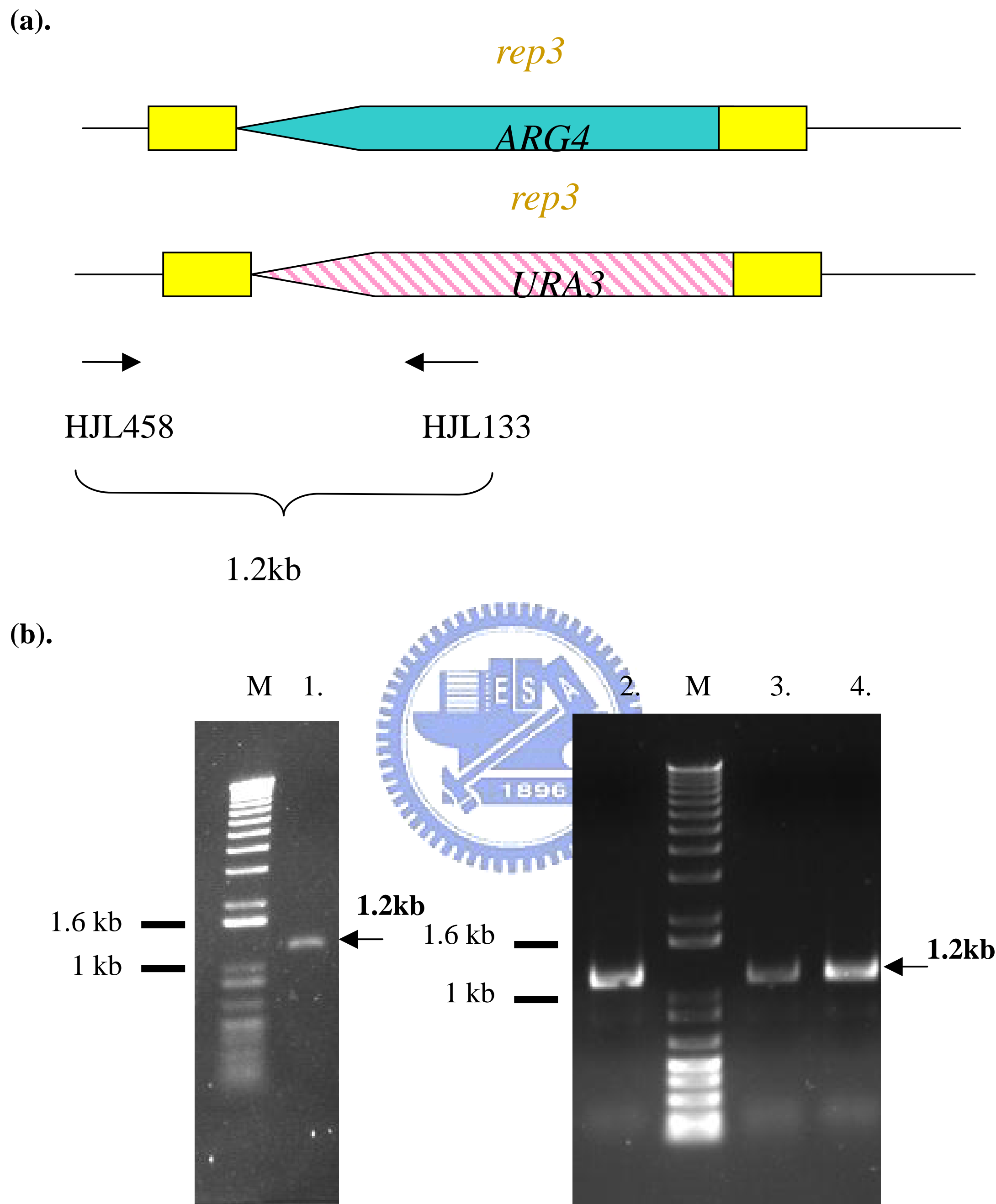


Figure 13. Assessing the of *rep3/rep3* homozygous mutant strain by PCR **(a).** Schematic drawing of assessing *rep3/rep3* homozygous mutant strain using primers HJL 133 and HJL 458. **(b).** Result of the PCR. M: 1kb plus DNA ladder, 1: BWP17 *rep3/rep3* homozygous mutant strain, 2,3,4 : BWP17 *tetR*-His *rep3/rep3* homozygous mutant strain. Arrows indicated the positions of the primers on the genome.

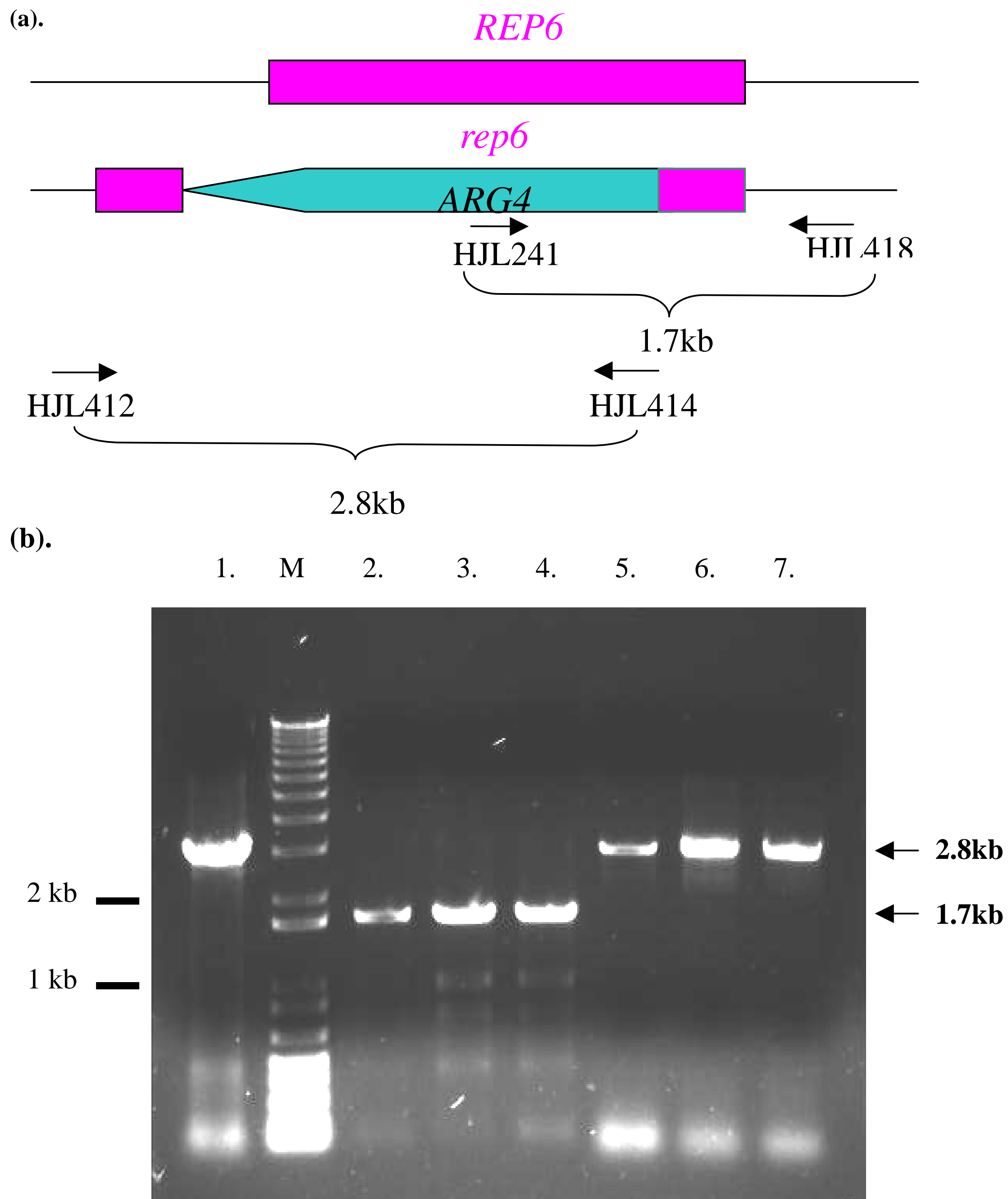


Figure 14. Assessing the of *REP6/rep6* heterozygous mutant strain by PCR (**a**). Schematic drawing of assessing *REP6/rep6* heterozygous mutant strain using primers HJL241 and HJL418; HJL414 and HJL412 **(b).** Result of the PCR. M: 1kb plus DNA ladder, 1: BWP17 *REP6/rep6* heterozygous mutant strain using primers HJL412 and HJL414 , 2-4 : BWP17 tetR-His *REP6/rep6* heterozygous mutant strain using primers HJL 241 and HJL 418, 5-6: BWP17 *tetR*-His *REP6/rep6* heterozygous mutant strain using HJL412 and HJL414. Arrows indicated the positions of the primers on the genome.