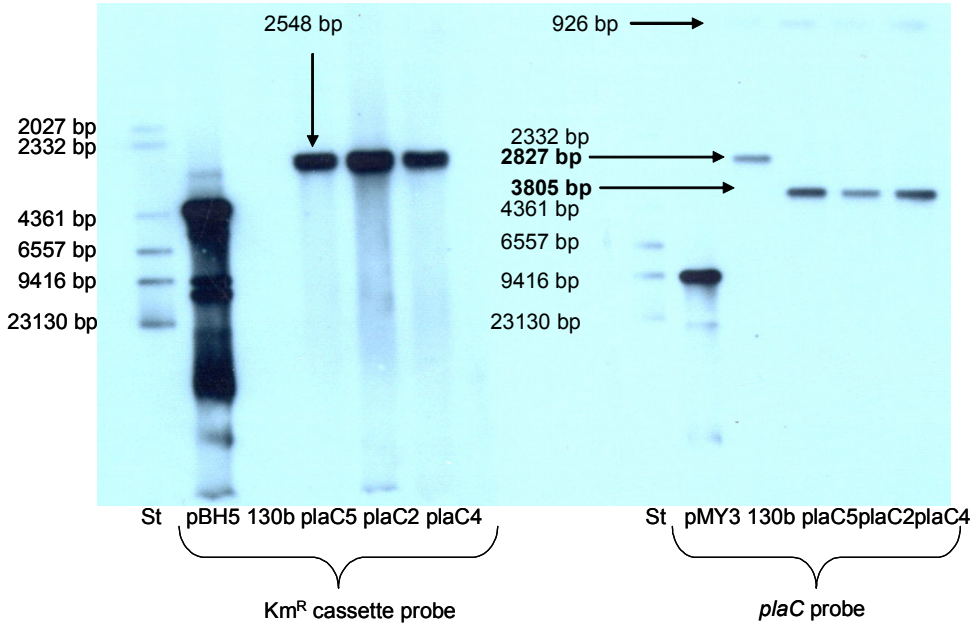
6. Appendix

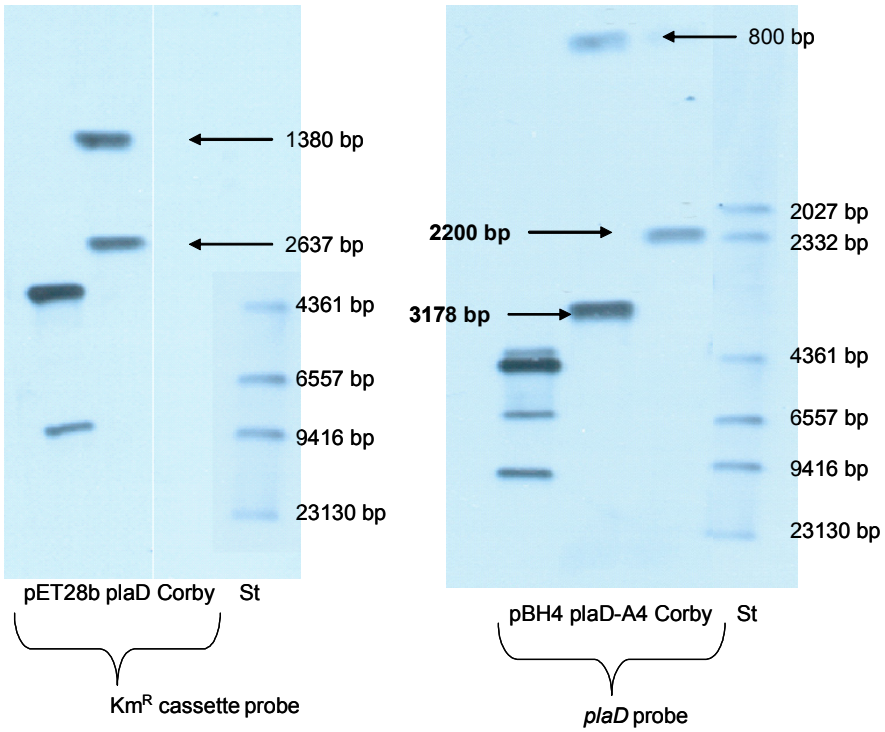
Southern blot analysis *of L. pneumophila* mutants

L. pneumophila mutants were analyzed for the presence of a Km^R or Gm^R cassette with specific primers by Southern blot analysis (Table 3.27). To this purpose genomic DNA was obtained and digested with appropriate restriction enzymes listed in Table 3.27. Another aliquot was digested with *Ava*I which has a restriction site in the Km^R cassette and Gm^R cassette but not in any of the analyzed genes. The presence of the resistance gene cassette was confirmed by hybridization of the blotted genomic DNA fragments with a dioxygenin (DIG)-labelled Km^R or Gm^R cassette probe. The insertion of the Km^R or Gm^R cassette into the gene of interest was marked by a 978 bp or 1024 bp shift of the mutant gene in comparison to the wild type gene, respectively. The results are discussed in the respective chapters and/or in the discussion section.

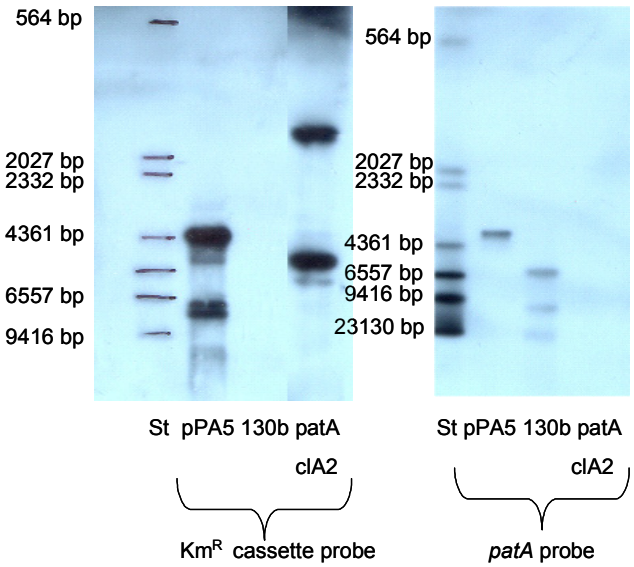
6.1 *L. pneumophila* 130b *plaC* mutants



6.2 *L. pneumophila* Corby *plaD* mutants

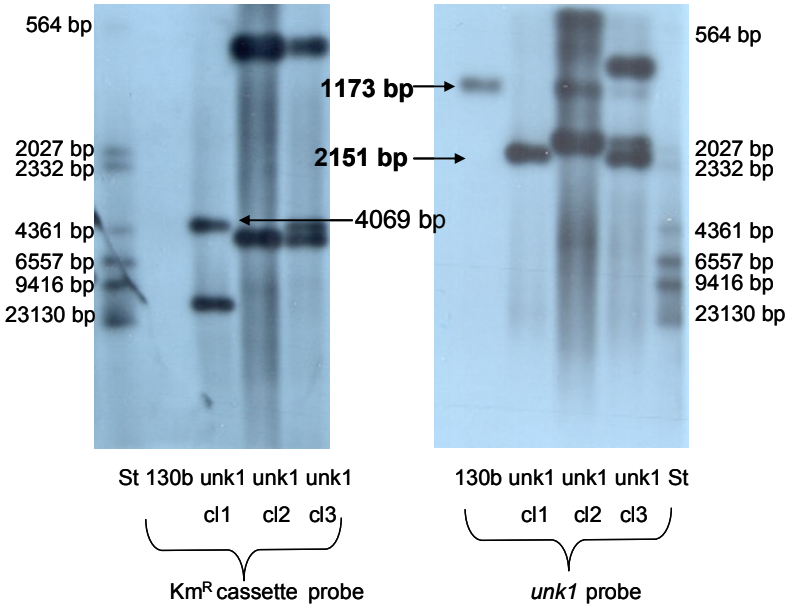


6.3 *L. pneumophila* 130b *patA* mutant

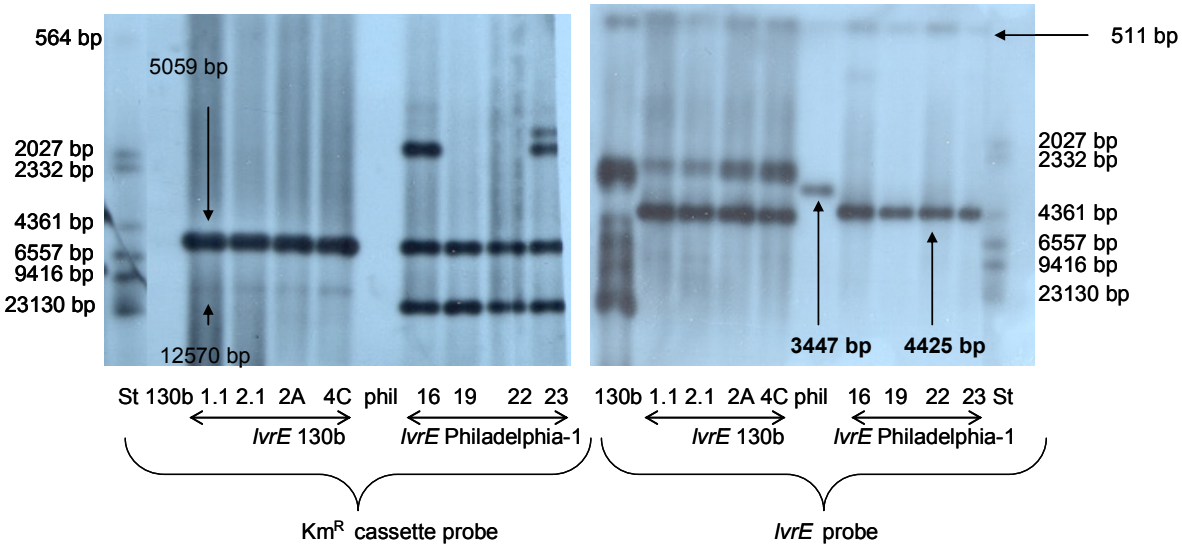


Note: The *patA* probe (derived from strain 130b) did not hybridize with the genome of the *patA* mutant which might be due to the fact that the *patA* genetic lokus in the 130b mutant had been replaced by that of strain Philadelphia-1 by allelic exchange with pPA5 (pGEMTez+$patA_{Phialdelphia\text{-}1}$::$Km^R$). The positive signal received with the control pPA5 might be attributed to a much higher DNA concentration of the plasmid. In conclusion, it was found that the *L. pneumophila* 130b *patA* mutant clA2 had a Km^R cassette integrated into its genome and PCR analysis furthermore showed an approximately 1 kb shift in the *patA* gene of this mutant (data not shown).

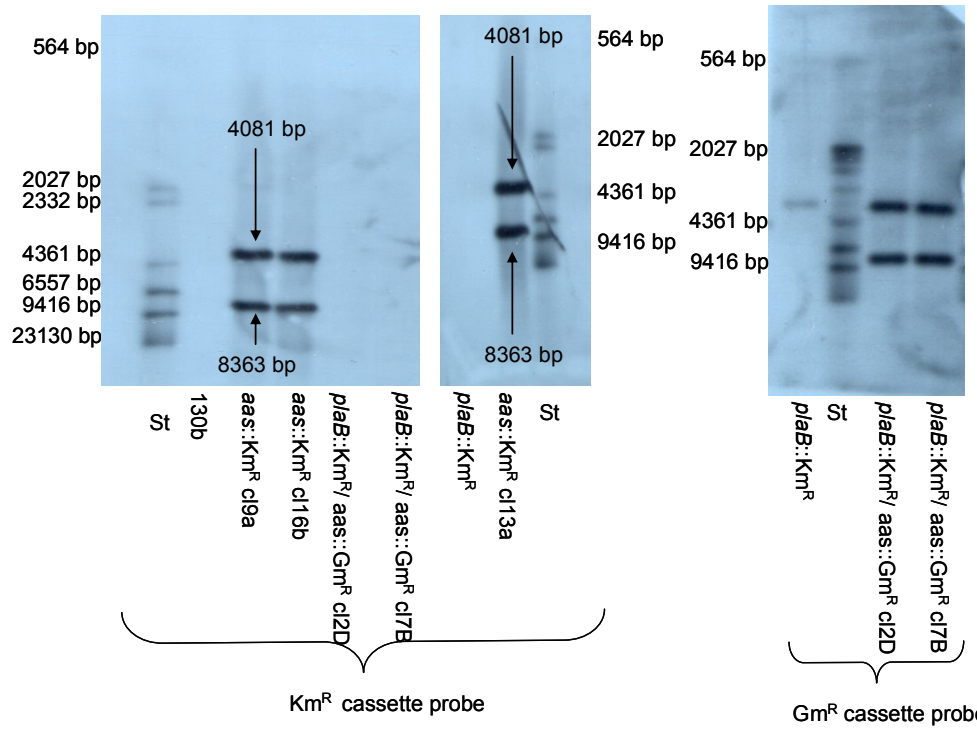
6.4 *L. pneumophila* 130b *unk1* mutants

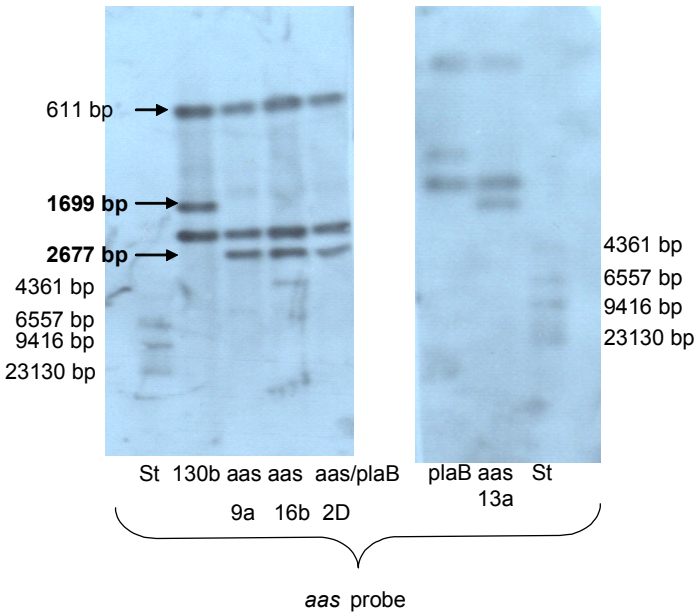


6.5 *L. pneumophila* 130b and Philadelphia-1 *lvrE* mutants



6.6 *L. pneumophila* 130b *aas* mutants





Note: The Km^R cassette probe amplified from pET28b did not hybridize with the genome of the *plaB* mutant (which is kanamycin resistant) and derivative clones, which might be due to the fact that the Km^R gene cassette in these mutants was not derived from pET28b unlike in all other mutants, which were used in this study. Furthermore, the Gm^R cassette probe hybridized with a fragment of the *plaB* mutant's genomic DNA possibly because it contained flanking regions of *lipB*. PCR analysis revealed that in the *plaB*/ *aas* double mutants both genes had intergrated a Km^R or Gm^R gene cassette, respectively and these mutants were kanamycin as well as gentamicin resistant (data not shown).