Substitution mapping was used to refine localization of low blood pressure (BP) QTL allele(s) within the congenic region of S.R-Edn3 rats, in which the q-terminal portion of rat chromosome 3 (RNO3) from Dahl salt-resistant (R) rats was introgressed into the Dahl salt-sensitive (S) strain. An F_2 (S x S.R-Edn3) population (n = 173) was screened to identify rats having crossovers within this congenic interval and six congenic substrains carrying smaller portions of R-rat derived RNO3 were bred. Two substrains with non-overlapping congenic regions, S.R(ET3x1) and S.R(ET3x6), had significantly lower BP (-12 and -13 mm Hg, respectively) compared to the S strain. This indicates that the RNO3 q-ter contains two distinct BP QTLs. In addition, the ET3x1, but not the S.R(ET3x6) or S.R(ET3x7) substrains, had significantly lower kidney weight (KW; -248, -57, and +42 mg, respectively) compared to S rats. More than 40 novel microsatellites, polymorphic between Dahl S and R rats, were developed in the q-terminal region of RNO3 to more precisely define the congenic regions of the congenic substrains derived from S.R-Edn3. The more proximal RNO3 q-terminal BP QTL-containing interval (QTL1) is defined by a 0.04 – 1.12 Mb portion of the overlap of the congenic regions of S.R(ET3x6) and S.R(ET3x2). This QTL1 interval does not overlap with the congenic interval of S.R(ET3x5), which lacks low BP QTL allele(s). The portion of S.R(ET3x1) congenic region not overlapping with the S.R(ET3x7) congenic region delimits the more distal RNO3 QTL-containing interval (QTL2) which is 0.78 – 1.20 Mb in size. Comparative mapping of the rat chromosomal intervals with mouse chromosome 2 (Mmu2) indicated similar sizes for the QTL1 and QTL2 regions in mice of 0.03 – 1.89 Mb and 0.76 – 1.17 Mb, respectively. The congenic regions of S.R(ET3x2) and S.R(ET3x3) contain both the QTL1 and QTL2 intervals, with both substrains having significantly lower BP (-21 and -32 mM Hg, respectively) compared to S rats. The observed BP differences suggest that the S.R(ET3x2) and S.R(ET3x3) substrains carry low BP alleles from both RNO3 q-ter BP QTLs which exert additive effects. Association of a KW difference with only one BP QTL suggests that the alleles responsible for the different BP QTLs may act in different tissues and/or pathways.