Insulin-like factor-I (IGF-I) and neuropeptide Y (NPY) are candidate genes related with reproductive traits in chicken. The aim of the present study, were to determine polymorphisms the IGF-I (5'untranslated region) and NPY genes in pure layer lines reared Ankara Poultry Research Institute by using PCR-RFLP method. For this purpose, 621 and 248 bp fragments were amplified for IGF-I and NPY genes, respectively. The PCR products were digested with PstI and DraI restriction enzymes, respectively, to detect of single nucleotide polymorphism (SNP) on IGF-I and NPY genes. All pure chicken lines were found polymorphic except Black and D-229 lines for the IGF-I gene. While the frequency of A allele ranged from 0.344 (COL) and 0.906 (RIRII) in brown layer pure lines for IGF-I gene, in white layer pure lines were found in the range between 0.781 (Maroon) and 1.000 (D-229, Brown). While all brown pure layer chicken lines were polymorphic for NPY gene in this research, only Maroon line was found monomorphic in white layer chicken lines. The frequency of the T allele for NPY gene ranged between 0.200 (BARI) and 0.985 (COL) in brown layers and between 0.397 (D-229) and 1.000 (Maroon) in white layers. According to applied chi-square test, no deviation from Hardy-Weinberg equilibrium was observed in polymorphic populations. As a result, polymorphisms were shown for the first time for IGF-I and NPY genes in pure layer lines reared at the Ankara Poultry Research Institute.