The nucleotide sequence of the matrix (M) gene of human parainfluenza virus 3 (HPIV3), strain 47885, was determined using overlapping cDNA clones derived from mRNA and genomic RNA. The sequence is presented as DNA in the mRNA sense beginning with the first nucleotide of M mRNA. The HPIV3 M gene is 1150 nucleotides long, excluding 5' terminal A residues. A single long open reading frame begins at nucleotide 33 and encodes a polypeptide of 353 amino acids (molecular weight 39499). The HPIV3 M protein is basic (net charge +20.5 at pH 7). Many arginine- and lysine residues are conserved among paramyxovirus M proteins (1-4). Two slightly hydrophobic stretches are found (amino acids 173-208 and 264-294); the second within a region, with a predicted secondary structure common among paramyxovirus M proteins, believed to interact with but not span the envelope (4-6). The HPIV3 M protein exhibits considerable homology with the M protein of Sendai virus (%62%) but less homology with the M proteins of measles virus (%18%) and NDV (%18%).