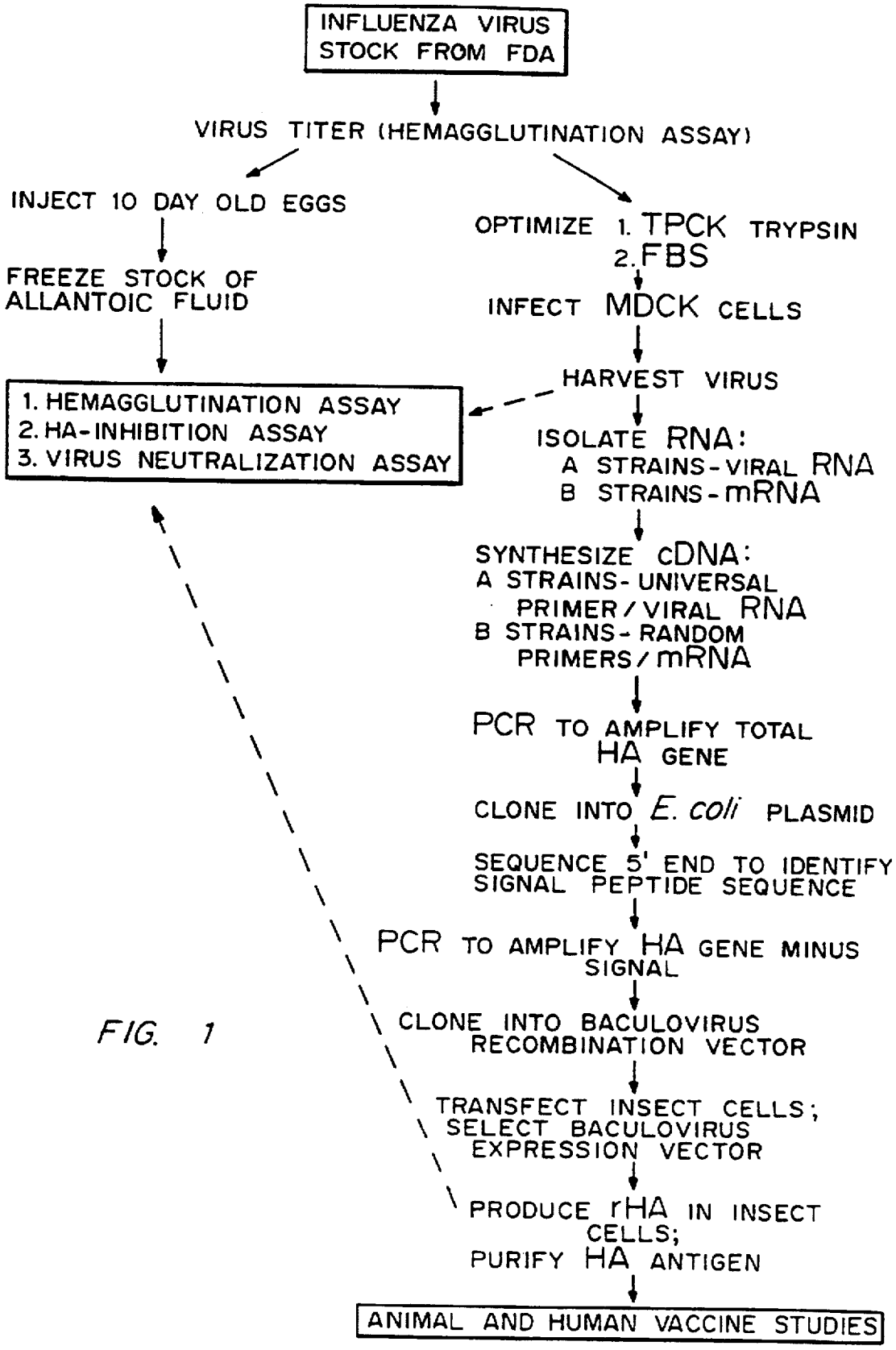
INFLUENZA VIRUS STOCK FROM FDA
VIRUS TITER (HEMAGGLUTINATION ASSAY)
INJECT 10 DAY OLD EGGS
FREEZE STOCK OF ALLANTOIC FLUID
OPTIMIZE 1. TPCK TRYPSIN 2. FBS
INFECT MDCK CELLS
HARVEST VIRUS
1. HEMAGGLUTINATION ASSAY
2. HA-INHIBITION ASSAY
3. VIRUS NEUTRALIZATION ASSAY
ISOLATE RNA:
A STRAINS - VIRAL RNA
B STRAINS - mRNA
SYNTHESIZE cDNA:
A STRAINS - UNIVERSAL PRIMER / VIRAL RNA
B STRAINS - RANDOM PRIMERS / mRNA
PCR TO AMPLIFY TOTAL HA GENE
CLONE INTO E. coli PLASMID
SEQUENCE 5' END TO IDENTIFY SIGNAL PEPTIDE SEQUENCE
PCR TO AMPLIFY HA GENE MINUS SIGNAL
CLONE INTO BACULOVIRUS RECOMBINATION VECTOR
TRANSFECT INSECT CELLS; SELECT BACULOVIRUS EXPRESSION VECTOR
PRODUCE rHA IN INSECT CELLS; PURIFY HA ANTIGEN
ANIMAL AND HUMAN VACCINE STUDIES

FIG. 1