Figure 1

Hamster IGF-1

Secreted mature hamster IGF-1 (70 amino acids)

```
  1 : tttgccacagctggaccagagaccctctgcggggctgagctggtggatgctcttcaattc
                G  P  E  T  L  C  G  A  E  L  V  D  A  L  Q  F

 61 : gtgtgtggaccaaggggcttttacttcaacaagcccacaggctatggctccagcattcgg
      V  C  G  P  R  G  F  Y  F  N  K  P  T  G  Y  G  S  S  I  R

121 : agggcacctcagacaggcattgtagatgagtgctgcttccggagctgtgatctgagaaga
      R  A  P  Q  T  G  I  V  D  E  C  C  F  R  S  C  D  L  R  R

181 : ctggagatgtactgtgcccccctcaagcctacaaaatcggcccgctctatccgtgcccag
      L  E  M  Y  C  A  P  L  K  P  T  K  S  A

241 : cg
```

Figure 2

Comparison between hamster and
human IGF-1 amino acid sequences

```
                1                   20                  34
                                    |                   |
Hamster     GPETLCGAEL VDALQFVCGP RGFYFNKPTG YGSSIRRAPQ
            |||||||||| |||||||||  |||||||||| |||| |||||
Human       GPETLCGAEL VDALQFVCGD RGFYFNKPTG YGSSSRRAPQ

                                          67
                                          |
Hamster     TGIVDECCFR SCDLRRLEMY CAPLKPTKSA
            |||||||||| |||||||||| |||||| |||
Human       TGIVDECCFR SCDLRRLEMY CAPLKPAKSA
```

Figure 3

Native E3E hamster IGF-1 and
active E3R, E3K, E3S hamster IGF-1

3 70

Hamster IGF1 **GPETLCGAEL VDALQFVCGP**.....

↓

Variation in amino acid charge

E3E (-) native form

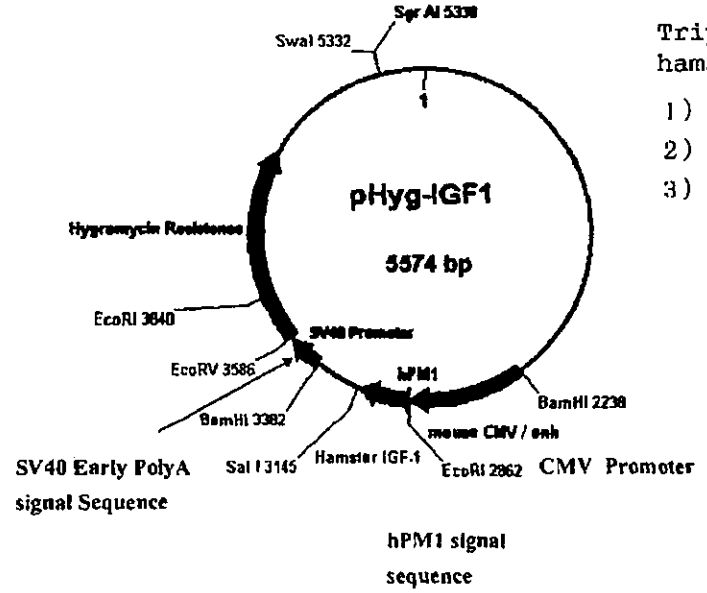
E3R (+) active form

E3K (+) active form

E3S (±) active form

Figure 4

Hamster IGF-1 (70 amino acids)-expression plasmid
containing triple hamster IGF-1 genes



Triple transferred
hamster IGF1 genes

1) E3E

2) E3S

3) E3K

Figure 5

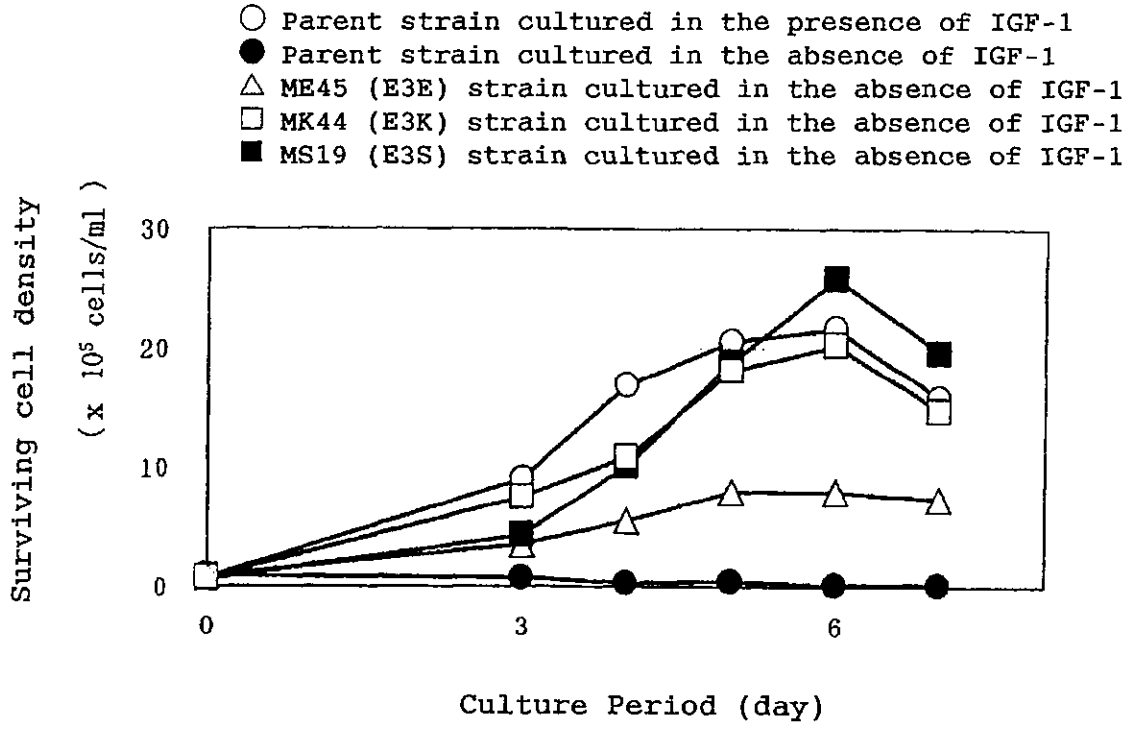

○ Parent strain cultured in the presence of IGF-1
● Parent strain cultured in the absence of IGF-1
△ ME45 (E3E) strain cultured in the absence of IGF-1
□ MK44 (E3K) strain cultured in the absence of IGF-1
■ MS19 (E3S) strain cultured in the absence of IGF-1
Surviving cell density
(x 10⁵ cells/ml)
30
20
10
0
0
3
6
Culture Period (day)

Figure 6

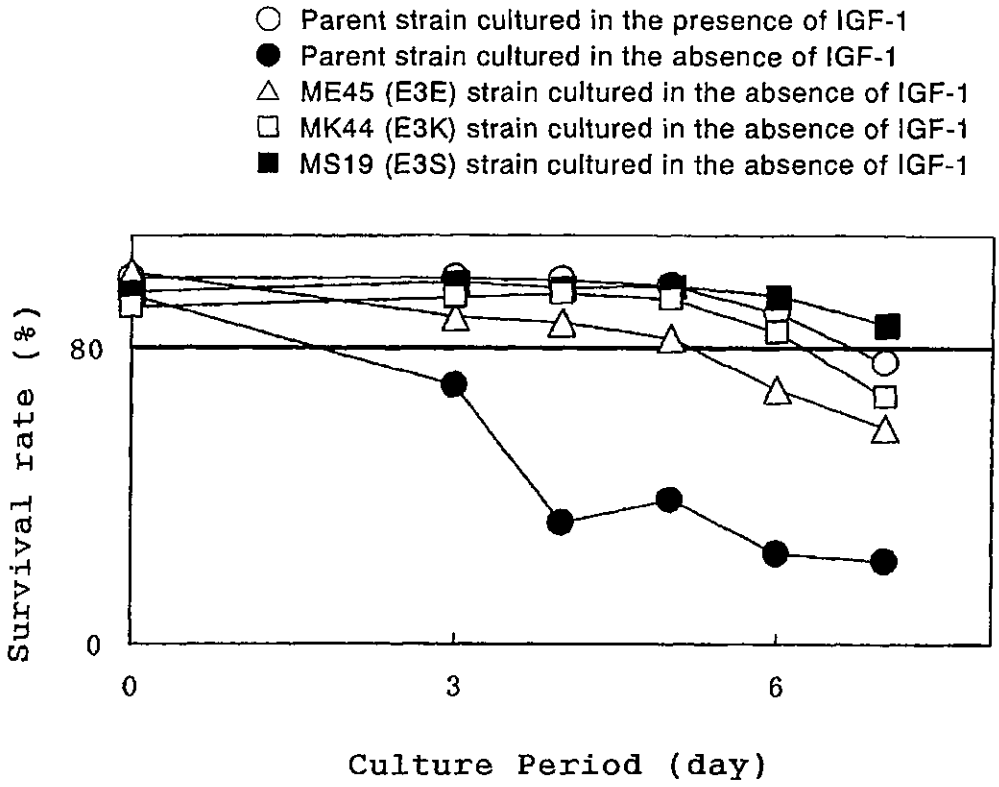

○ Parent strain cultured in the presence of IGF-1
● Parent strain cultured in the absence of IGF-1
△ ME45 (E3E) strain cultured in the absence of IGF-1
□ MK44 (E3K) strain cultured in the absence of IGF-1
■ MS19 (E3S) strain cultured in the absence of IGF-1
Survival rate (%)
80
0
0
3
6
Culture Period (day)

Figure 7

Amounts of anti-IL6R antibodies produced in fed-batch cultures

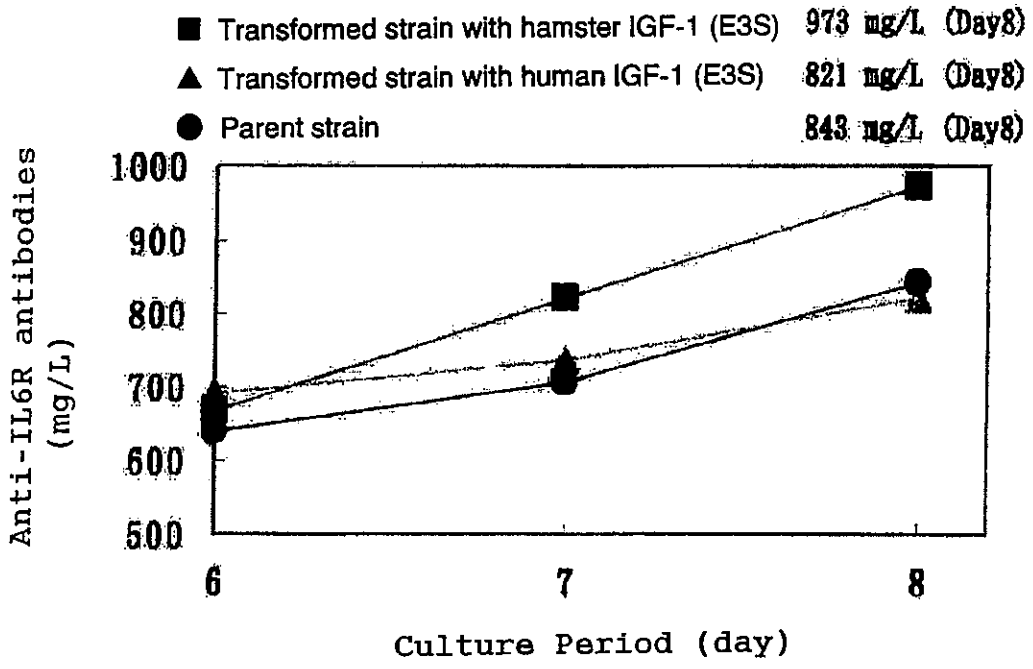


Figure 8

Amounts of anti-IL6R antibodies produced in fed-batch cultures

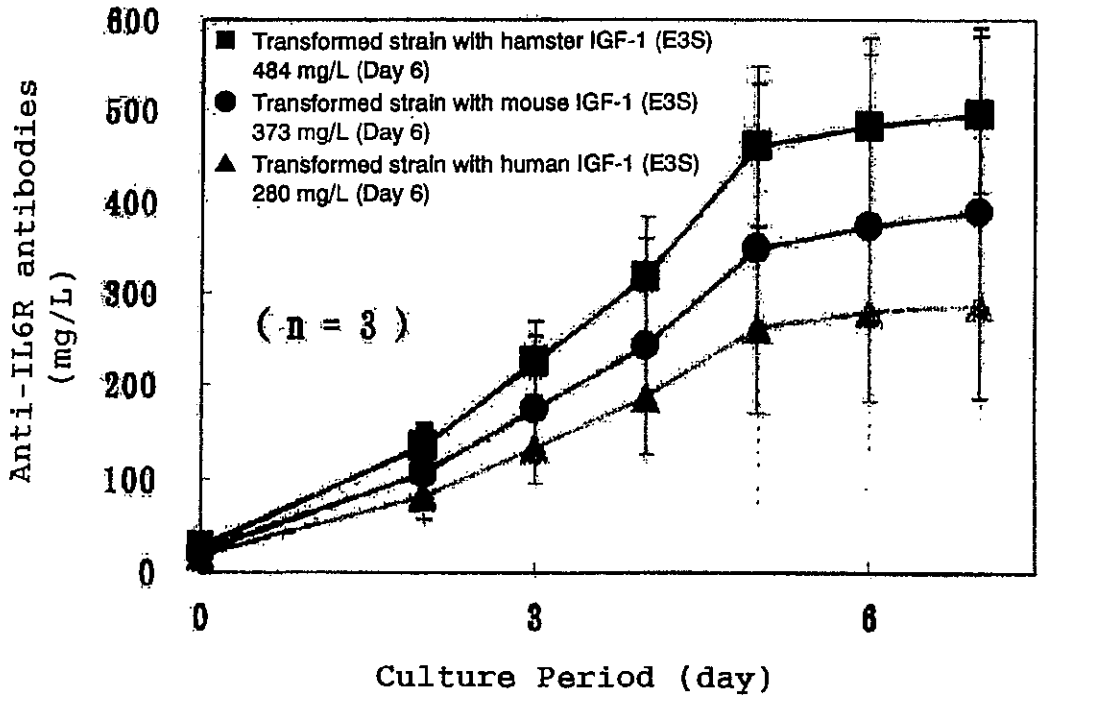


Figure 9

Amounts of anti-IL6R antibodies produced in fed-batch cultures of the best strains

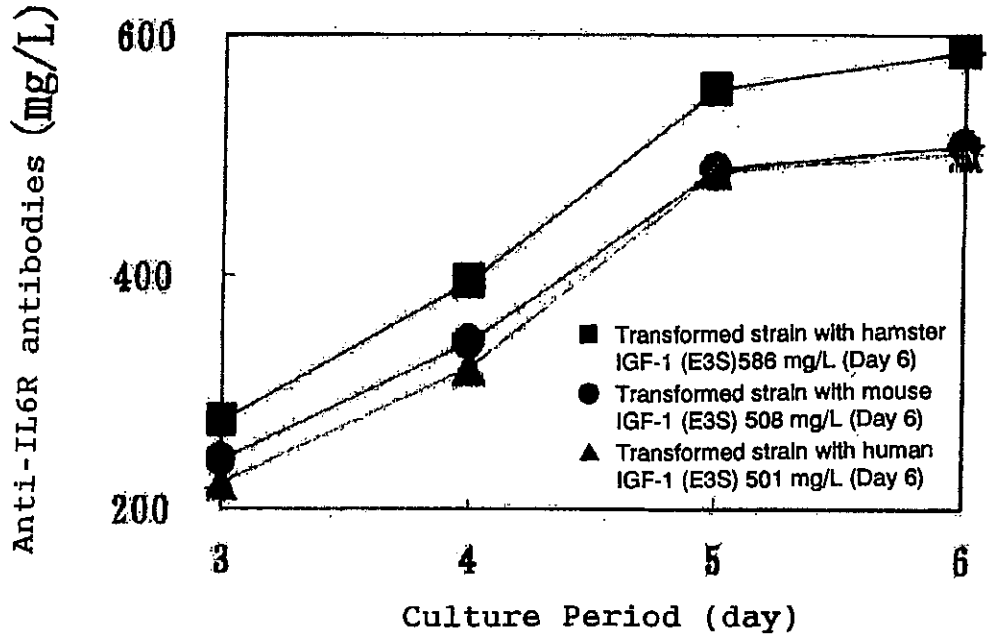
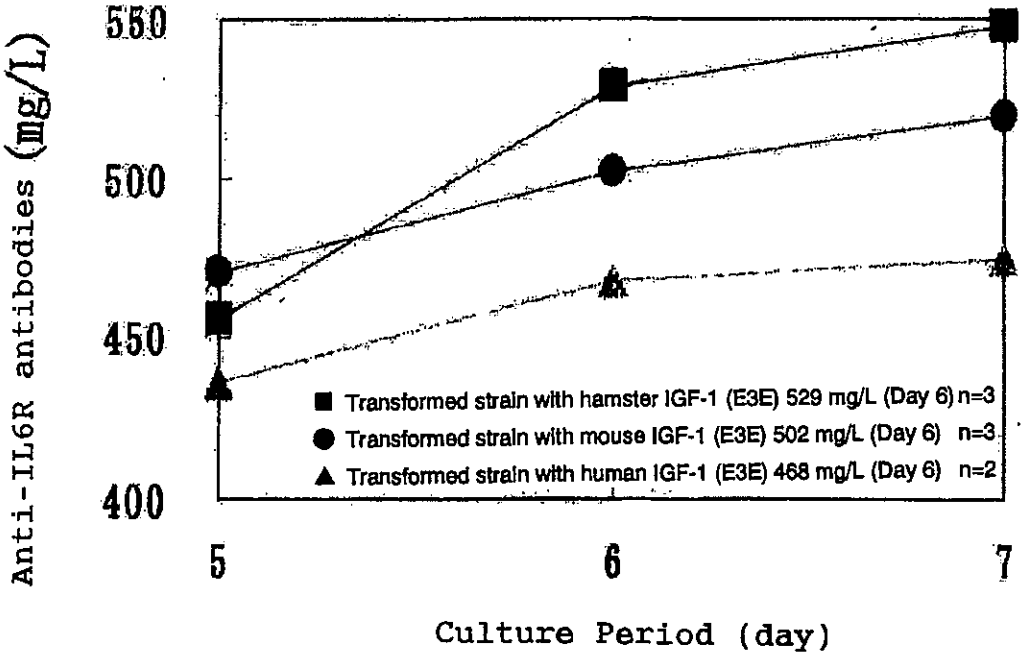


Figure 10

Amounts of anti-IL6R antibodies produced in fed-batch cultures