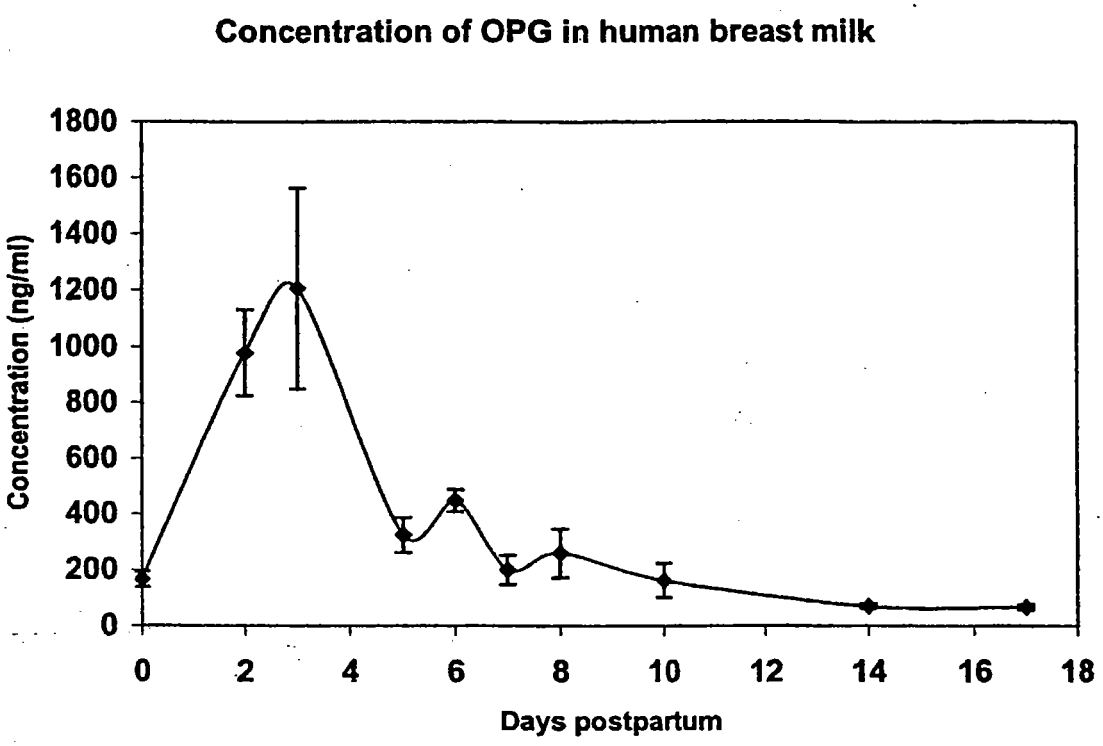


Fig. 1: Concentration of OPG in human breast milk at different times during lactation

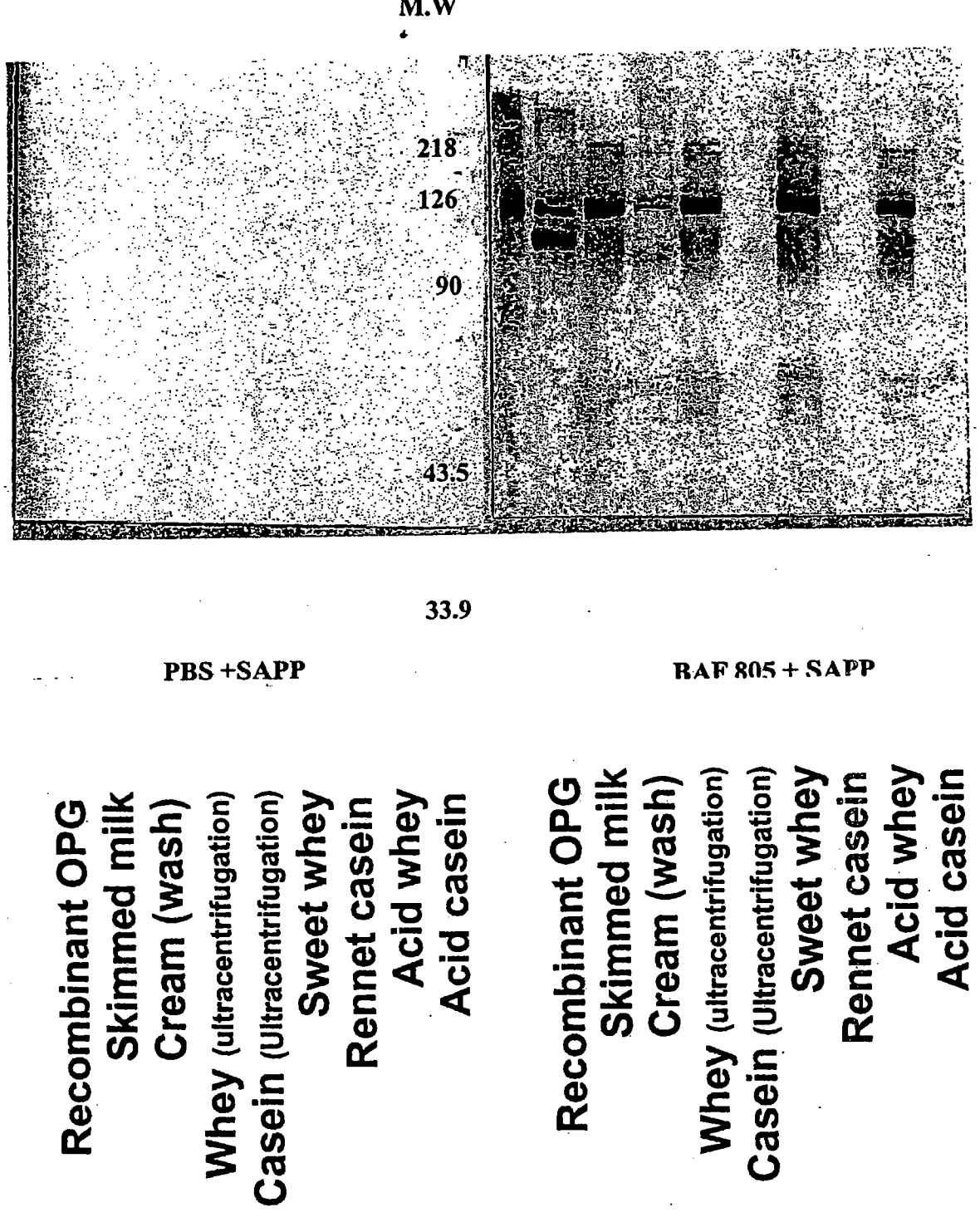


Fig. 2: Western blot analysis of human milk fractions under reducing conditions using 10% SDS-

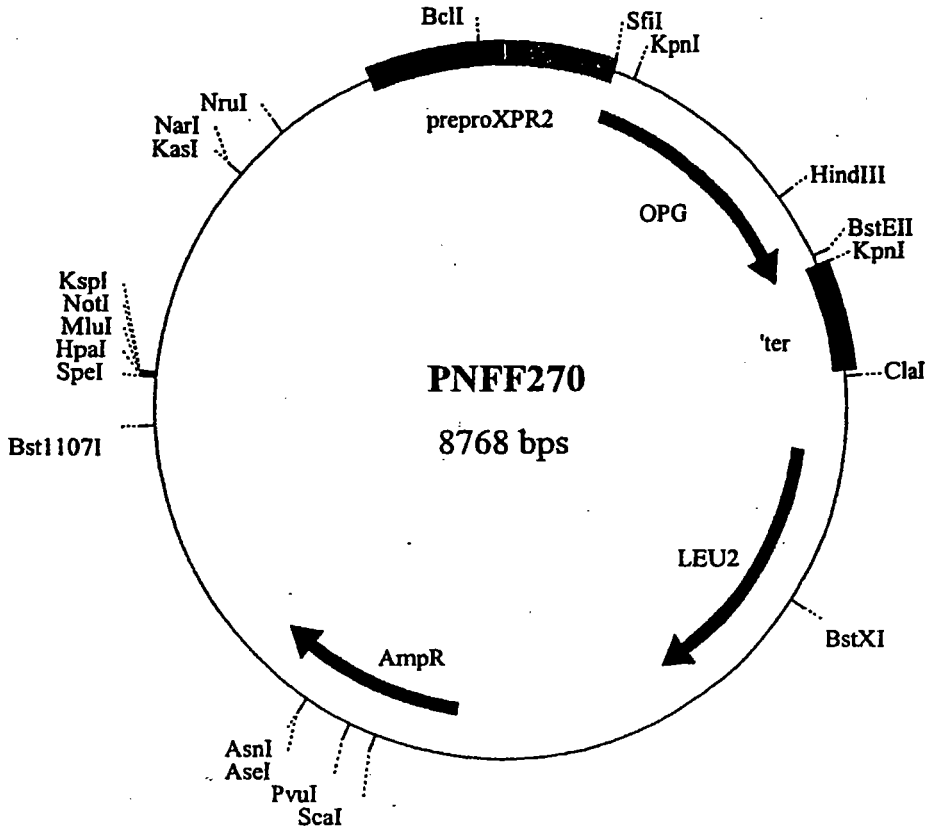


Figure 3. The resriction map of the plasmid which was integrated into the genomic DNA of *Yarrowia* transformants.

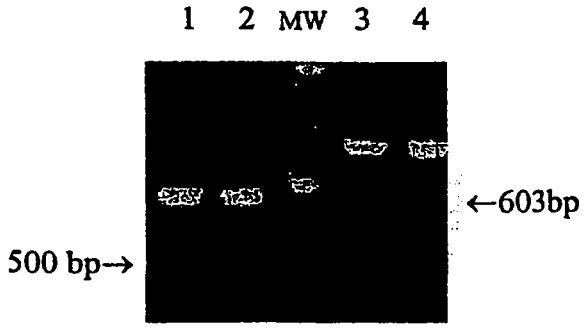


Figure 4. RT-PCR analysis of human breast milk cells and human mammary gland epithelial cells, MCF-7.

Lanes 1 and 2 :β-actin (expected size band: 460 bp)
Lanes 3 and 4 : OPG (expected size band: 603 bp)

1. Human breast milk cells
2. MCF-7
3. Human breast milk cells
4. MCF-7

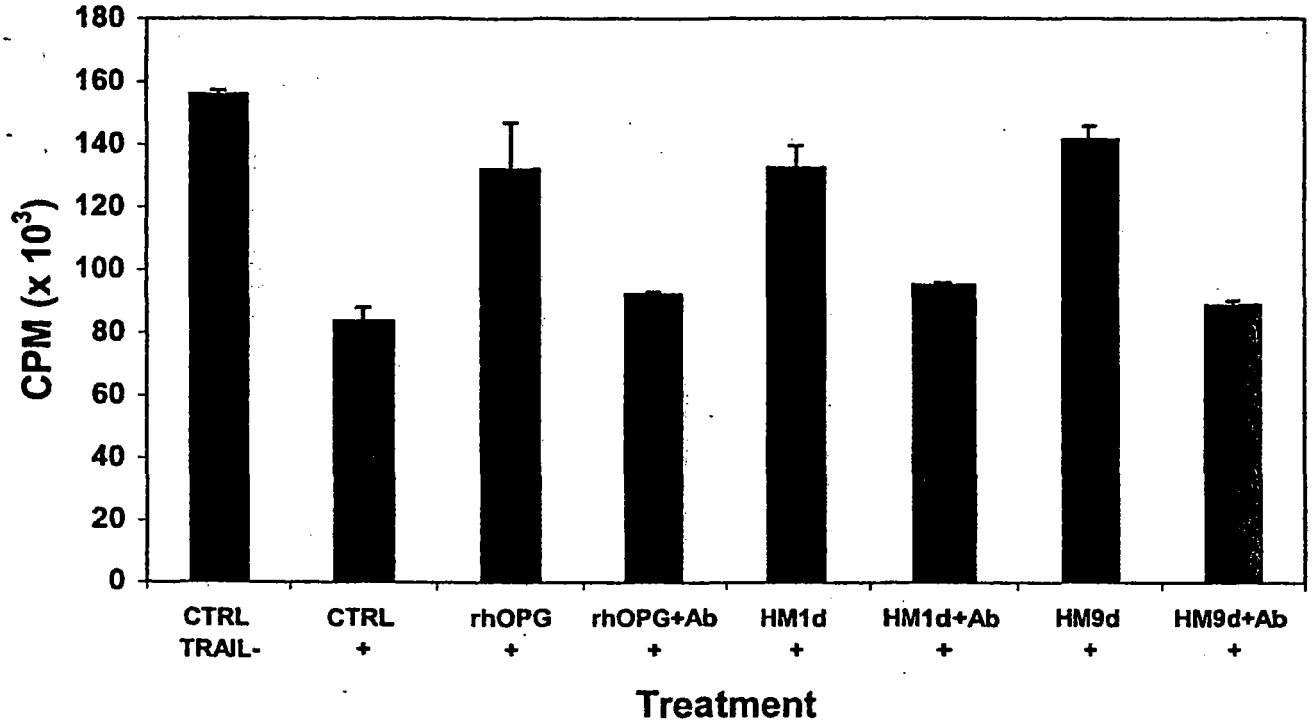


Figure 5: OPG inhibits TRAIL-induced apoptosis of Jurkat cells. The figure depicts a single representative experiment of Jurkat cells treated with 20ng/ml of TRAIL and recombinant human OPG (rhOPG) or human milk (HM) at a final dilution of 1/80. The HM in the above experiment was from a single mother at either 1 day or 9 days postpartum. Antibody (Ab) against OPG was used at a concentration of 20µg/ml. Cell proliferation was measured by ^{3}H-thymidine incorporation. In control wells (CTRL), cells were exposed to culture medium with or without TRAIL.

MKLATAFTILTAVLAAPLAAPAPAPDAAPAAVPEGPAAAAYSSILSVVAKQSKKFKHHKR
DLDEKDQFIVVFDSSATVDQIASEIQKLDSLVDEDSSNGITSALDLPVYTDGSGFLGFVG
KFNSTIVDKLKESSVLTVEPDTIVSLPEIPASSAAKRETFPPKYLHYDEETSHQLLCDKC
PPGTYLKQHCTAKWKTVCAPCPDHYYTDSWHTSDECLYCSPVCKELQYVKQECNRTHNRV
CECKEGRYLEIEFCLKHRSCPPGFGVVQAGTPERNTVCKRCPDGFFSNETSSKAPCRKHT
NCSVFGLLLTQKGNATHDNICSGNSESTQKCGIDVTLCEEAFFRFAVPTKFTPNWLSVLV
DNLPGTKVNAESVERIKRQHSSQEQTFQLLKLWKHQNKAQDIVKKIIQDIDLCENSVQRH
IGHANLTFEQLRSLMESLPGKKVGAEDIEKTIKACKPSDQILKLLSLWRIKNGDQDTLKG
LMHALKHSKTYHFPKTVTQSLKKTIRFLHSFTMYKLYQKLFLEMIGNQVQSVKISCL

Figure 6. The protein encoded by the OPG plasmid inserted in *Y. Lipolytica*. The mature OPG is indicated in bold print.

```
1   TCCGGCCTCTTCGGCCgccaagcgaGAAACGTTTCCTCCAAAGTACCTTCATTATGACGA   60
    1                xpr2          E  T  F  P  P  K  Y  L  H  Y  D  E   12

   61  AGAAACCTCTCATCAGCTGTTGTGTGACAAATGTCCTCCTGGTACCTACCTAAAACAACA  120
   13   E  T  S  H  Q  L  L  C  D  K  C  P  P  G  T  Y  L  K  Q  H   32

  121  CTGTACAGCAAAGTGGAAGACCGTGTGCGCCCCTTGCCCTGACCACTACTACACAGACAG  180
   33   C  T  A  K  W  K  T  V  C  A  P  C  P  D  H  Y  Y  T  D  S   52

  181  CTGGCACACCAGTGACGAGTGTCTATACTGCAGCCCCGTGTGCAAGGAGCTGCAGTACGT  240
   53   W  H  T  S  D  E  C  L  Y  C  S  P  V  C  K  E  L  Q  Y  V   72

  241  CAAGCAGGAGTGCAATCGCACCCACAACCGCGTGTGCGAATGCAAGGAAGGGCGCTACCT  300
   73   K  Q  E  C  N  R  T  H  N  R  V  C  E  C  K  E  G  R  Y  L   92

  301  TGAGATAGAGTTCTGCTTGAAACATAGGAGCTGCCCTCCTGGATTTGGAGTGGTGCAAGC  360
   93   E  I  E  F  C  L  K  H  R  S  C  P  P  G  F  G  V  V  Q  A  112

  361  TGGAACCCCAGAGCGAAATACAGTTTGCAAAAGATGTCCAGATGGGTTCTTCTCAAATGA  420
  113   G  T  P  E  R  N  T  V  C  K  R  C  P  D  G  F  F  S  N  E  132

  421  GACGTCATCTAAAGCACCCTGTAGAAAACACACAAATTGCAGTGTCTTTGGTCTCCTGCT  480
  133   T  S  S  K  A  P  C  R  K  H  T  N  C  S  V  F  G  L  L  L  152

  481  AACTCAGAAAGGAAATGCAACACACGACAACATATGTTCCGGAAACAGTGAATCAACTCA  540
  153   T  Q  K  G  N  A  T  H  D  N  I  C  S  G  N  S  E  S  T  Q  172

  541  AAAATGTGGAATAGATGTTACCCTGTGTGAGGAGGCATTCTTCAGGTTTGCTGTTCCTAC  600
  173   K  C  G  I  D  V  T  L  C  E  E  A  F  F  R  F  A  V  P  T  192

  601  AAAGTTTACGCCTAACTGGCTTAGTGTCTTGGTAGACAATTTGCCTGGCACCAAAGTAAA  660
  193   K  F  T  P  N  W  L  S  V  L  V  D  N  L  P  G  T  K  V  N  212

  661  CGCAGAGAGTGTAGAGAGGATAAAACGGCAACACAGCTCACAAGAACAGACTTTCCAGCT  720
  213   A  E  S  V  E  R  I  K  R  Q  H  S  S  Q  E  Q  T  F  Q  L  232
                                  C
  721  GCTGAAGTTATGGAAACATCAAAACAAAGACCAAGATATAGTCAAGAAGATCATCCAAGA  780
  233   L  K  L  W  K  H  Q  N  K  D  Q  D  I  V  K  K  I  I  Q  D  252
                                  A
  781  TATTGACCTCTGTGAAAACAGCGTGCAGCGGCACATTGGACATGCTAACCTCACCTTCGA  840
  253   I  D  L  C  E  N  S  V  Q  R  H  I  G  H  A  N  L  T  F  E  272

  841  GCAGCTTCGTAGCTTGATGGAAAGCTTACCGGGAAAGAAAGTGGGAGCAGAAGACATTGA  900
  273   Q  L  R  S  L  M  E  S  L  P  G  K  K  V  G  A  E  D  I  E  292

  901  AAAAACAATAAAGGCATGCAAACCCAGTGACCAGATCCTGAAGCTGCTCAGTTTGTGGCG  960
  293   K  T  I  K  A  C  K  P  S  D  Q  I  L  K  L  L  S  L  W  R  312

  961  AATAAAAAATGGCGACCAAGACACCTTGAAGGGCCTAATGCACGCACTAAAGCACTCAAA 1020
  313   I  K  N  G  D  Q  D  T  L  K  G  L  M  H  A  L  K  H  S  K  332
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1021  GACGTACCACTTTCCCAAAACTGTCACTCAGAGTCTAAAGAAGACCATCAGGTTCCTTCA  1080

 333   T  Y  H  F  P  K  T  V  T  Q  S  L  K  K  T  I  R  F  L  H   352

1081  CAGCTTCACAATGTACAAATTGTATCAGAAGTTATTTTTAGAAATGATAGGTAACCAGGT  1140
 353   S  F  T  M  Y  K  L  Y  Q  K  L  F  L  E  M  I  G  N  Q  V   372

1141  CCAATCAGTAAAAATAAGCTGCTTATAACTAGTATCACTAGT  1182
 373   Q  S  V  K  I  S  C  L                    380
```

Figure 7. Sequence of milk OPG.