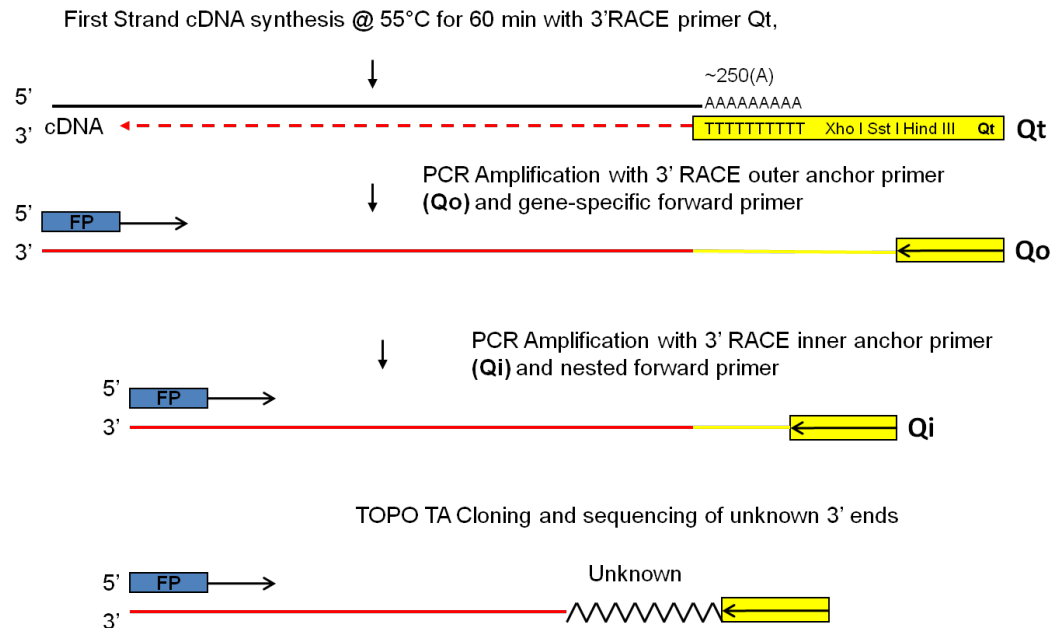


3' RACE



Supplemental Figure 1. Schematic of 3' RACE. cDNA synthesis was carried out with a specialized **Qt** primer, that contains a poly(dT) stretch for poly(A) binding which is preceded by restriction enzyme sites and a unique adapter sequence. An 'outer' race (**Qo**) primer was used in the first round of PCR amplification and a gene-specific forward primer. A second round of PCR is required to increase specificity, with a nested forward primer and the (**Qi**) 'inner' anchor primer. The many registers in which the **Qt** primer can bind the poly(A) give rise to a heterogenous population of PCR products, which requires cloning of a singular PCR product before sequencing.

Genomic sequence for the PRTN3 gene

GCCTCCACCACACTCCATGCCAGGAGCTCCCTTTTCGTGACAGCCACAGGTGTCCTCAGACCTCGCCAGGGTCCCTTG
 GGGGCAGAATCACCTCTTGATTGAGAATCCTGGCCTTAAAGGGGGAAGAAAACCCCCAGACAAAACCTGGCAGGAGGAT
 GTTCGCTGGGGCCTCAGGTGAGGCACTGAGGGCACTCGTGGTGGGGCCTGCCTTCCCAGCTCTGCATCCTGTCCCC
 TGCTACGTCCTGTGTCTGTCCTTTCCAGCTCACCCACCCACGGGACACACCTGCCACGGCCGGCTTTGCTGGGGCC
 ACAGGGACCTCGTCAGGGAGGCGTCTCCTTTGTTGGGTGGCACGTGGGCATGTGTTGCTCTGAGGGCTGTGGCCATGTTG
 CCCACCTGGCCAGGGACCCCGACTTGGGTGGGTGACAGCCAGCTCCCCGCCCCACAAAGGTTGGGACCTTGAGCC
 CAAAGCCCCACCTCCTCCCCGGAGTCCGTTCTGACTCCAGGCTCCAAAGGCAAAAGGAGGAAGTGGGGACCCAGCC
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 CGTGCCCATCCATCCAGCTCCAGGCCCGGTGGATTGTGGGAAATATCCACCACGAGGTCCATCCAAAGCCCTTCT
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 CCGGGGGAGACCGCTCCTTGACACCAGGCCACTCCTCCTCCCCGCC

PR3 promotor region

Exon 1 (red)
PR3 FP underlined

Intron I
PR3-002
FP underlined (green)

Exon 2
PR3 RP underlined
Intron II (spliced out)

Exon 3
PR3-002
RP (green)

Supplemental Figure 2, Continued

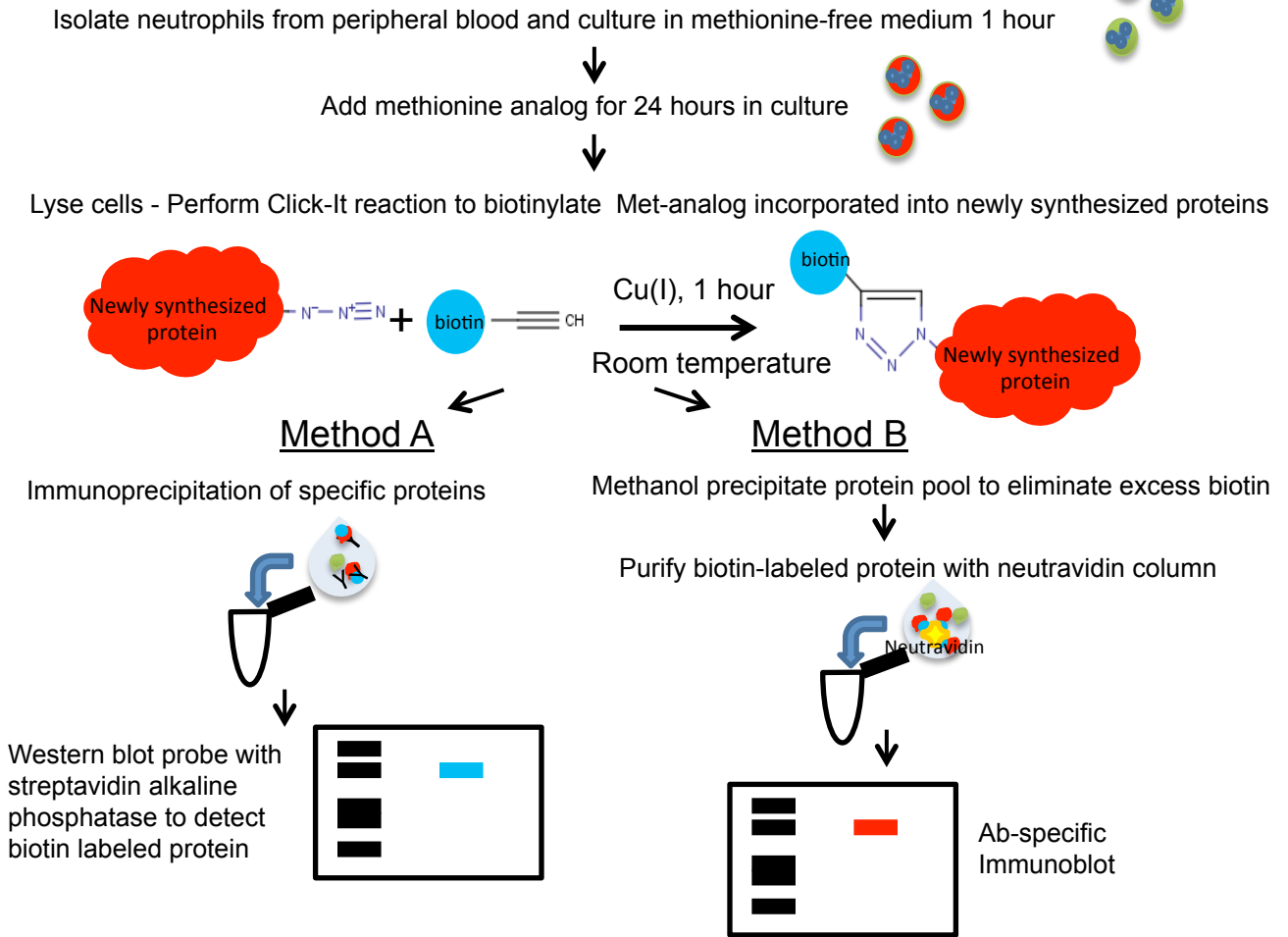
A detailed description of the RT-PCR Protocol used to detect PR3 mRNA versus PRTN3-002 mRNA.

- 1) *PR3* forward primer sequence resides in exon 1 (5'-CCC ACC ATG GCT CAC-3') and reverse primer in exon 2 (5'-CAA GGT GCC TCC GCA GAA-3') producing a 188 nt amplicon.
- 2) *PRTN3-002* forward primer sequence resides in intron 1 (5'-GTTGCAGATCGGGAGACG-3') and reverse primer in exon 3 (5'-GTTCTCCGCGTCGTAGTTGT-3') producing a 298 nt amplicon.
- 3) Full length transcript amplification for *PR3* FP(5'- GACCCACCATGGCTCAC-3') and *PRTN3-2* (5'-GTTGCAGATCGGGAGACG-3'). The same reverse primer was used for both 5'-GGAGTTTCAACGTTTATTGAGGTCAC-3'.
- 4) Primer sequences for cyclophilin were - forward primer 5'- AGG CAG GAA AAG CAA GGA GCCA-3' and reverse primer RP 5'- TCACTGCAGGTAGTCTGCGCC-3'.

Detailed RT-PCR protocol

The reverse transcription (RT) reaction was performed on 600 ng of mRNA in a 15 µl reaction of 0.75 µl of SS III Reverse Transcriptase, 0.75 µl of 10mM dNTPs, 0.2 µl of random hexamer primer (IDT Tech), 0.75 µl of DTT, 3 µl of 5xFS buffer, 1.5 µl of DMSO, 1.5 µl of Betaine, and 0.5 µl of RNasin Plus RNase inhibitor. Random hexamers, dNTPs, and mRNA were heated (65°C/ 5 minutes) and cooled immediately (4°C/ 5 minutes) before addition of the remaining reagents. RT conditions for all templates were 50 minutes at 50.0°C, 5 minutes at 60.0°C, 5 minutes at 65.0°C, 5 minutes at 85.0°C, and 4°C. A 50 µL PCR reaction was performed with 10 µl of 5xGC PCR Buffer, 1 µl of 10 mM dNTPs, 1 µl each of 10 µM forward and reverse primers (IDT Tech), 0.5 µl of Phusion Hot Start DNA polymerase (Finnzymes), 2.5 µl DMSO, 30 µl of nuclease free water, and 4 µl of cDNA from the reverse transcription reaction. Thermal cycler profile was 2 minutes at 98 °C, "touchdown" PCR for 10 cycles (98°C, 20 seconds; 68°C, 30 seconds, decreasing by 1°C per cycle; 72°C, 30 seconds), followed by 25 cycles (98°C, 20 seconds; 58°C, 30 seconds; 72°C, 30 seconds), with a final 5 minute extension at 72°C. Product was analyzed by agarose gel electrophoresis. PCR primer sequences were generated by Primer3 (v. 0.4.0) online tool.

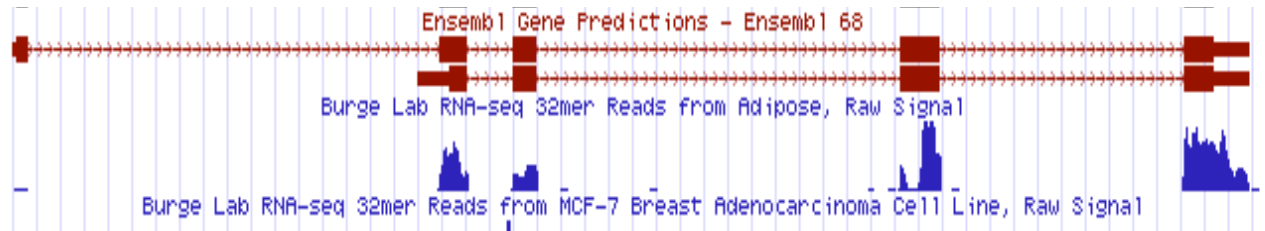
Supplemental Figure 3



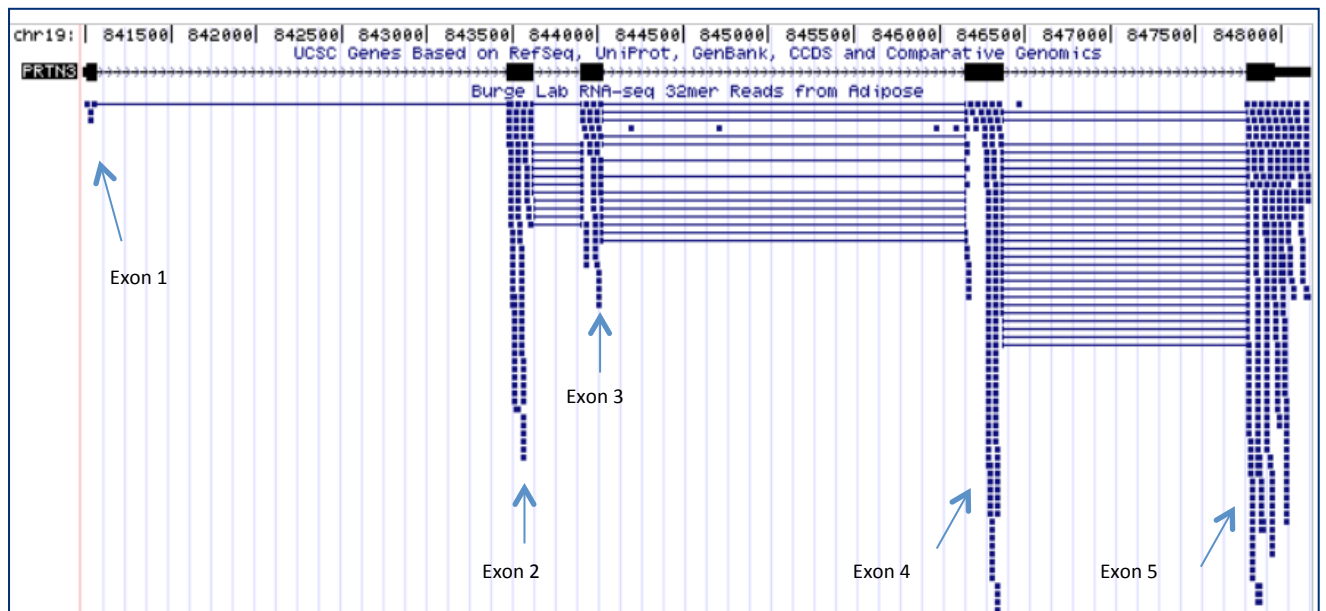
Supplemental Figure 3. Schematic of neutrophil protein synthesis analysis. Leukocytes were isolated from whole blood using HetaSep followed by a Histopaque 1077 gradient to isolate neutrophils. Red blood cells were lysed in hypotonic buffer. Neutrophils were incubated in a 5mL polypropylene tube in a volume of 500ul (6.6×10^6 cells/ mL) in methionine free RPMI1640 supplemented with 10% dialyzed FBS 1 hr to deplete methionine pools. Methionine analog L-azidohomoalanine (Invitrogen) (40uM) was added to medium and neutrophils were incubated 20 hours/37°C, 5% CO₂ under gentle agitation. Cells were lysed in 1% SDS 50mM Tris-HCl lysis buffer supplemented with EDTA Free Protease Inhibitor Cocktail (Roche), incubated on ice for 20 minutes and vortexed for 5 minutes. Lysate was sonicated to fragment DNA, centrifuged and transferred to a clean tube to remove insoluble cellular debris. Cell lysate volume was increased to 1mL using an IP buffer of NaCl, 1M HEPES, EDTA and .1% IPEGAL. Newly synthesized protein, which incorporated the methionine analog with an azide group, was biotinylated through the use of a biotin alkyne (Invitrogen) and the reaction was allowed to proceed at RT while rotating end over end for one hour. **Method A:** 4ug of anti-PR3 antibody (Santa Cruz H-60) or 4ug of anti-biotin antibody (Abcam, Cambridge, MA) was incubated overnight at 4C. Magnetic Protein A/G beads (40ul) (Pierce, 88802) were added 1 hour followed by 5X washes in IP buffer. Protein was eluted with 0.1% TFA for 45 minutes or 40ul of LDS loading buffer and boiling for 5 minutes respectively. pH was adjusted to 7.0 using 1M Tris HCl. **Method B:** Excess biotin was removed by a methanol chloroform precipitation or a desalting column. Biotinylated proteins were applied to streptavidin magnetic beads and incubated for 30 minutes at RT under gentle agitation. The beads were applied to a magnetic field and washed 6X in PBS supplemented with 1% BSA and 0.1% Tween 20. Samples were suspended in LDS loading buffer (Sigma) and electrophoresed on SDS-PAGE and blotted onto nitrocellulose membranes, blocked with 10% BSA in TBST for 1 hr, probed with streptavidin conjugated to alkaline phosphatase at a 1:1000 dilution or an antibody against PR3 (Supp Fig. 5) (MPO (Dako Carpinteria, CA)), or COX5B (Abcam), and visualized using Western Blue Stabilized Alkaline Phosphatase Substrate (Promega, Madison, WI)

Supplemental Figure 4

PRTN3 gene locus



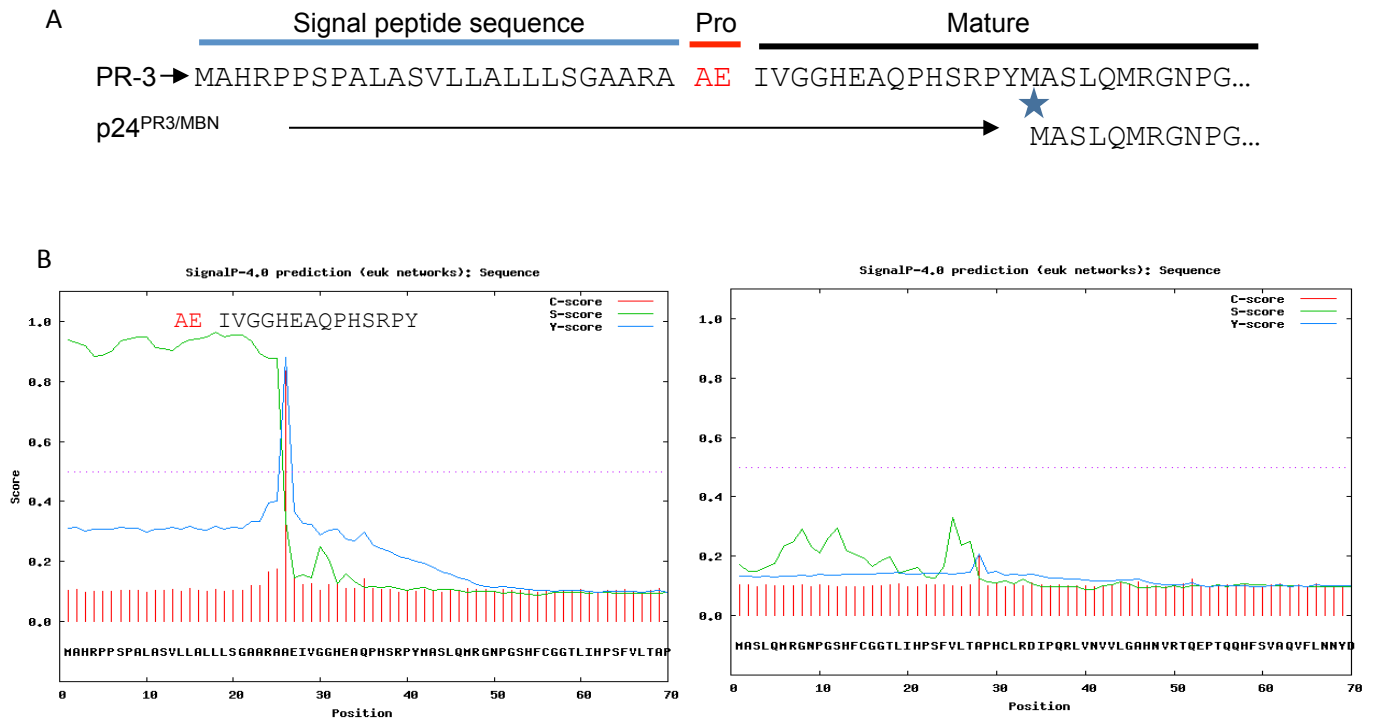
PRTN3



PRTN3-23-001	ENST00000234347	1012 nt
PRTN3-23-002	ENST00000544537	1041 nt

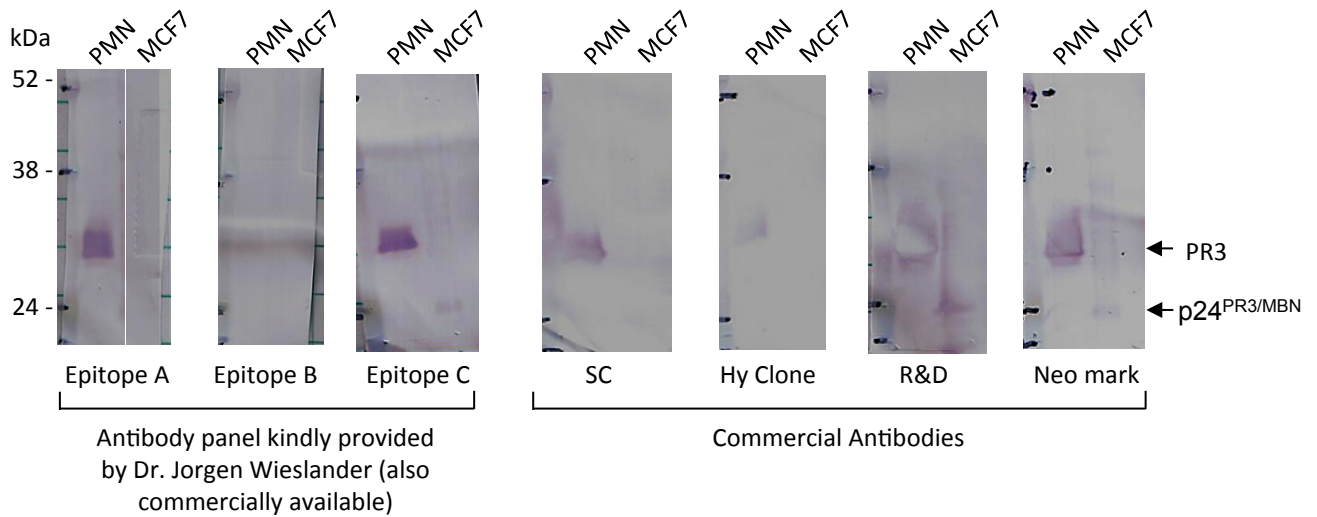
Supplemental Figure 3. Ensembl entry identifying a unique transcript from the *PRTN3* gene locus, expressed in adipose tissue and MCF7 breast epithelial cell.

Supplemental Figure 5



Supplemental Figure 6. In silico translation of *PRTN3-002* transcript indicates the protein lacks the signal peptide sequence present in PR3 that directs newly produced protein to the Golgi for posttranslational glycosylation. (B) bSignalP 4.0:Online tool verified sequence as a signal peptide: <http://www.cbs.dtu.dk/services/SignalP/> Thomas Nordahl Petersen, Søren Brunak, Gunnar von Heijne & Henrik Nielsen *Nature Methods*, **8**:785-786, 2011.

Supplemental Figure 6



Antibody	Cat #	Species
Epitope A	PR3.4A3	Mouse
Epitope B	PR3.4A5	Mouse
Epitope C	PR3.6A6	Mouse
Santa Cruz	Sc-28818	Rabbit
Hy Clone	HM2172	Mouse
R&D	AF6134	Sheep
Neo mark	MS-1333-P1	Mouse

Supplemental Figure 7. Identification of three anti-PR3 antibodies that are also reactive with the 24kDa p24^{PR3/MBN} by western blot.

Supplemental Table 2. Primers for northern probe, RT-PCR, 3'RACE, and Ligation anchored RT-PCR

Primer Name	Sequence	Product length
Northern probe		
PR3 336 exon 3	5'-GGT GTT TCT GAA CAA CTA CGA C-3'	292bp
PR3 627 exon 5	5'-TCC GAA GCA GAT GCC GGC CTT-3'	
Taqman and RT-PCR primers		
PR3 FOR ds exon 4	5'-TGT CAC CGT GGT CAC CTT CTT-3'	147bp
PR3 REV ds exon 5	5'-CCC CAG ATC ACG AAG GAG TCT AT-3'	
PR3 probe exon 4	FAM-TTG CAC TTT CGT CCC TCG CCG-TAMRA	
PR3 sense (exon 1 FOR)	5'-GAC CCC ACC ATG GCT CAC-3'	189bp
PR3 antisense	5'-CAA GGT GCC TCC GCA GAA-3'	
PRTN3-002 sense	5'-ATC GGG AGA CGG AGG CTC-3'	298bp
PRTN3-002 antisense	5'-CTC CGA GCA CCA CGT TCA-3'	
Cyclophilin FOR	5'-AGG CAG GAA AAG CAA GGA GCC A-3'	582bp
Cyclophilin REV	5'-TCA CTG CAG GTA GTC TGC GCC-3'	
PR3 sense (exon 1 FOR)	5'-GAC CCC ACC ATG GCT CAC-3'	986bp
PR3 exon 5, 3'UTR REV	5'-GGA GTT TCA ACG TTT ATT GAG GTC AC-3'	
PRTN3-002 intron 1 FOR	5'-GTT GCA GAT CGG GAG ACG-3'	1023bp
PR3 exon 5, 3'UTR REV	5'-GGA GTT TCA ACG TTT ATT GAG GTC AC-3'	
3' RACE primers		
PR3 374 exon 3 FOR	5'-ACG ACG TTC TCC TCA TCC AG-3'	
PR3 547 exon 4 FOR	5'-GTC ACC GTG GTC ACC TTC TT-3'	~500bp and ~580bp
PR3 617 exon 5 FOR	5'-TCT GCT TCG GAG ACT CAG GT-3'	~420bp and ~500bp
PR3 634 exon 5 FOR	5'-GGT GGC CCC CTG ATC TGT GAT G-3'	~400bp and ~480bp
Qo	5'-CCA GTG AGC AGA GTG ACG-3'	
Qi	5'-GAG GAC TCG AGC TCA ACG-3'	
Qt	5'-CCA GTG AGC AGA GTG ACG AGG ACT CGA GCT CAA GCT TTT TTT TTT TTT TTT T-3'	