Cis versus *Trans* Splicing: Both Mediated by Spliceosomes

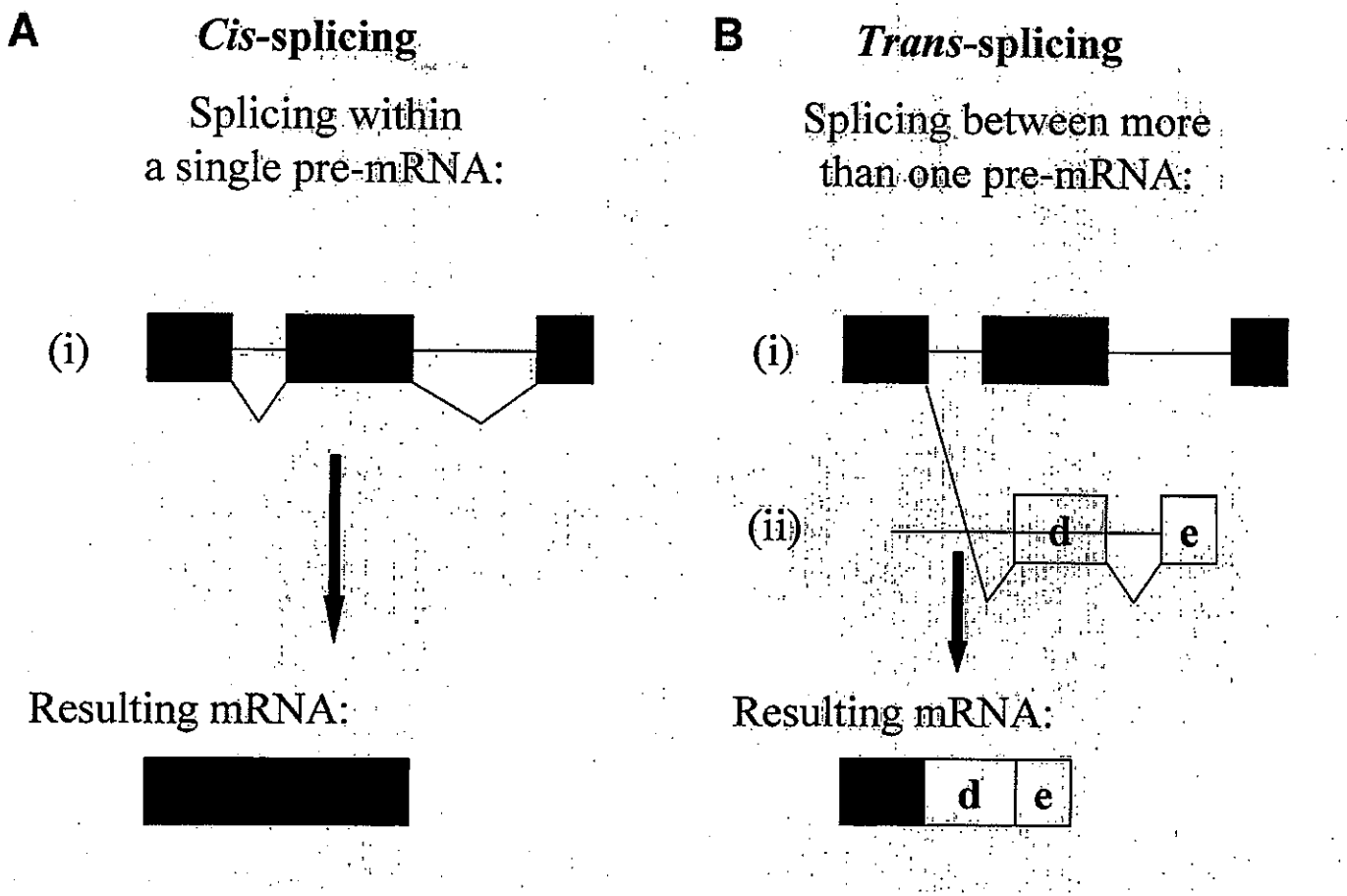


Figure 1

PTMs: Pre-Trans-splicing Molecules

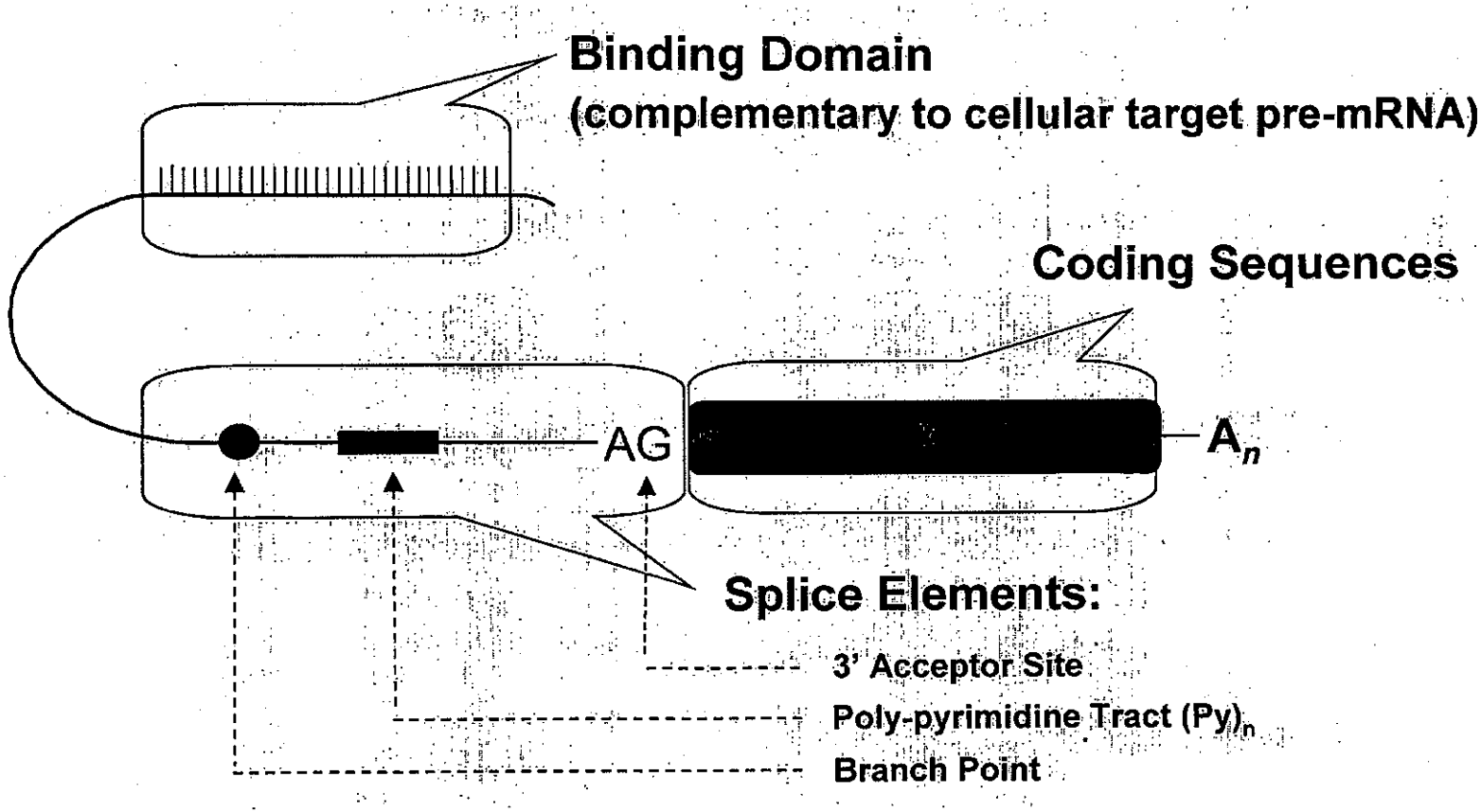


Figure 2

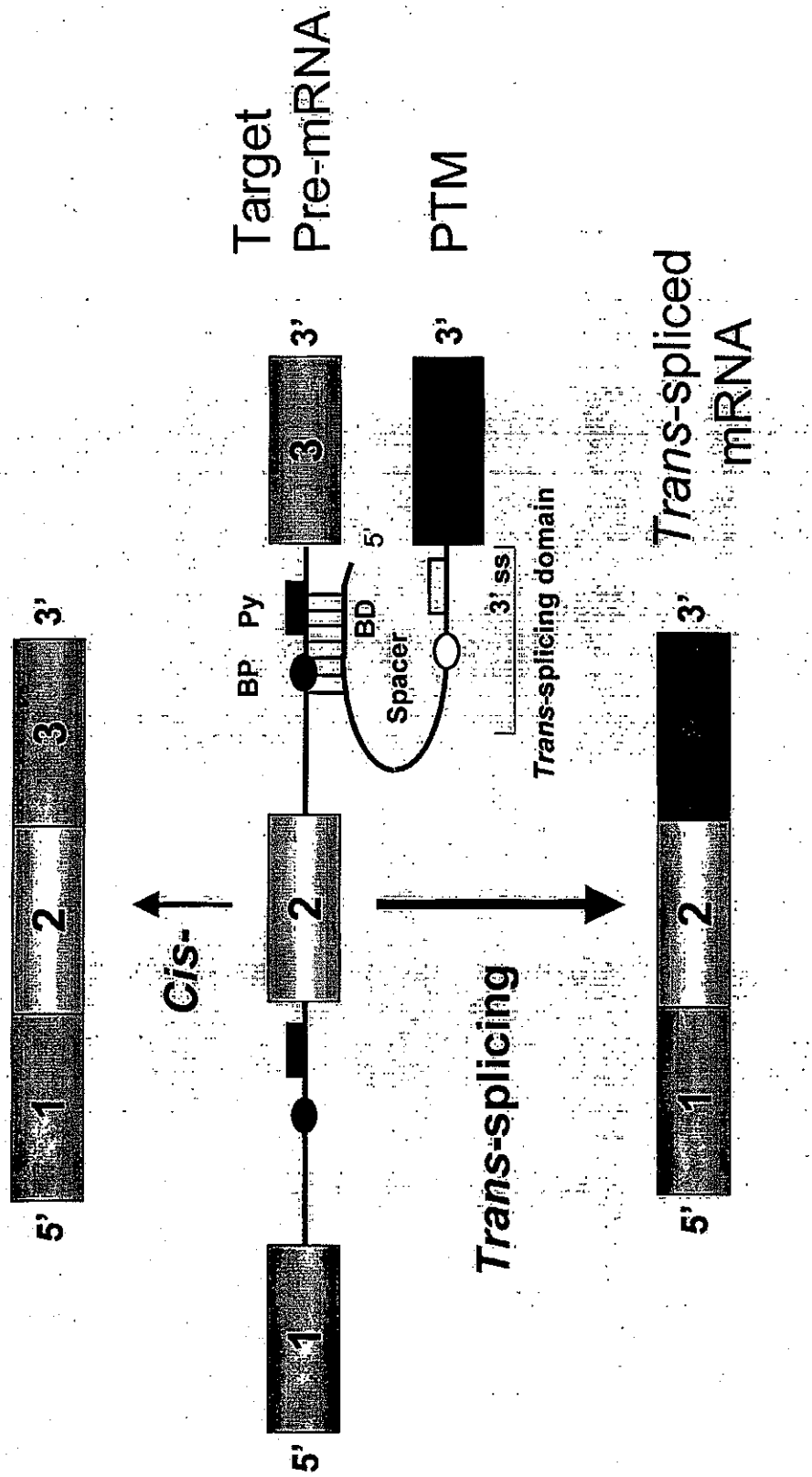
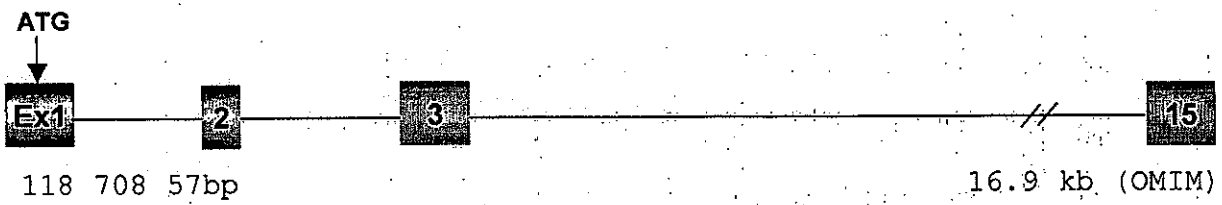

Target Pre-mRNA
PTM
Trans-spliced mRNA
Cis-
Trans-splicing
Trans-splicing domain
Spacer
BD
BP
Py
3' ss
5'
3'
1
2
3

Figure 3

Rationale for Selecting Albumin as Target



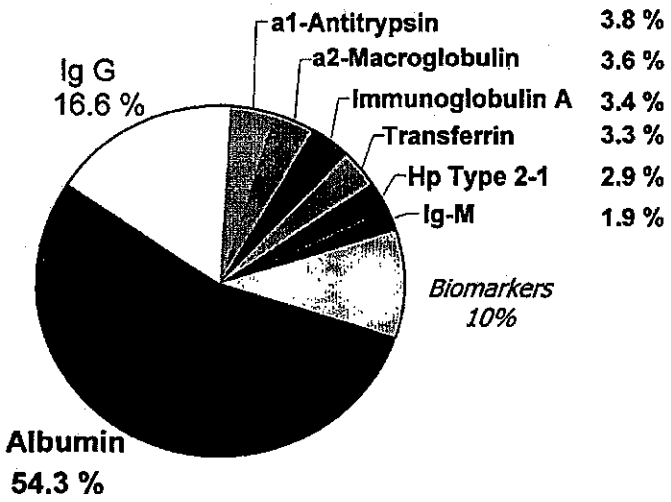
Gene: 16.96 kb, 15 exons and 14 introns
mRNA: 2.21 kb
Exon 1: 118 bp; majority codes for signal peptide

- **Abundance**, albumin protein comprises about one-half of the blood serum protein. Normal range ~35-50 mg/ml.

- Apo A-1 normal range ~1-1.5 mg/ml

Figure 4

The Human Plasma Proteome



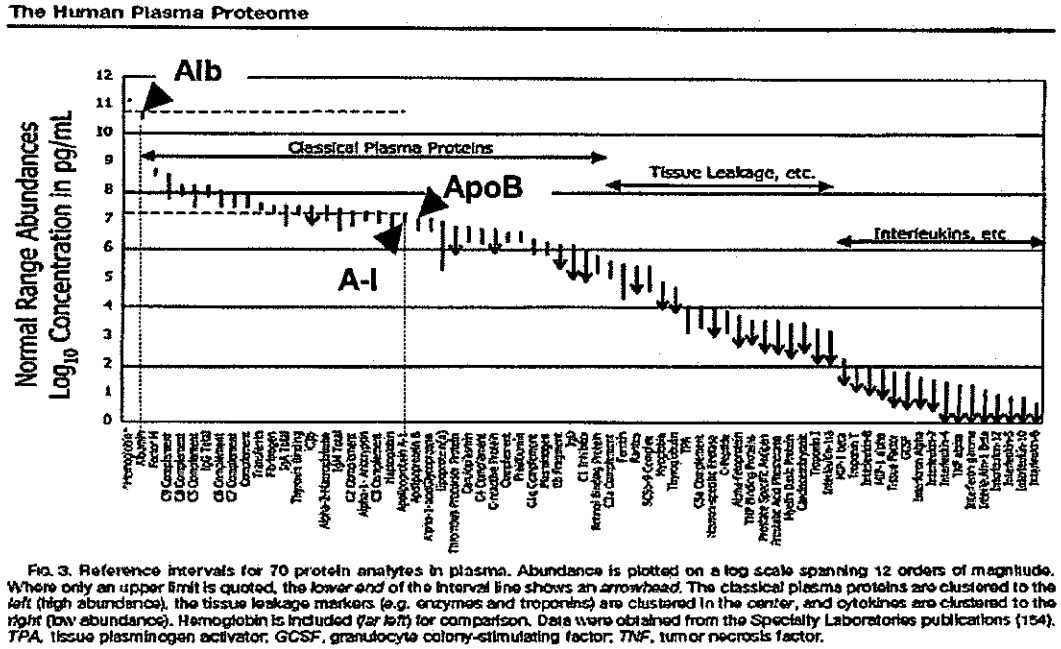


FIG. 3. Reference intervals for 70 protein analytes in plasma. Abundance is plotted on a log scale spanning 12 orders of magnitude. Where only an upper limit is quoted, the *lower end* of the interval line shows an *arrowhead*. The classical plasma proteins are clustered to the *left* (high abundance), the tissue leakage markers (*e.g.* enzymes and troponins) are clustered in the *center*, and cytokines are clustered to the *right* (low abundance). Hemoglobin is included (*far left*) for comparison. Data were obtained from the Specialty Laboratories publications (154). *TPA*, tissue plasminogen activator; *GCSF*, granulocyte colony-stimulating factor; *TNF*, tumor necrosis factor.

Anderson and Anderson, Molec. Cell. Proteomics 2002 1: 845

Figure 5

Trans-splicing sc Mab into Tumor Antigen

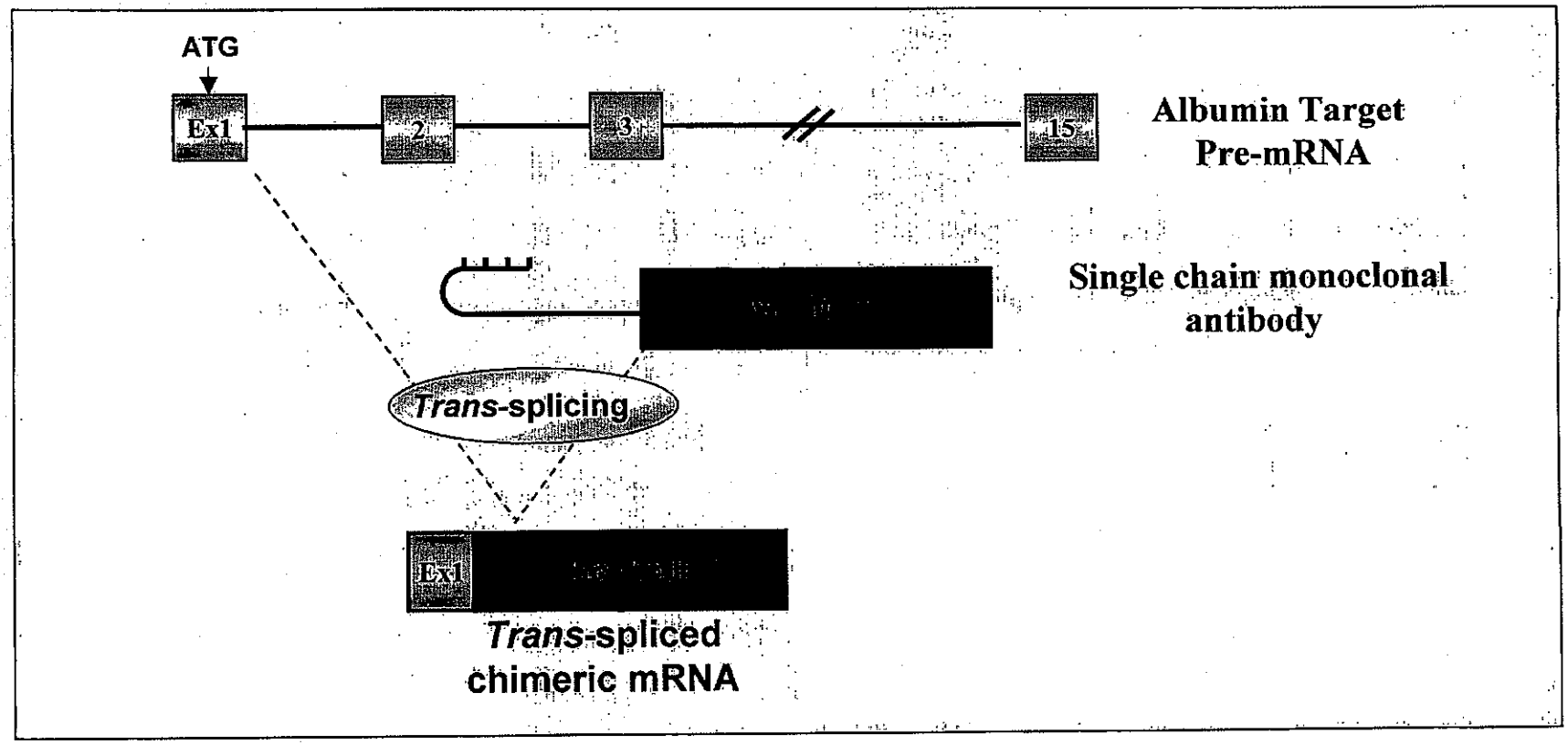


Figure 6

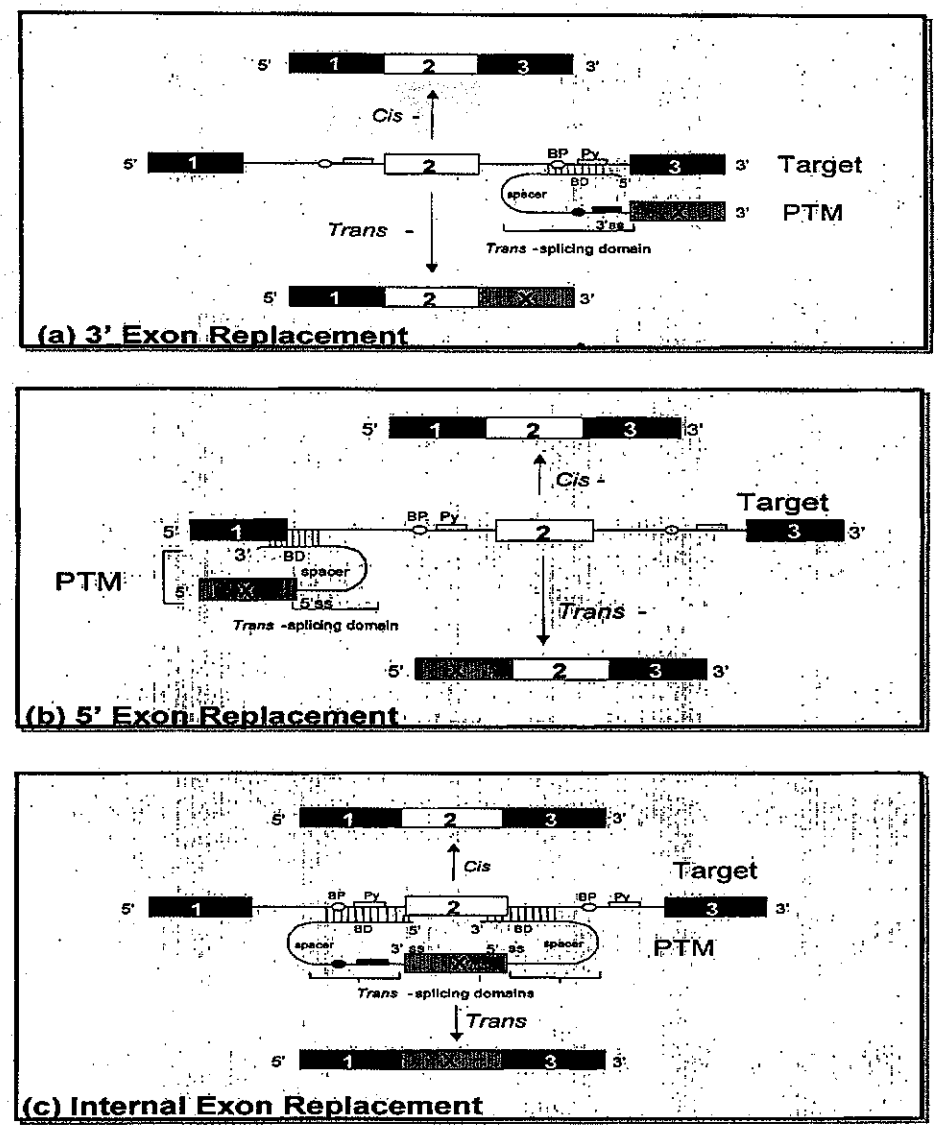

5'
1
2
3
3'
Cis -
BP
Py
Target
BD
spacer
PTM
3'ss
Trans -splicing domain
Trans -
X
(a) 3' Exon Replacement
5'
3'
Cis -
Target
BP
Py
PTM
BD
spacer
5'ss
Trans -splicing domain
Trans -
(b) 5' Exon Replacement
Cis
Target
PTM
Trans -splicing domains
Trans
(c) Internal Exon Replacement

Figure 7

Albumin Targeting Strategy to Produce Single Chain Antibodies *In Vivo*

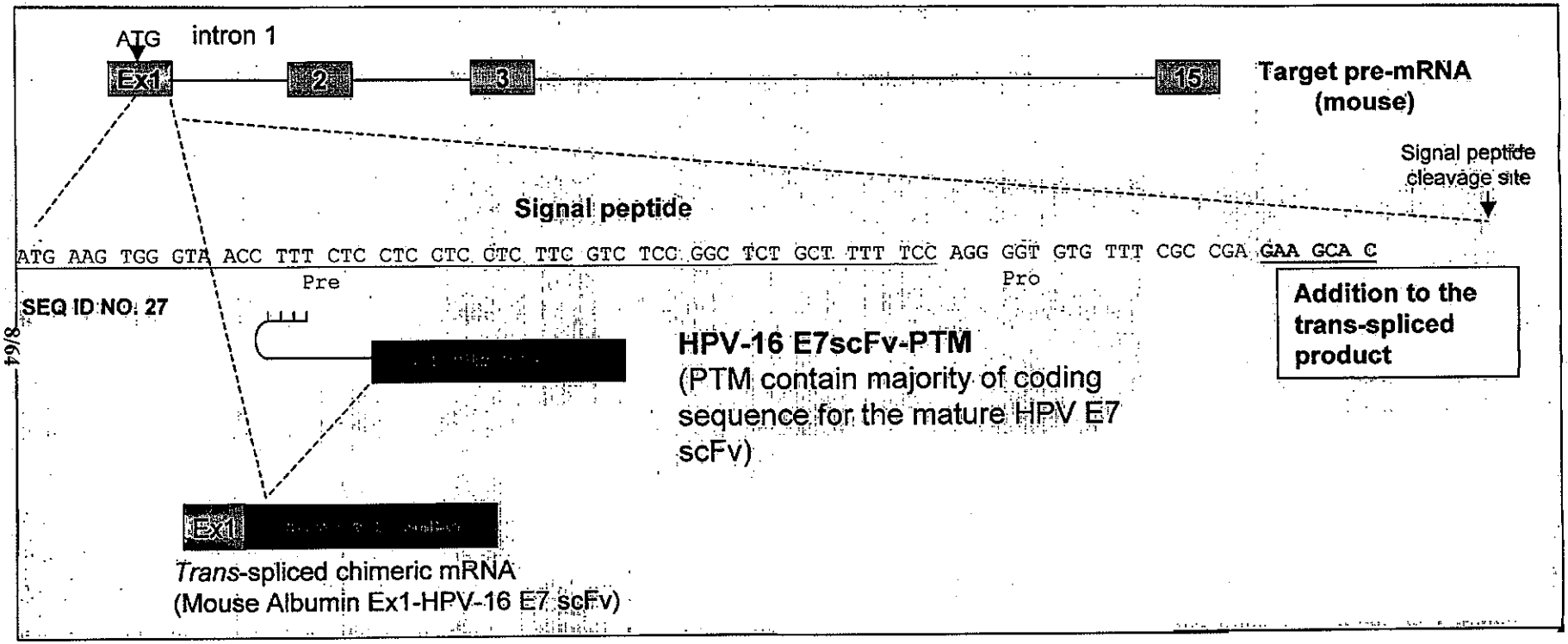


Figure 8

Mouse Albumin Exon 1-HPV-16 anti-E7 scFv cDNA

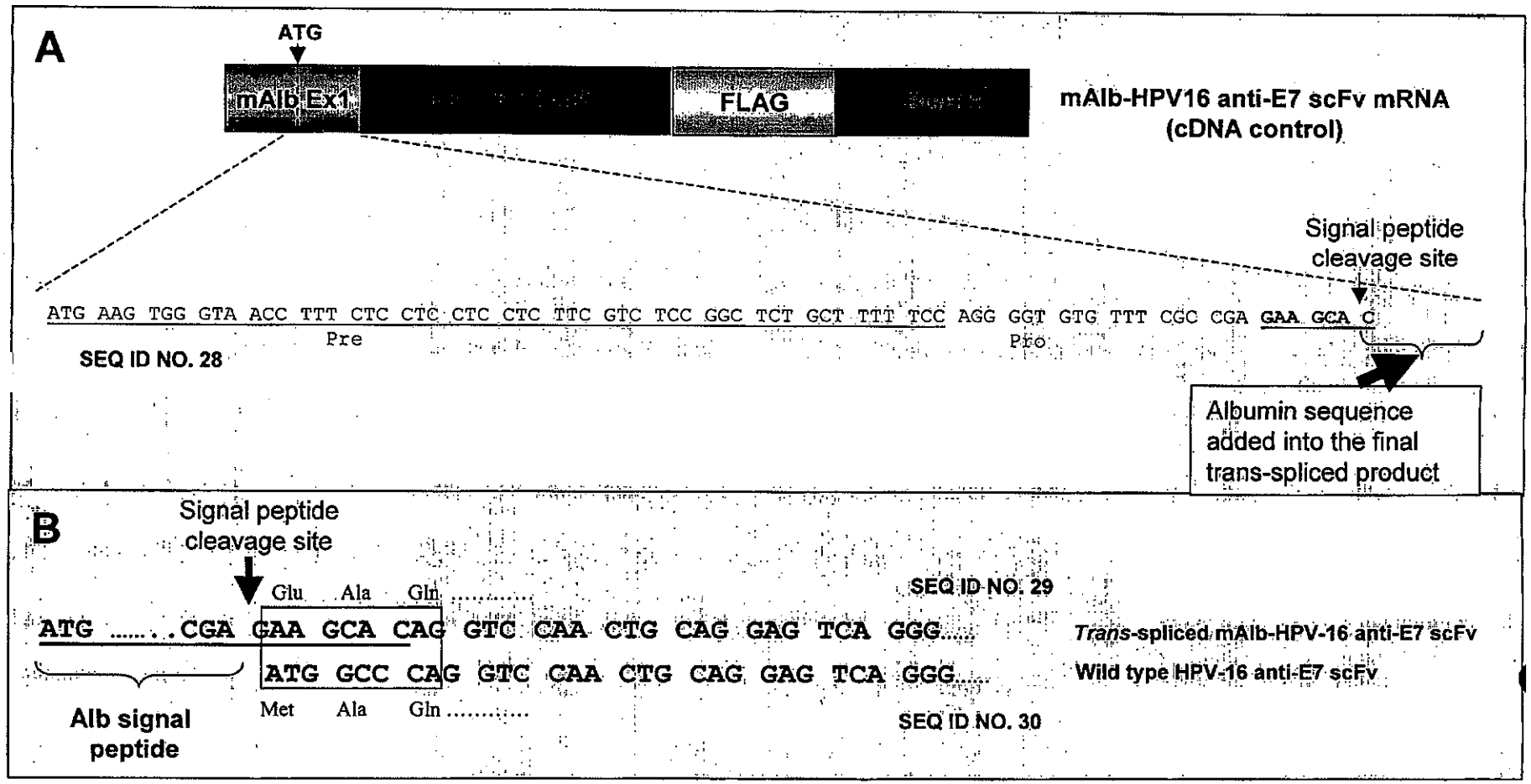
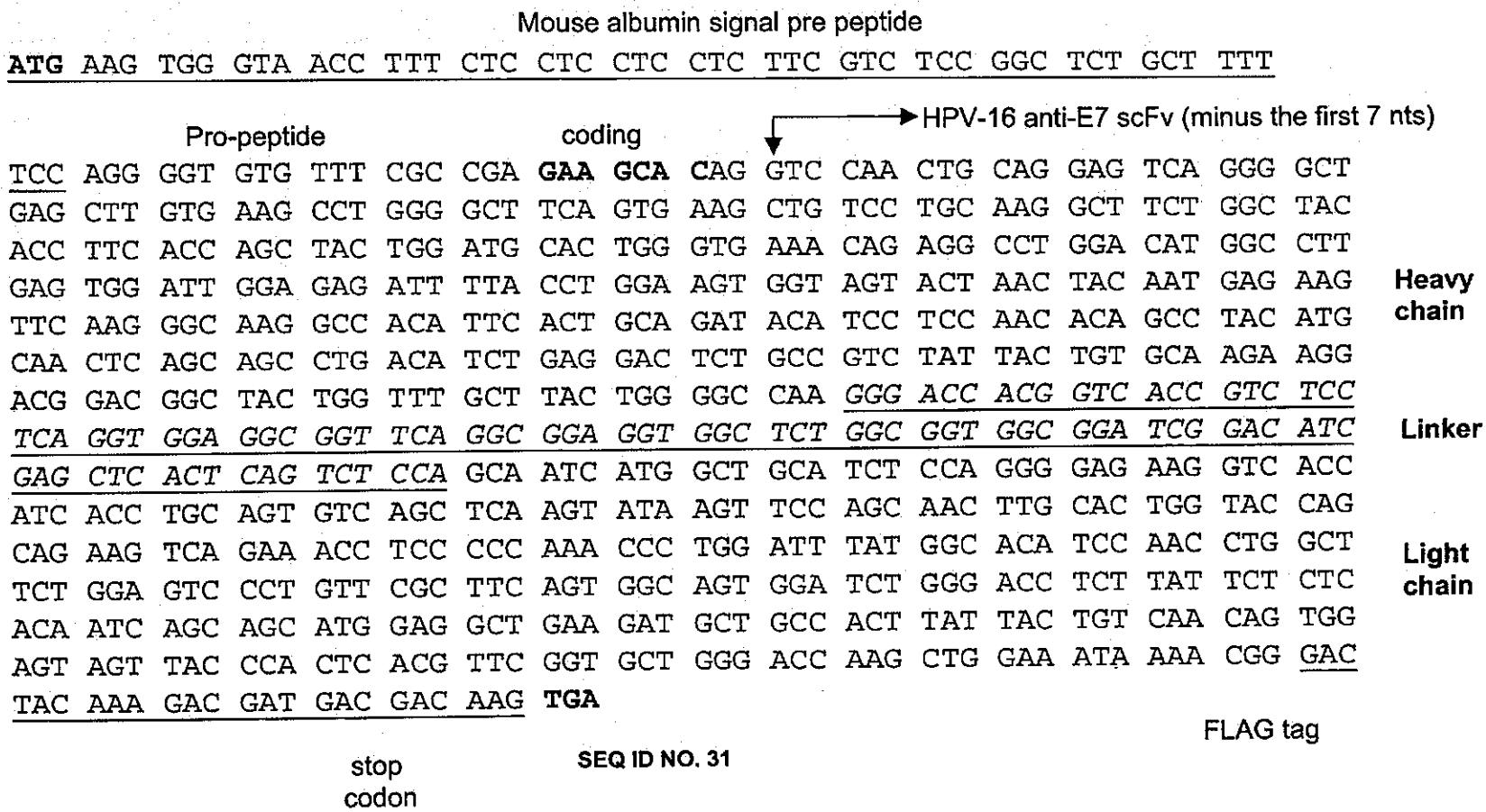


Figure 9



SEQ ID NO. 31

Figure 10

Strategy to Eliminate Albumin Sequence in the Final Product

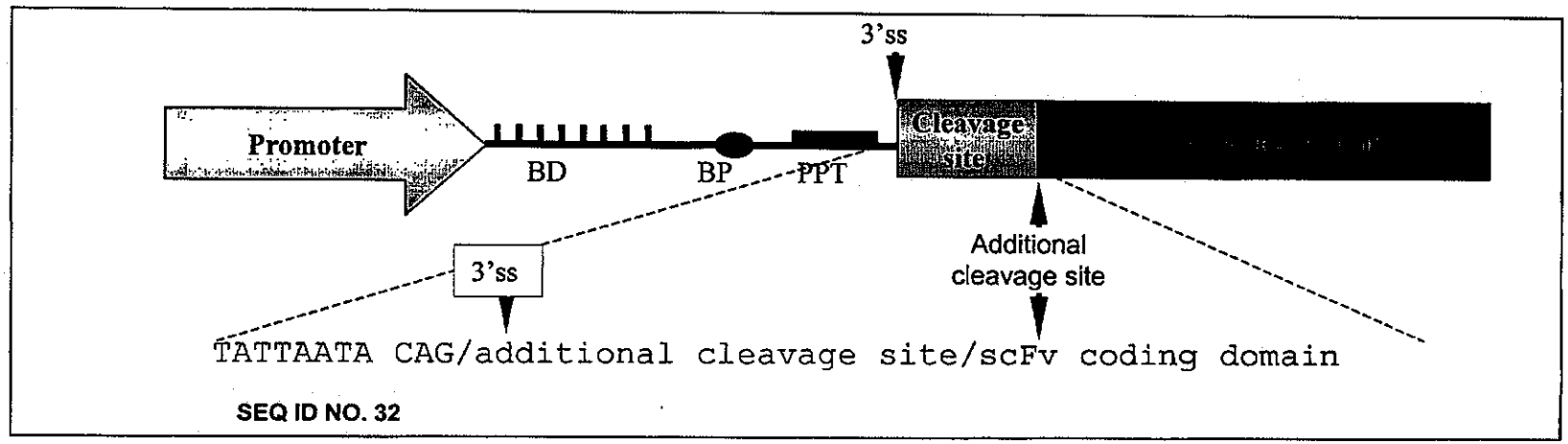


Figure 11

Strategy to Eliminate Albumin Sequence in the Final Product

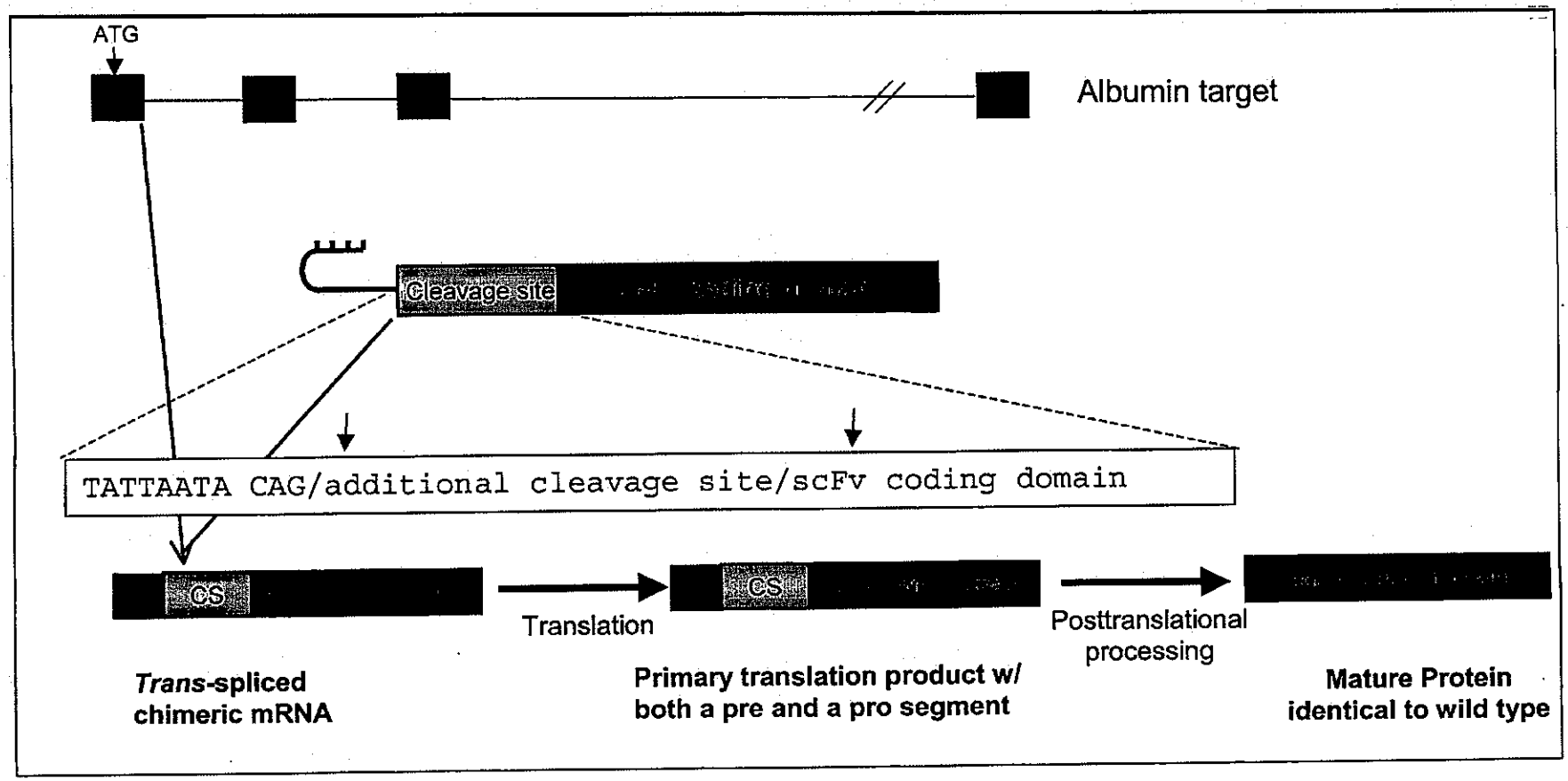


Figure 12

Mouse Alb-HPV E7 scFv cDNA is Translated, Processed and Secreted in Hepatoma and Cos-7 Cells

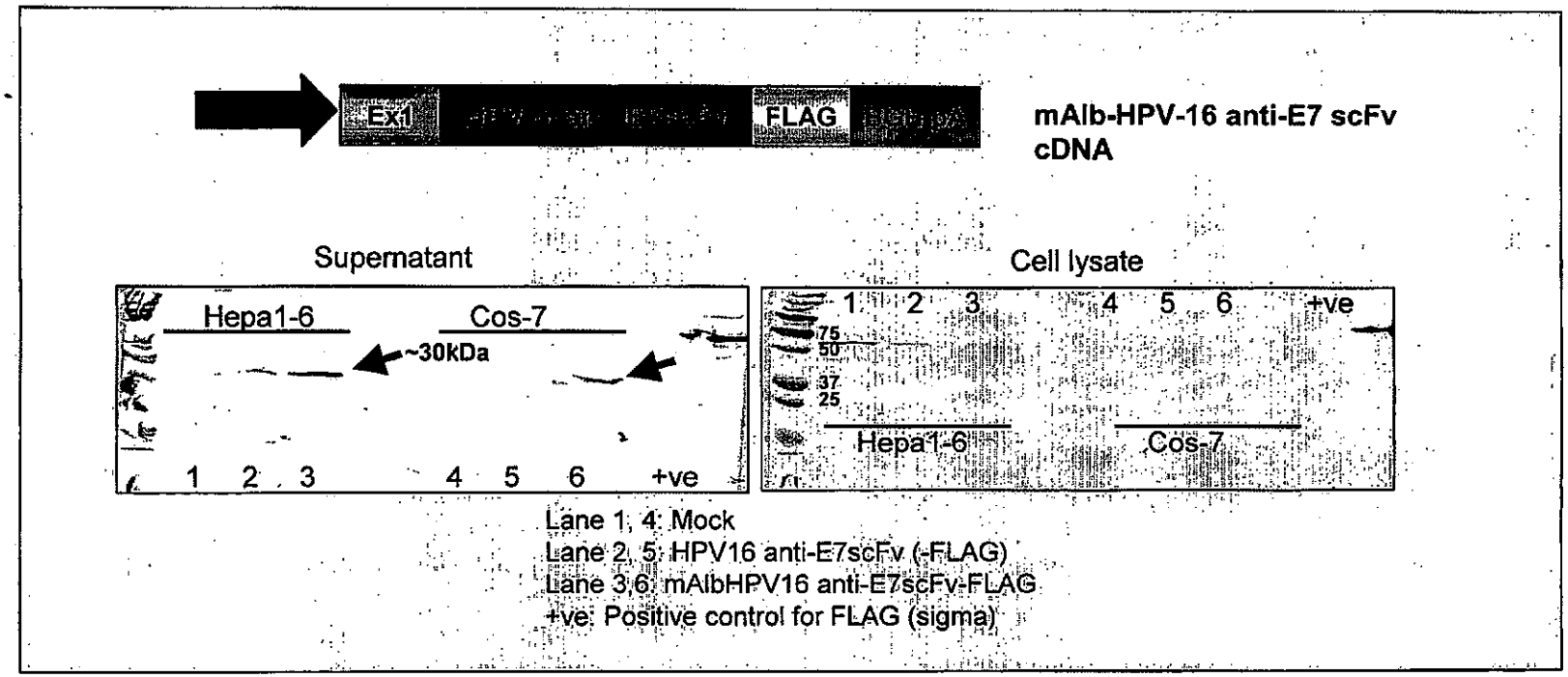


Figure 13

Trans-spliced mAlb-HPV16 anti-E7 scFv is Functional

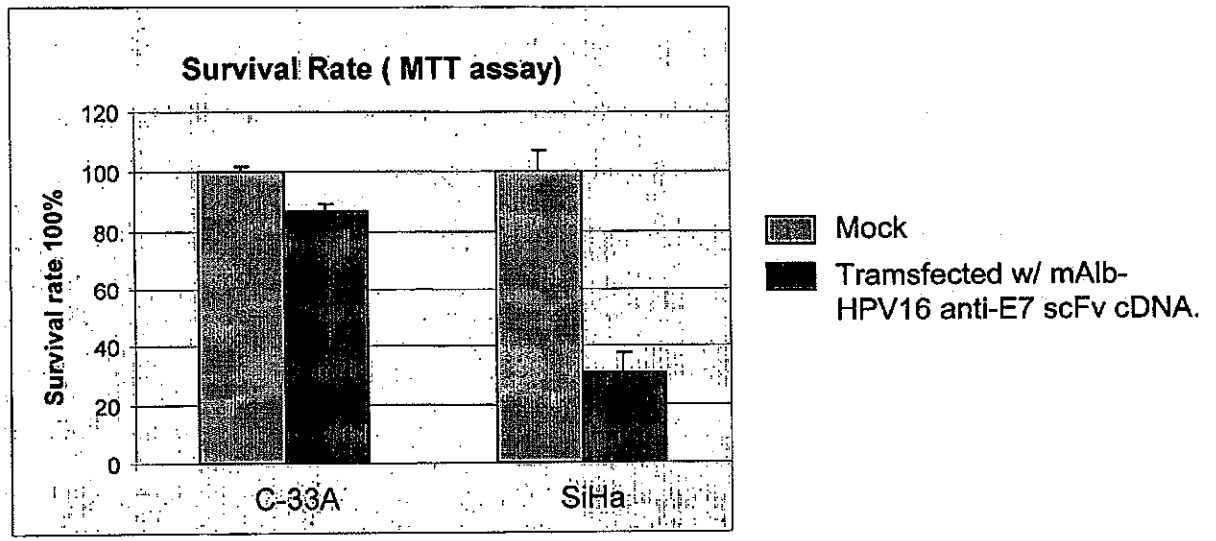


Figure 14

PTM and Target Constructs

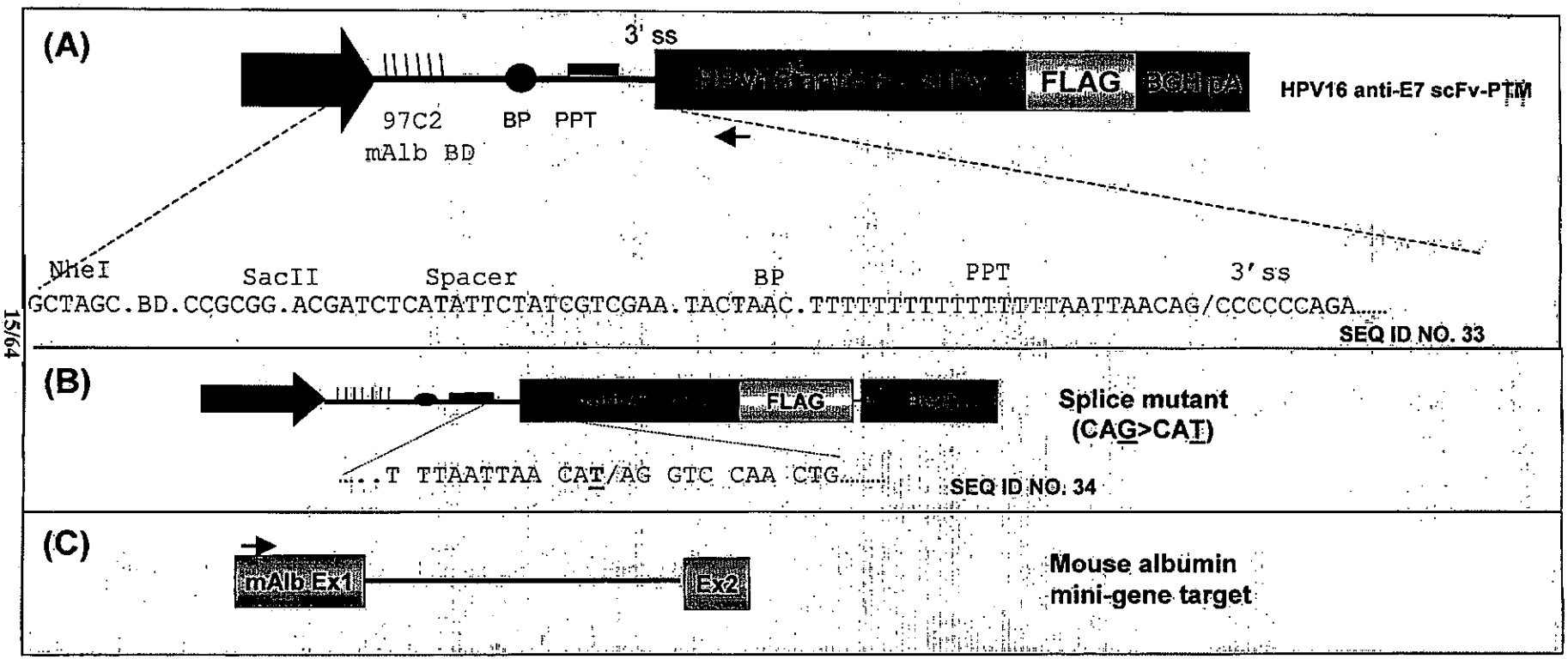


Figure 15

Trans-splicing to Albumin Pre-mRNA Target is Accurate

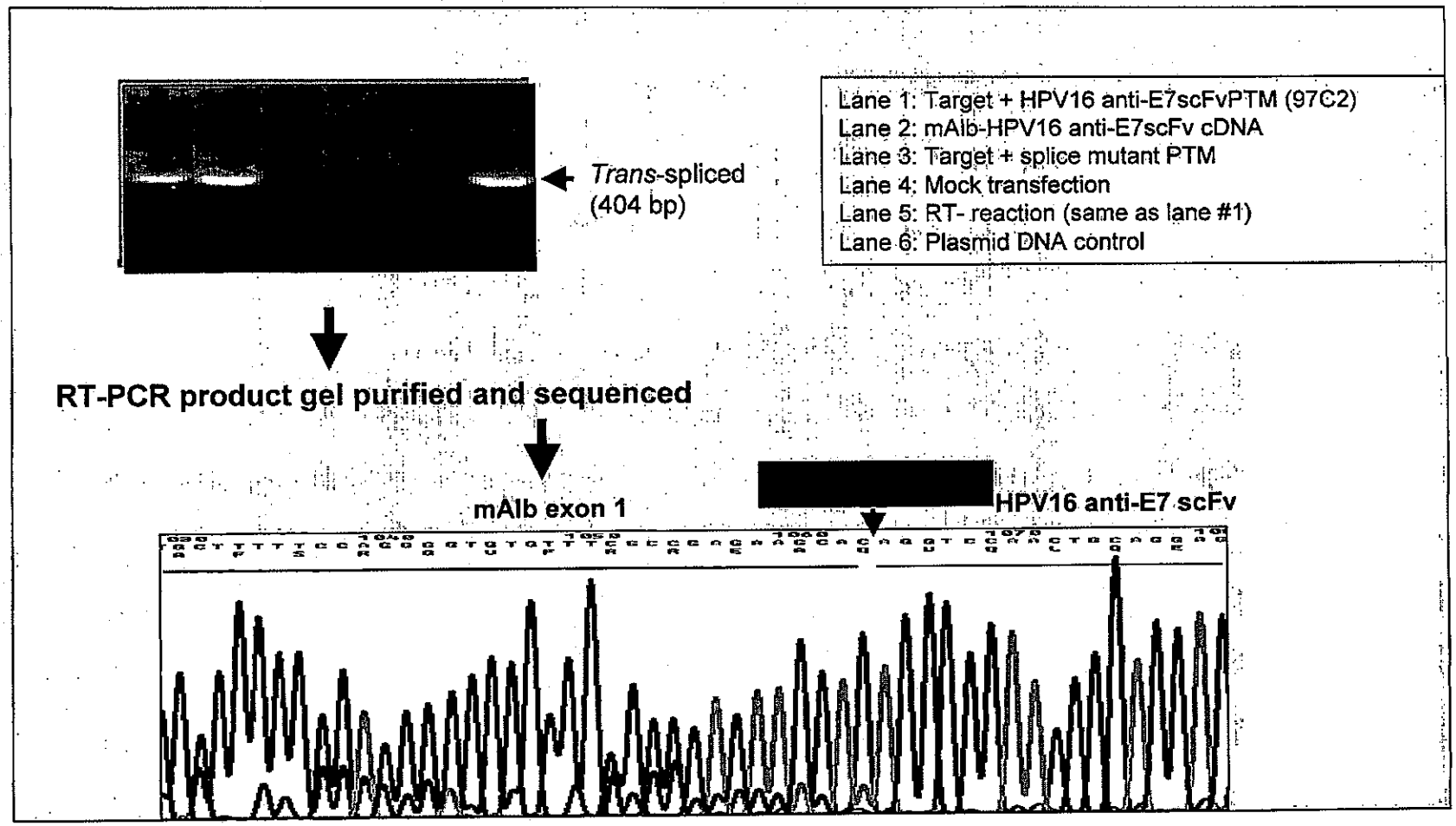


Figure 16

Production of HPV16 anti-E7 scFv in Mice

Western Results

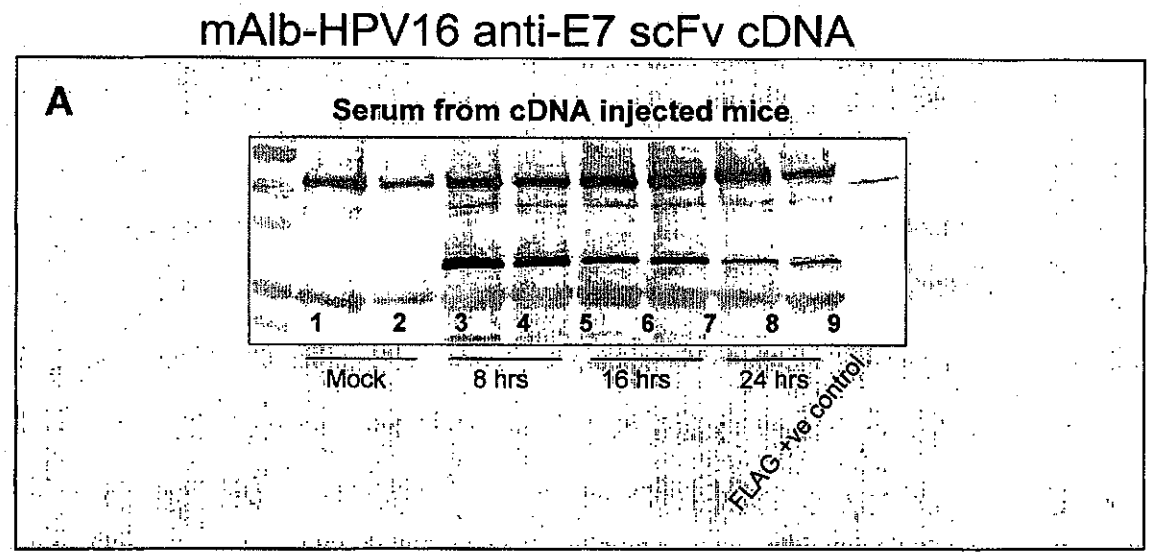


Figure 17A

Production of HPV16 anti-E7 scFv in Mice

Western Results

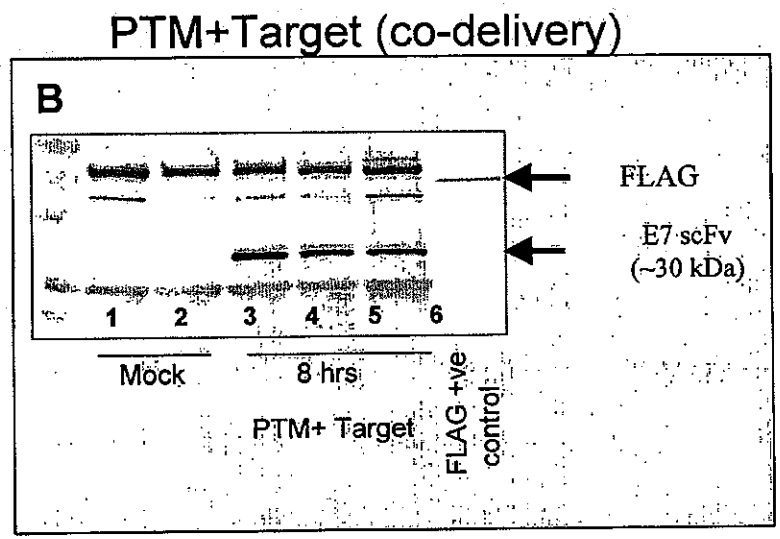


Figure 17B

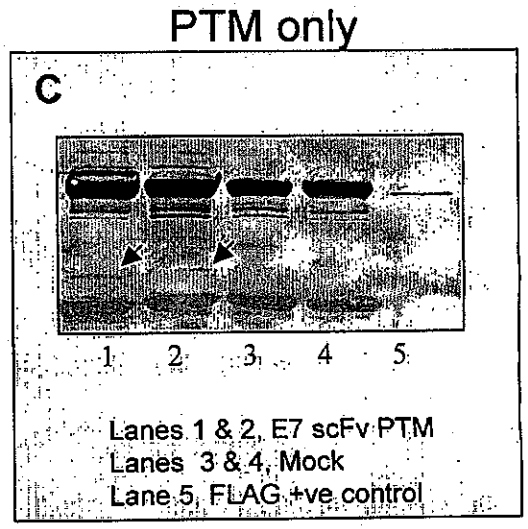
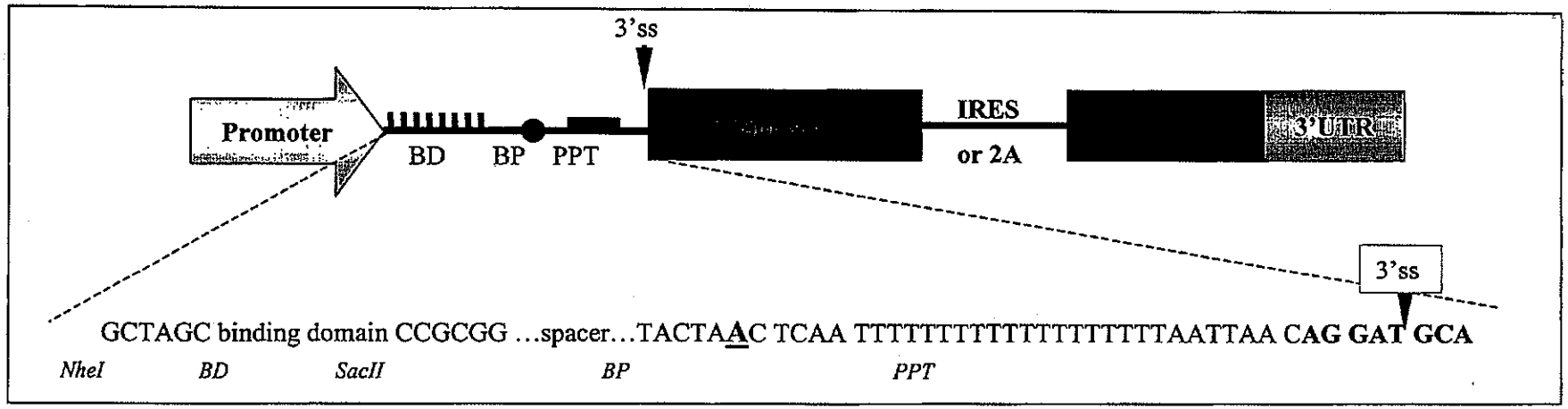


Figure 17C

Structure of a Bicistronic PTM



SEQ ID NO. 35 SEQ ID NO. 36 SEQ ID NO. 37

Figure 18

Albumin Targeting Strategy to Increase hApoAI Expression

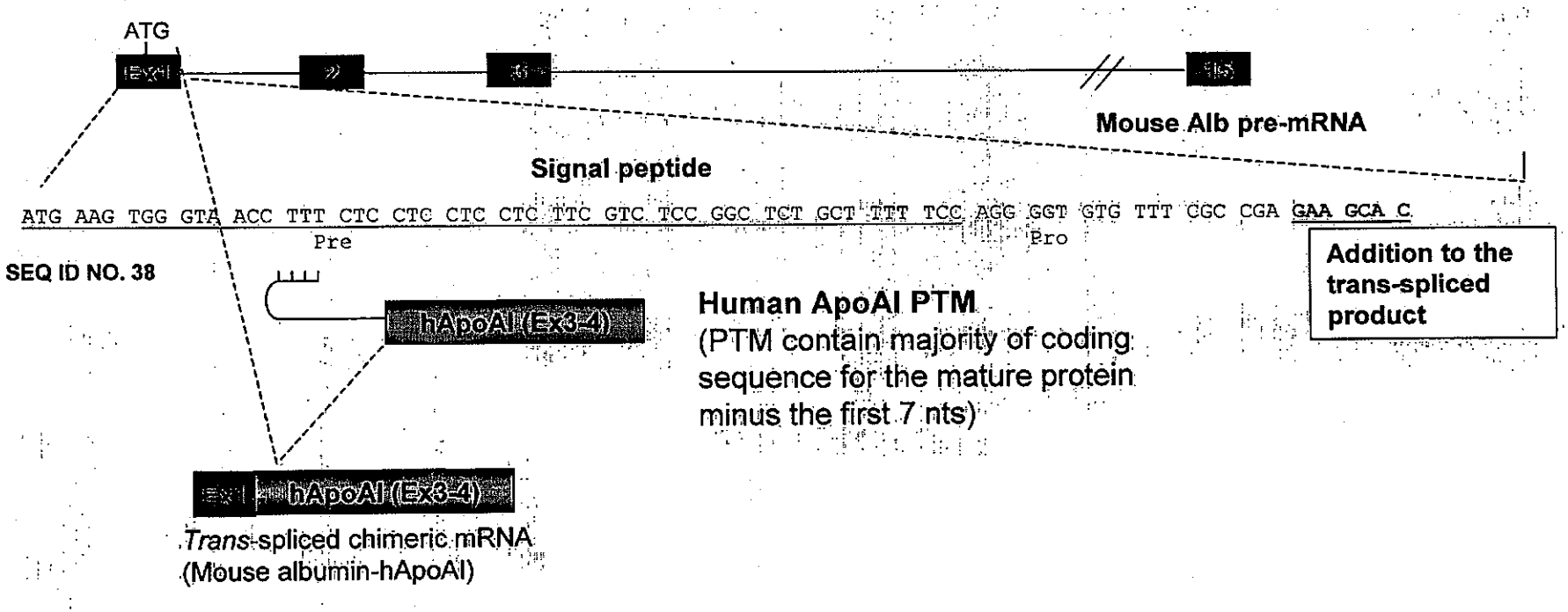


Figure 19 *trans*-splicing into human albumin to increase expression of human ApoAI. Nucleotides in bold, indicate the human albumin sequence (7 nts) that are added to final trans-spliced product.

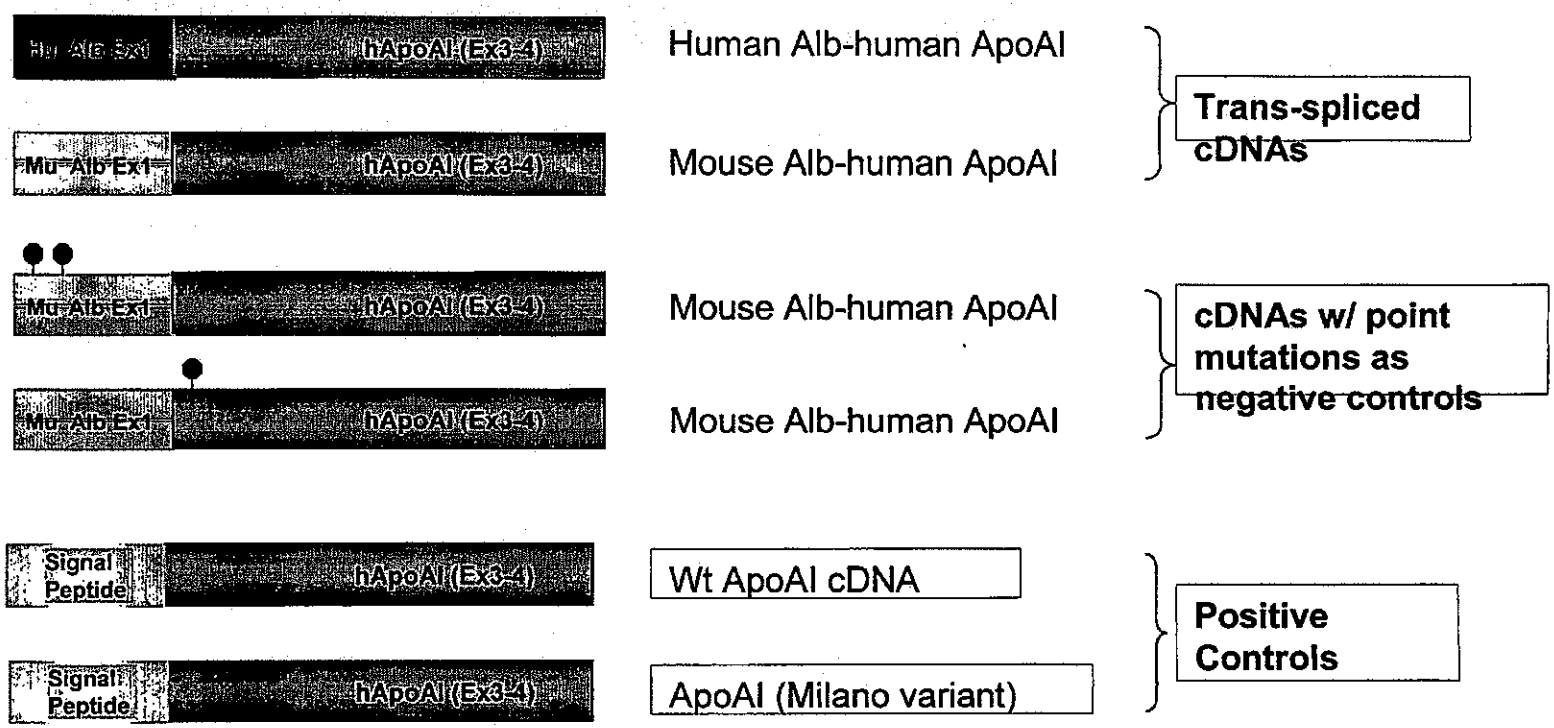


Figure 20 Schematic drawings of human and mouse albumin-human Apo A-I cDNA constructs (test constructs), similar constructs with point (deletion) mutants (negative controls) and wild type human Apo A-I and milano variants (positive controls).
Indicates point mutation (deletion) that result in premature termination. No full-length protein was detected on Western blot.

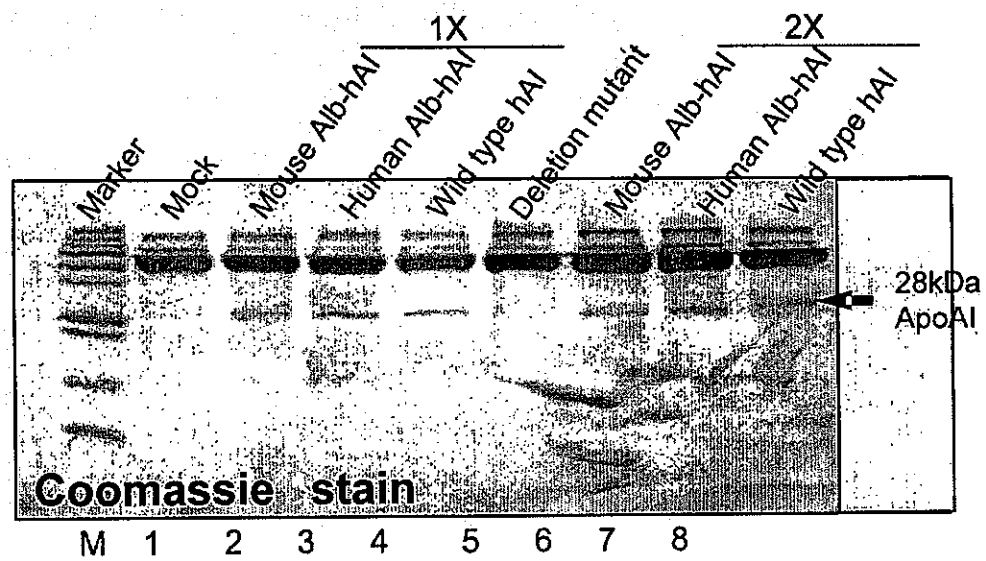


Figure 21 SDS gel showing the production of human Apo A-I protein in 293 cells.

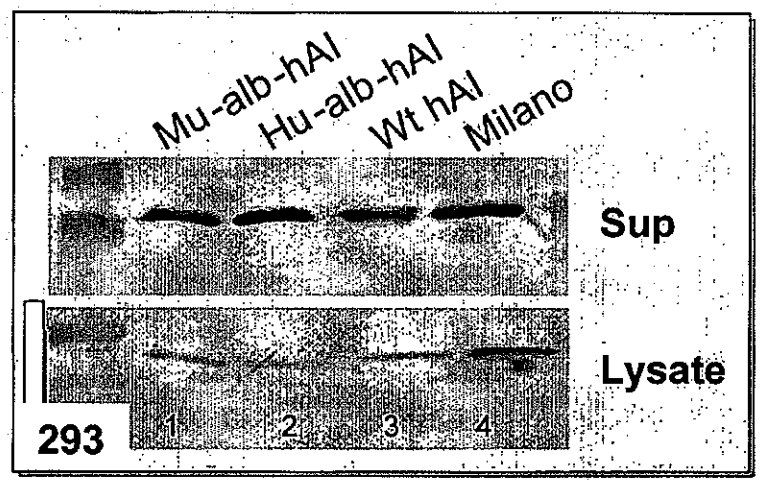


Figure 22 Western blot showing the expression and secretion of mature human Apo A-I protein in 293 cells. Lane 1, mouse Alb-hAI; lane 2, human Alb-hAI; lane 3, wt Apo A-I and lane 4, milano variant. Upper panel, protein in supernatant and lower panel, protein in cell lysate.

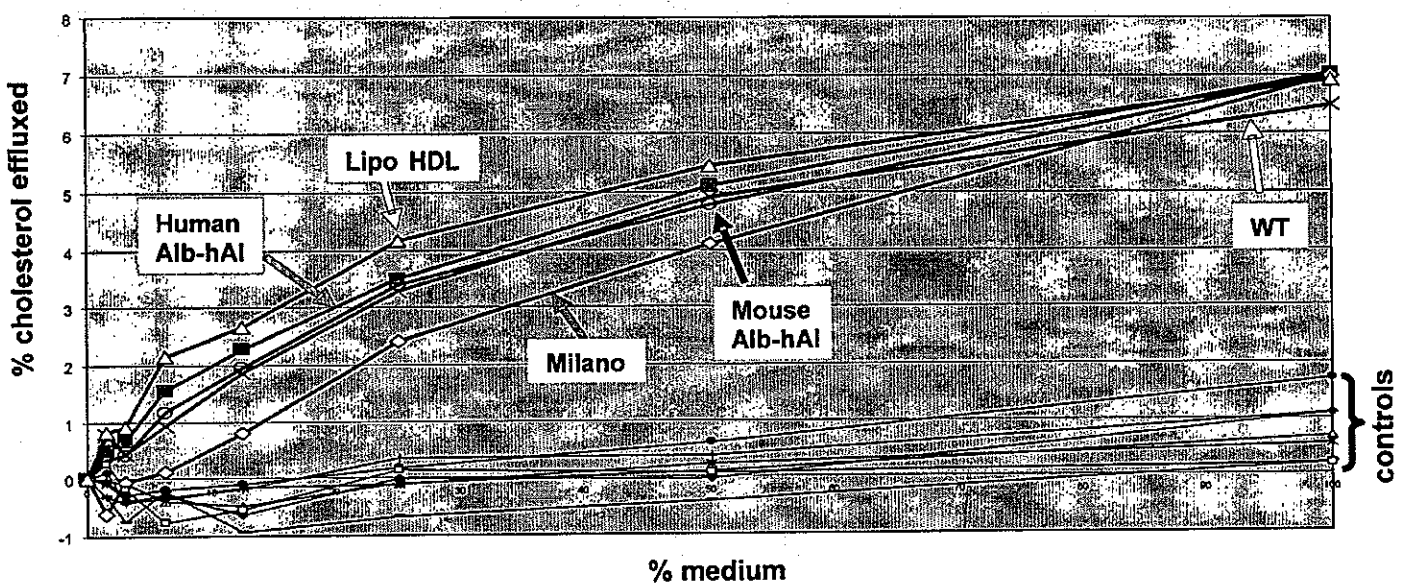


Figure 23 Cholesterol efflux from HeLa control and ABC1 cells with conditioned media from albumin-hApoAI expressing 293 cells.

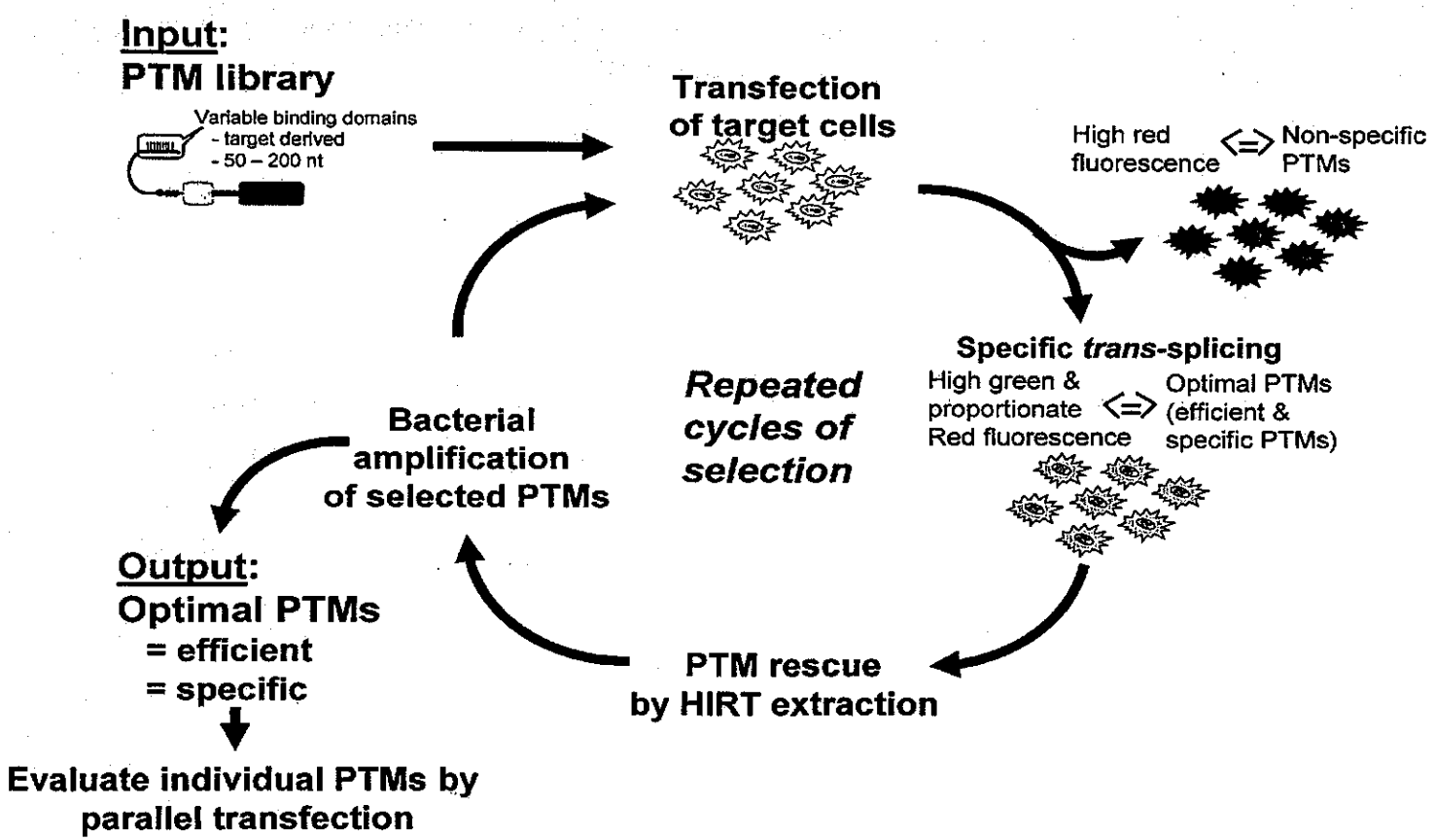
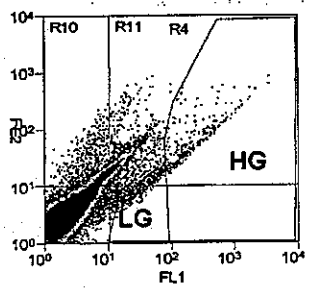


Figure 24A Schematic illustration of FACS-based PTM selection strategy.

Previous Protocol:

1. Library in **pQC** vector backbone (w/ Maz)
2. Cells collected as **single** fraction (mean 10^1-10^4)
3. After first round, routinely tested **20-40** clones by parallel transfection
4. Selected the winner from these clones



Current (modified) Protocol:

1. Library in **pc3.1B** vector backbone (pc3.1 with **Maz** to reduce cryptic *cis*-splicing to amp gene, and, is a high copy number plasmid).
2. Cells collected as **two** fractions, **LG** (mean 10^1-10^2) and **HG** (mean 10^2-10^4)
3. After first round, pre-tested **40** clones from each fraction and found that the **HG fraction had higher (& brighter) GFP positive cells than LG fraction (2:1)**
4. Tested **~100 PTMs from HG fraction** by parallel transfection
5. Selected **20** PTMs for further analysis.
6. Also, tested the effect of target conc. to "**better**" discriminate the winner from the pool. Based on the results, **reducing the target** conc. that is comparable to stables produced better results.
7. The lead candidate BDs were selected from **140** clones.

Figure 24B Comparison between previously described HCS vs. the current HCS steps.

5'GFP-AlbIn1Ex2 Pre-mRNA Target Sequence

SEQ ID NO. 39

ATGGCTCAGTCAAAGCACGGTCTAACAAAAGAAATGACAATGAAATACCGTATGGAAGGGTGCGTCGATGGACATA
AATTTGTGATCACGGGAGAGGGCATTGGATATCCGTTCAAAGGGAAACAGGCTATTAATCTGTGTGTGGTCGAAGG
TGGACCATTGCCATTTGCCGAAGACATATTGTCAGCTGCCTTTATGTACGGAAACAG/

5′ splice junction

SEQ ID NO. 40

GTAAGAAATCCATTTTTCTATTGTTCAACTTTTATTCTATTTTCCCAGTAAAATAAAGTTTTAGTAAACTCTGCAT
CTTTAAAGAATTATTTTGGCATTTATTTCTAAAATGGCATAGTATTTTGTATTTGTGAAGTCTTACAAGGTTATCT
TATTAATAAAATTCAAACATCCTAGGTAAAAAAAAAAAAAGGTCAGAATTGTTTAGTGACTGTAATTTTCTTTTGC
GCACTAAGGAAAGTGCAAAGTAACTTAGAGTGACTGAAACTTCACAGAATAGGGTTGAAGATTGAATTCATAACTA
TCCCAAAGACCTATCCATTGCACTATGCTTTATTTAAAAACCACAAAACCTGTGCTGTTGATCTCATAAATAGAAC
TTGTATTTATATTTATTTTCATTTTAGTCTGTCTTCTTGGTTGCTGTTGATAGACACTAAAAGAGTATTAGATATT
ATCTAAGTTTGAATATAAGGCTATAAATATTTAATAATTTTTAAAATAGTATTCTTGGTAATTGAATTATTCTTCT
GTTTAAAGGCAGAAGAAATAATTGAACATCATCCTGAGTTTTTCTGTAGGAATCAGAGCCCAATATTTTGAAACAA
ATGCATAATCTAAGTCAAATGGAAAGAAATATAAAAAGTAACATTATTACTTCTTGTTTTCTTCAGTATTTAACAA
TCCTTTTTTTTCTTCC

3′ splice junction

SEQ ID NO. 41

CTTGCCCAG/ACAAGAGTGAGGTTGCTCATCGGTTTAAAGATTTGGGAGAAGAAAATTTCAAAGCCTT

Figure 25

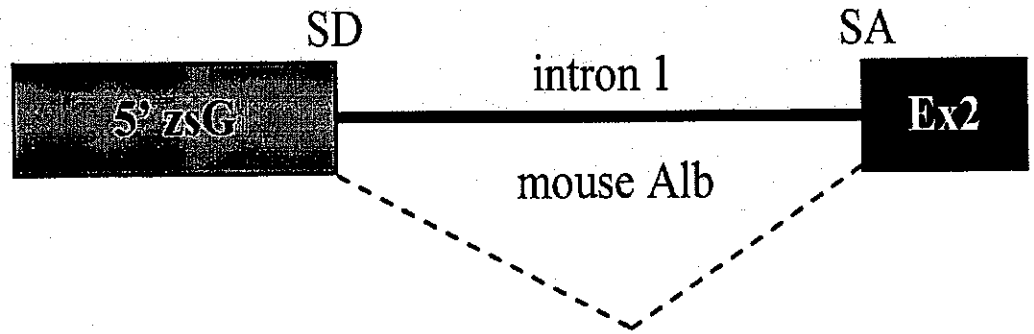


Figure 26 Schematic diagram of the pre-mRNA target used in the HCS. Abbreviations: SD, splice donor site; SA, splice acceptor site. Dotted lines indicate target *cis*-splicing.

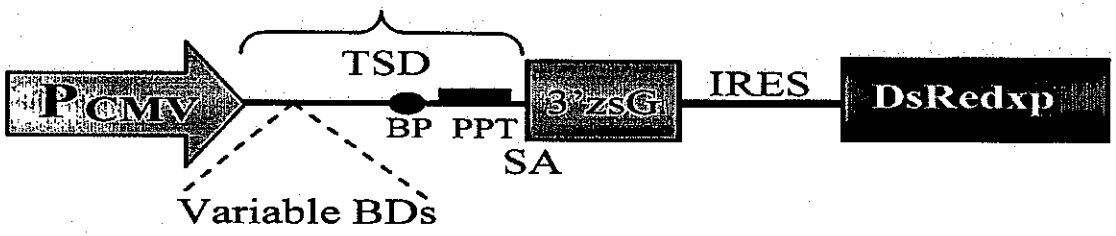


Figure 27 Schematic illustration of the PTM cassette used in the HCS. PTM cassette consists of a *trans*-splice domain including (TSD): variable BDs, short spacer, BP, PPT, 3' half of the coding sequence for zsG, IRES followed by the full length coding sequence for second reporter DsRedExpress. Abbreviations: 3'zsG, 3' half of the zsGreen fluorescent protein coding sequence; IRES, internal ribosome entry site, BD, binding domain; BP, branch point; PPT, polypyrimidine tract. SA, splice acceptor site.

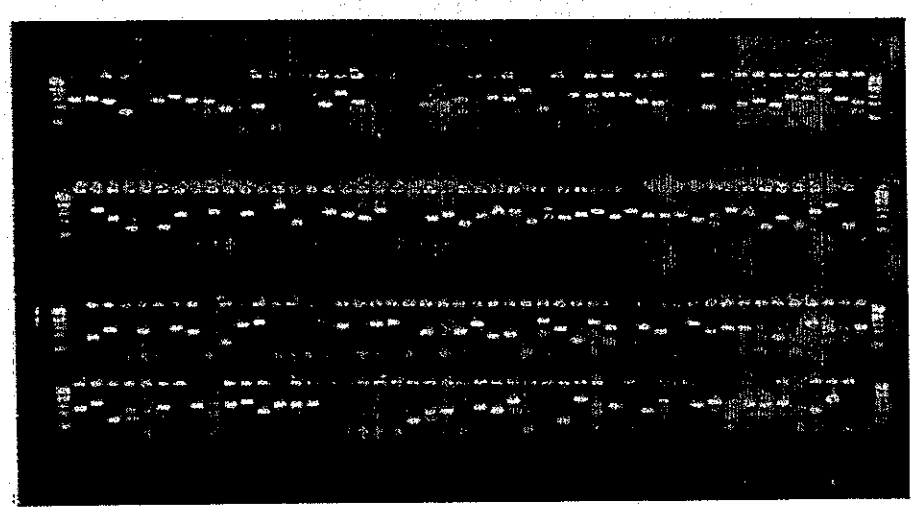
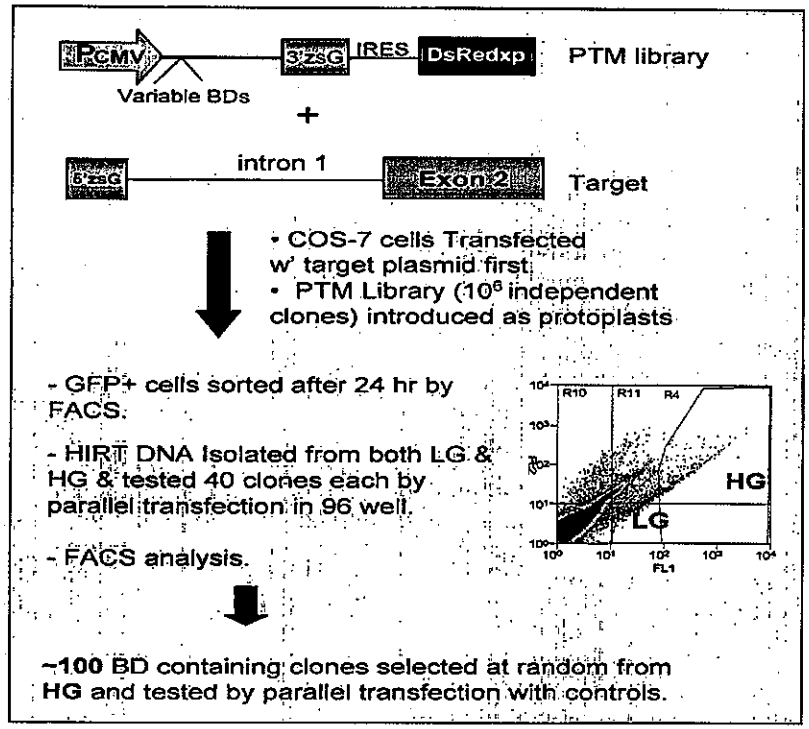


Figure 28 PCR screen showing the cloning efficiency and diversity of the mouse albumin BD library.

HCS for Mouse Albumin Target - Summary



Total cell sorted: 5.4 x10^6

	Low Green fraction (GFP Mean 10-100)	High Green fraction (GFP Mean >100)
Cell collected	2.15 x10^4, 0.4%	7300, 0.135%
GFP Mean	33.5	235
plasmid # recovered from HIRT	3.3 x10^5	2.76x 10^4
BD containing clones (% by PCR)	57.9	44
BD # recovered/cell	8.86	1.67*

* : 100% recovery.

Figure 29 Schematic illustration of HCS steps.

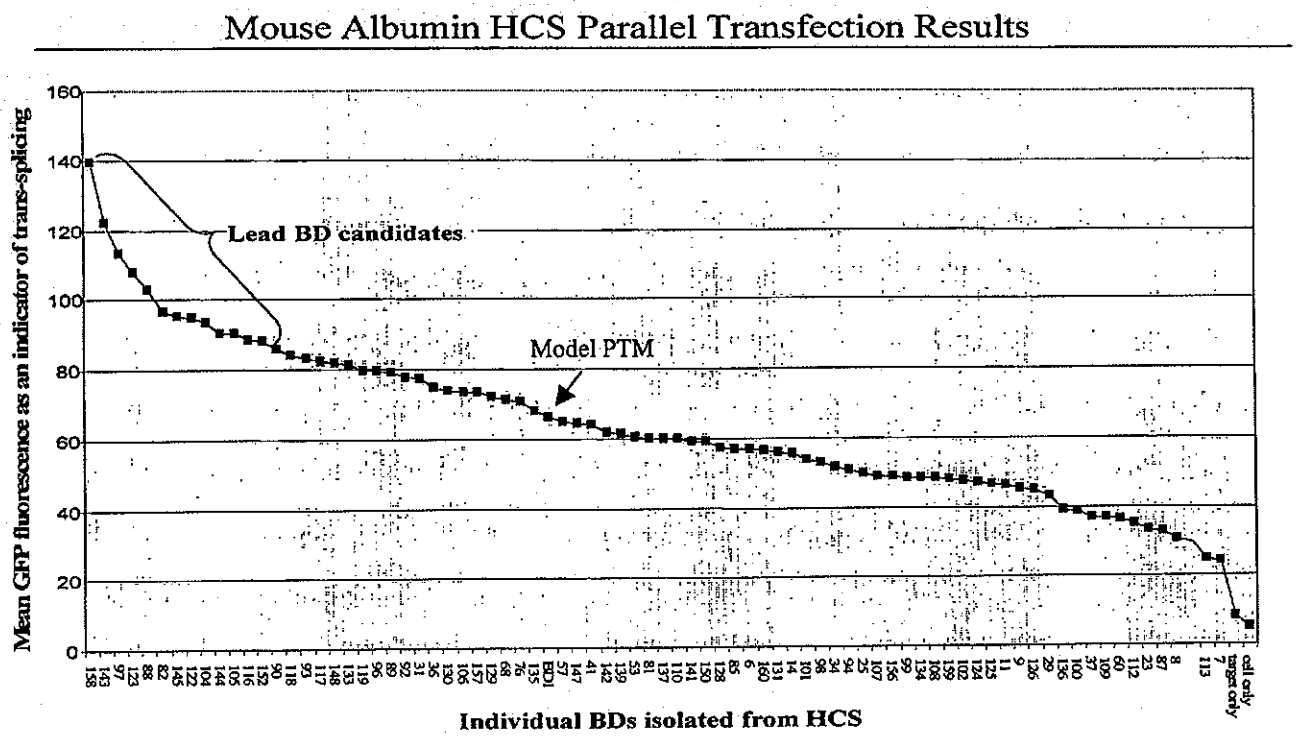


Figure 30 *Trans*-splicing efficiency of PTMs selected from HCS for mouse albumin target.

High Capacity Screen Results for Mouse Albumin Target

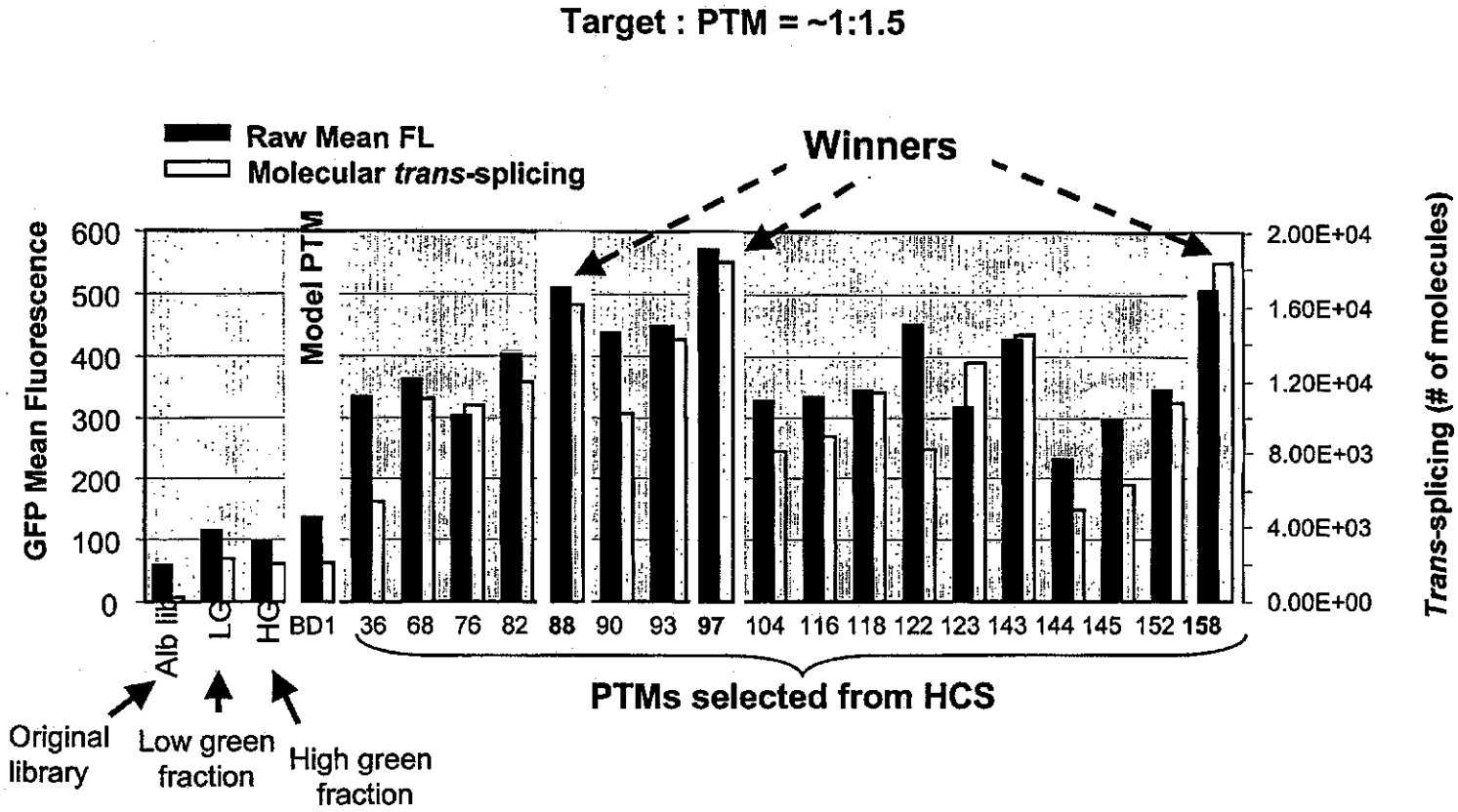
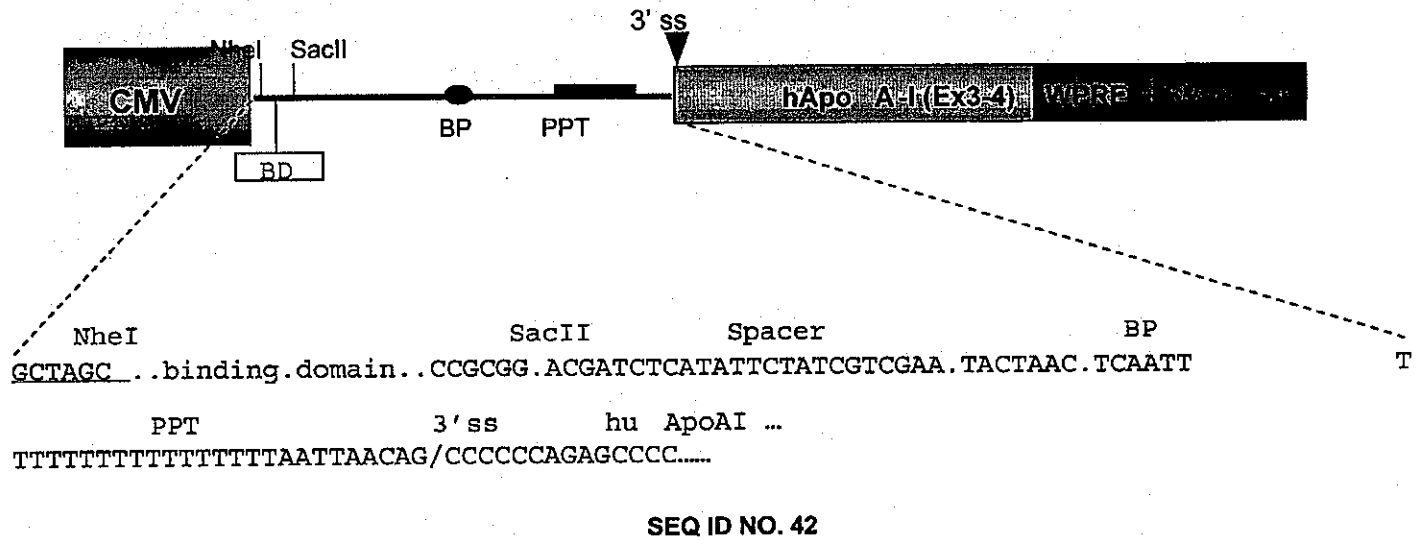


Figure 31 *Trans*-splicing efficiency of top 20 PTMs selected from the HCS assessed by FACS and qRT-PCR.



SEQ ID NO. 42

Figure 32 Schematic illustration of human Apo A-I PTM expression cassette used for *in vitr* POP studies.

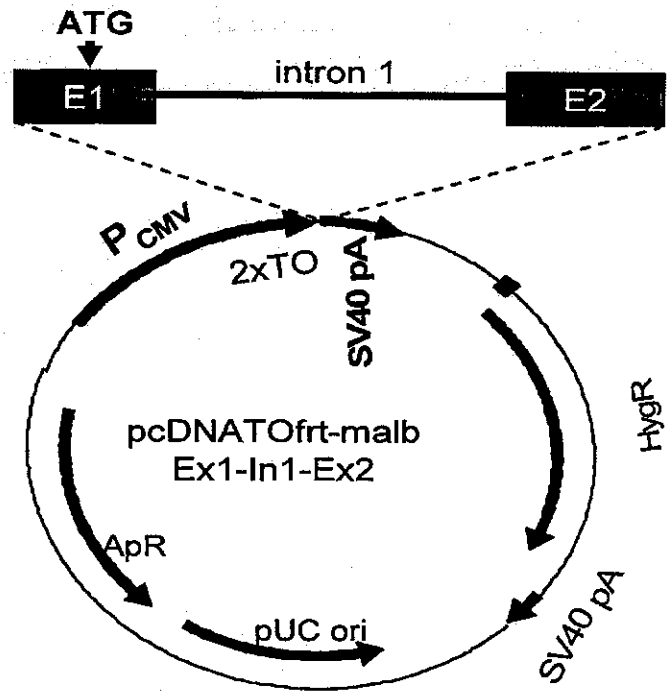


Figure 33 Schematic diagram of the mouse albumin mini-gene pre-mRNA target.

Nucleotide and amino acid sequences of ApoAI wild type

ApoAI wild type:

```
AGAGACTGCG AGAAGGAGGT CCCCCACGGC CCTTCAGG ATG AAA GCT GCG GTG CTG ACC TTG GCC GTG CTC TTC CTG ACG GGG AGC CAG GCT CGG CAT TTC TGG CAG
                                          m   k   a   a   v   l   t   l   a   v   l   f   l   t   g   s   q   a   r   h   f   w   q

CAA GAT GAA CCC CCC CAG AGC CCC TGG GAT CGA GTG AAG GAC CTG GCC ACT GTG TAC GTG GAT GTG CTC AAA GAC AGC GGC AGA GAC TAT GTG TCC CAG TTT      q    d
e   p   p   q   s   p   w   d   r   v   k   d   l   a   t   v   y   v   d   v   l   k   d   s   g   r   d   y   v   s   q   f

GAA GGC TCC GCC TTG GGA AAA CAG CTA AAC CTA AAG CTC CTT GAC AAC TGG GAC AGC GTG ACC TCC ACC TTC AGC AAG CTG CGC GAA CAG CTC GGC CCT GTG  e    g   s
a   l   g   k   q   l   n   l   k   l   l   d   n   w   d   s   v   t   s   t   f   s   k   l   r   e   q   l   g   p   v

ACC CAG GAG TTC TGG GAT AAC CTG GAA AAG GAG ACA GAG GGC CTG AGG CAG GAG ATG AGC AAG GAT CTG GAG GAG GTG AAG GCC AAG GTG CAG CCC TAC CTG  t    q   e
f   w   d   n   l   e100   k   e   t   e   g   l   r   q   e   m   s   k   d   l   e   e   v   k   a   k   v   q   p   y   l

GAC GAC TTC CAG AAG AAG TGG CAG GAG GAG ATG GAG CTC TAC CGC CAG AAG GTG GAG CCG CTG CGC GCA GAG CTC CAA GAG GGC GCG CGC CAG AAG CTG CAC d    d   f
q   k   k   w   q   e   e   m   e   l   y   r   q   k   v   e   p   l   r   a   e   l   q   e   g   a   r   q   k   l   h

                                                    nt 555 (C>T converts Wild type to Milano)
GAG CTG CAA GAG AAG CTG AGC CCA CTG GGC GAG GAG ATG CGC GAC CGC GCG CGC GCC CAT GTG GAC GCG CTG CGC ACG CAT CTG GCC CCC TAC AGC GAC GAG e    l   q
e   k   l   s   p   l   g   e   e   m   r   d   r   a   r   a   h   v   d   a   l   r   t   h   l   a   p   y   s   d   e

CTG CGC CAG CGC TTG GCC GCG CGC CTT GAG GCT CTC AAG GAG AAC GGC GGC GCC AGA CTG GCC GAG TAC CAC GCC AAG GCC ACC GAG CAT CTG AGC ACG CTC  l    r   q
r   l   a   a   r   l   e   a   l   k   e   n   g   g   a   r   l   a   e   y   h   a   k   a   t   e   h   l   s   t   l

AGC GAG AAG GCC AAG CCC GCG CTC GAG GAC CTC CGC CAA GGC CTG CTG CCC GTG CTG GAG AGC TTC AAG GTC AGC TTC CTG AGC GCT CTC GAG GAG TAC ACT  s    e   k
a   k   p   a   l   e   d   l   r   q   g   l   l   p   v   l   e   s   f   k   v   s   f   l   s   a   l   e   e   y   t

AAG AAG CTC AAC ACC CAG TGA GGCGCCCGCC GCCGCCCCCC TTCCCGGTGC TCAGAATAAA CGTTTCCAAA GTGGG
 k   k   l   n   t   q  stop
```

SEQ ID NO. 43

ApoAI-Milano: Molecular analysis have confirmed a Arg → Cys substitution at position 173 amino acid that converts the wild type apoAI into apoAI Milano variant.
At the nucleotide level, a single nucleotide substitution **C → T** was confirmed.

Figure 34

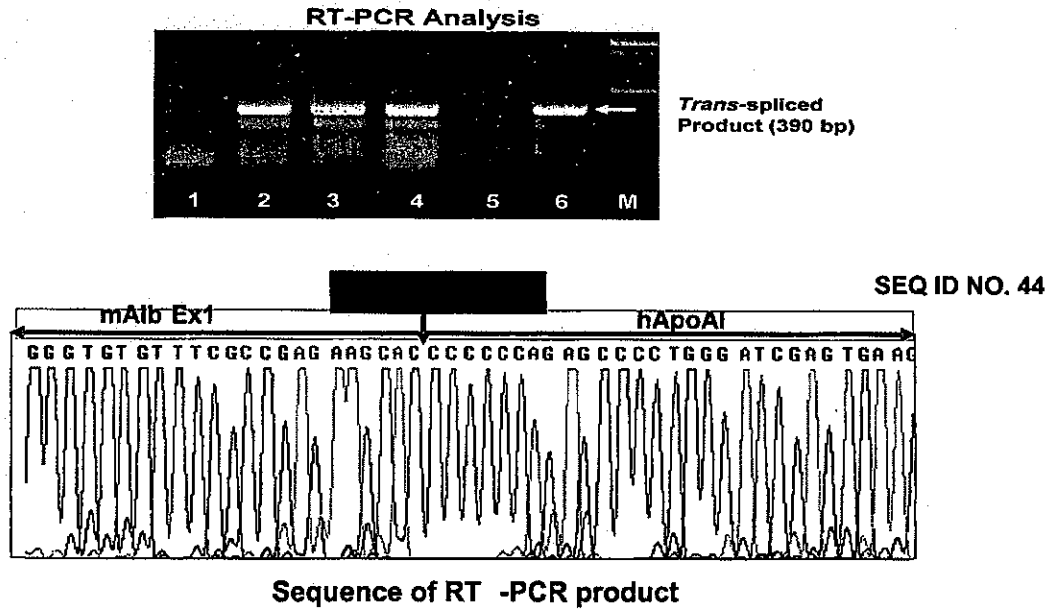


Figure 35 Evidence of precise *trans*-splicing of mAlbPTMs into albumin exon 1 in stable cells

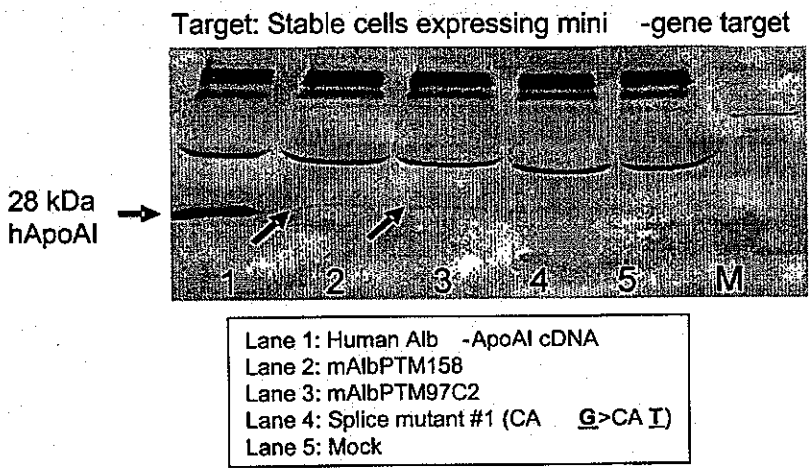
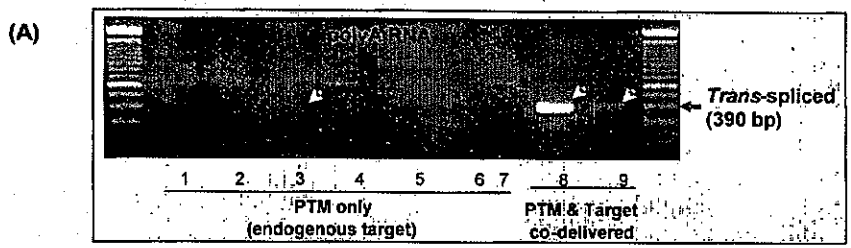


Figure 36 Detection of *trans*-spliced human Apo A-I protein by Western blot.



Splice junction sequence:

Mouse alb Ex 1 Splice junction ApoAI **SEQ ID NO. 45**

AGG GGT GTG TTT CGC CGA GAA GCA C/CC CCC CAG AGC CCC TGG GAT CGA GTG AAG

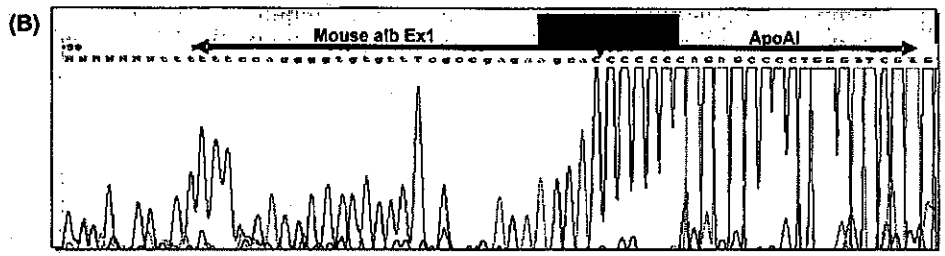


Figure 37 PTM mediated *trans*-splicing into endogenous albumin exon 1 in mice.

Human Albumin Targeting Strategy to Increase hApoAI Expression

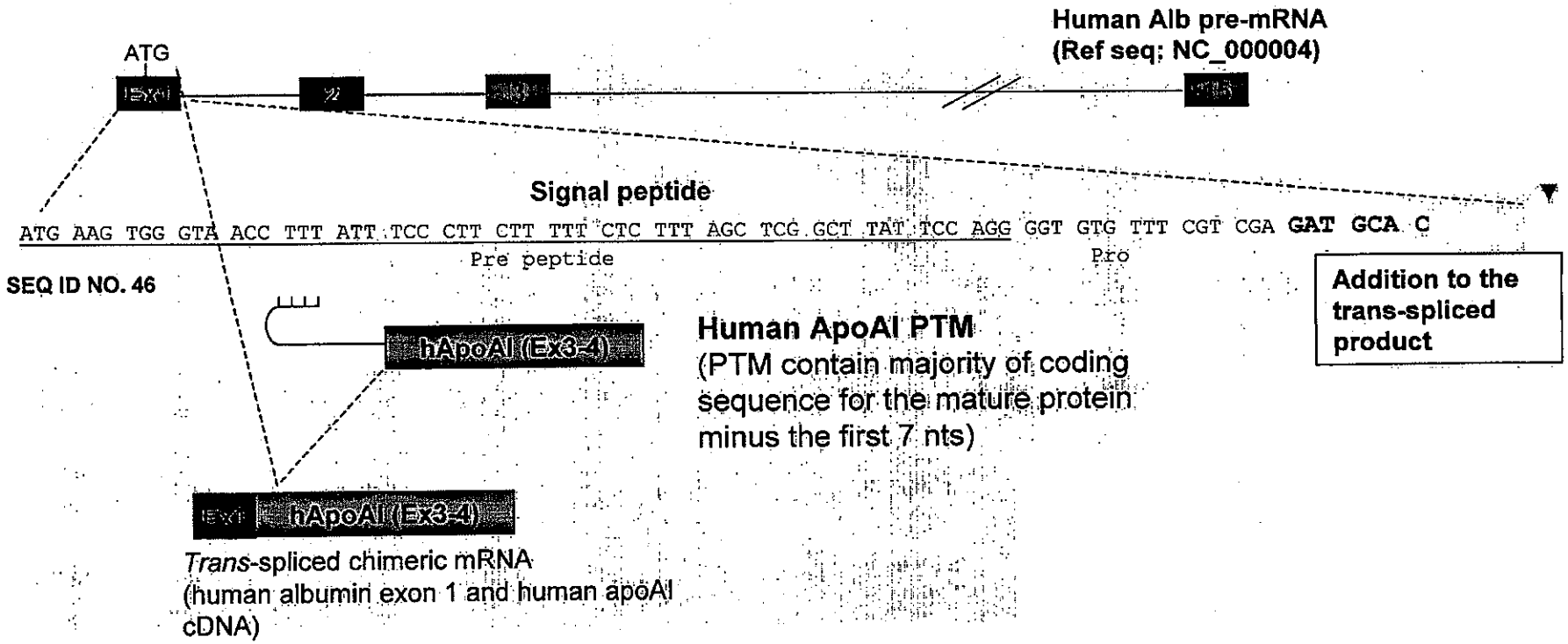


Figure 38 *trans*-splicing into human albumin to increase expression of human ApoAI. Nucleotides in bold, indicate the human albumin sequence (7 nts) that are added to final trans-spliced product.

Strategies to eliminate albumin sequence in the final *trans*-spliced product.

PTM ApoAI sequences (human NP_000030. apolipoprotein A-I...[gi:4557321])
» SIGNAL PEPTIDE CLEAVAGE AT RER
« PRO PEPTIDE CLEAVAGE AT GOLGI

Pre Pro Pro hApoAI

1. MKWVTFISLLFLFSSAYS»RGVFRR«DA**P**RGVFRR«*DEPP*.....ApoAI sequence
   cDNA construct designed to include additional albumin propeptide (underlined) followed by the entire mature coding sequence for ApoAI. Proline (in bold) was used as a junction amino acid.

2. MKWVTFISLLFLFSSAYS»RGVFRR«DA**P**RHFWQQ«*DEPP*.....ApoAI sequence
   cDNA construct designed to include human ApoAI pro-peptide (underlined) in addition to albumin pro-peptide followed by the entire mature coding sequence for ApoAI. Proline (in bold) was used as a junction amino acid.

3. MKWVTFISLLFLFSSAYS»RGVFRR«DARHFWQQ«*DEPP*.....ApoAI sequence
   cDNA construct designed to include human ApoAI pro-peptide (underlined) in addition to albumin pro-peptide followed by the entire mature coding sequence for ApoAI. The additional ApoAI pro-peptide was linked directly into albumin sequence without Proline.

With the presence of a second pro-peptide (albumin or ApoAI), these chimeric proteins will now undergo a second cleavage resulting in the final trans-spliced product that is identical to the wild type ApoAI sequence i.e., without any albumin sequence.

Similarly, PTMs can also be engineer to include peptidase cleavage site(s) or signal peptides which after trans-splicing would be recognized and cleaved to release the final product that is identical to ApoAI sequence i.e., without any albumin sequence.

Figure 39. Schematic showing the strategies to eliminate albumin sequence in the final *trans*-spliced product.

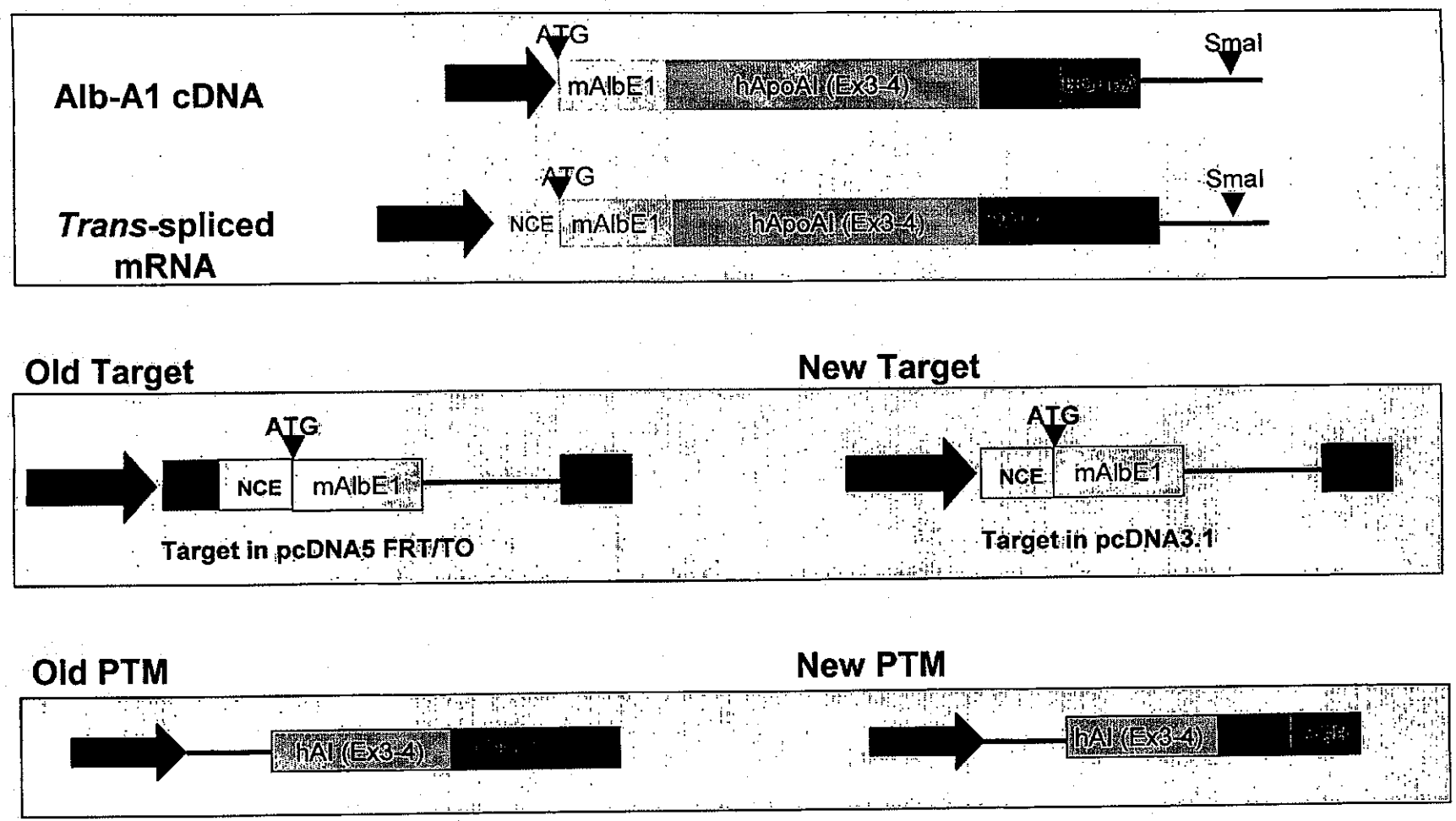


Figure 40 Schematic drawings of albumin-human Apo A-I cDNA, trans-spliced mRNA, old and new PTM and targets used in the current study. NCE, non-coding exon; hAI, human Apo A-I and Ex, exon.

Strategy to Eliminate Albumin Sequence in the Final Product

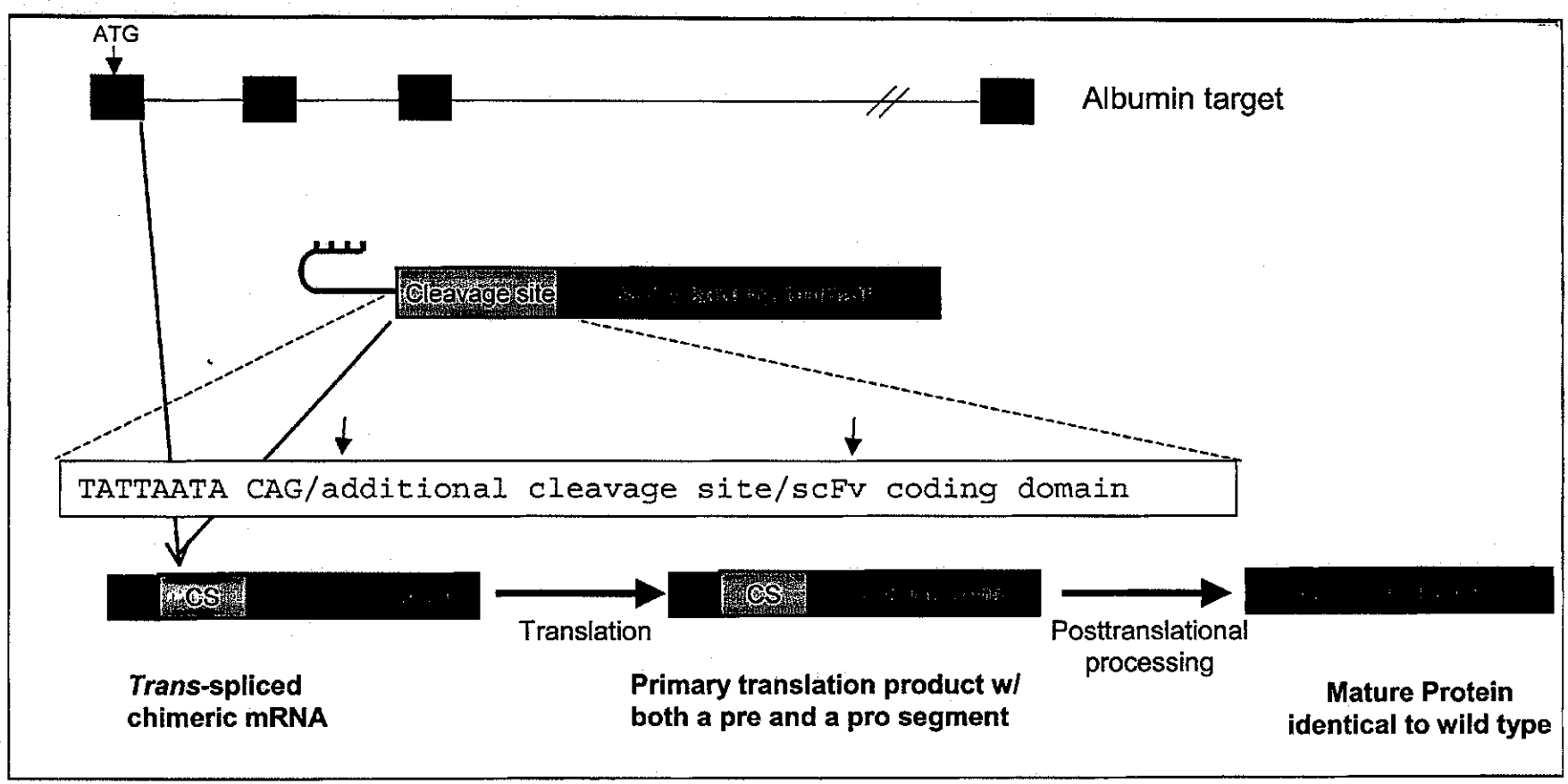
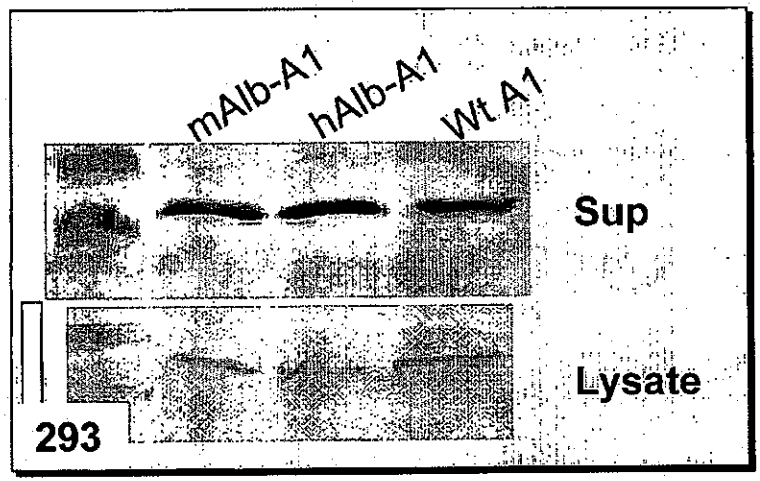


Figure 41

Alb-ApoA1 Processed & Secreted Properly

Western analysis of mouse and human Alb-hApoA1 *trans*-spliced protein: Test for producti & secretion in 293 & HepG2 cells.





Conclusion:
With both mouse and human Alb-A1 cDNAs, majority of the protein detected in the supernatant indicating proper processing and secretion.

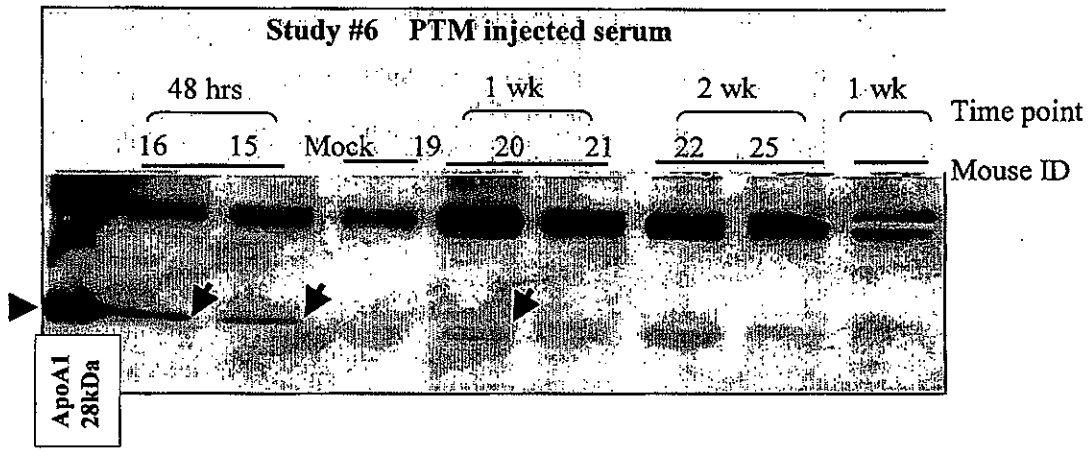
Figure 42

Human ApoAI Produced in Mice

Study #6

- Minicircle-PTM (75 µg/mouse)
- Time point: 48-2 wks
- Mice: C57BL/6 males

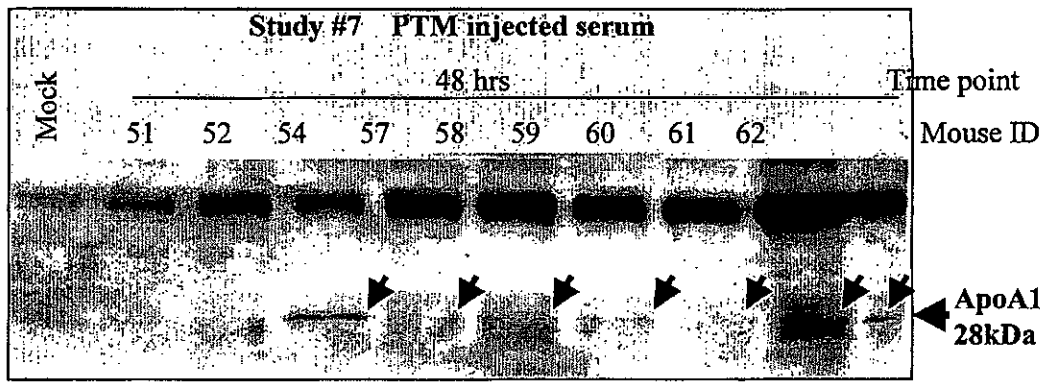
- 50 µl serum immuno-precipitated using monoclonal antibody to human ApoA1 followed by Western w/ the same antibody.



Study #7

- Minicircle-PTM (75 µg/mouse)
- Time point: 48 hrs
- Mice: C57BL/6 females

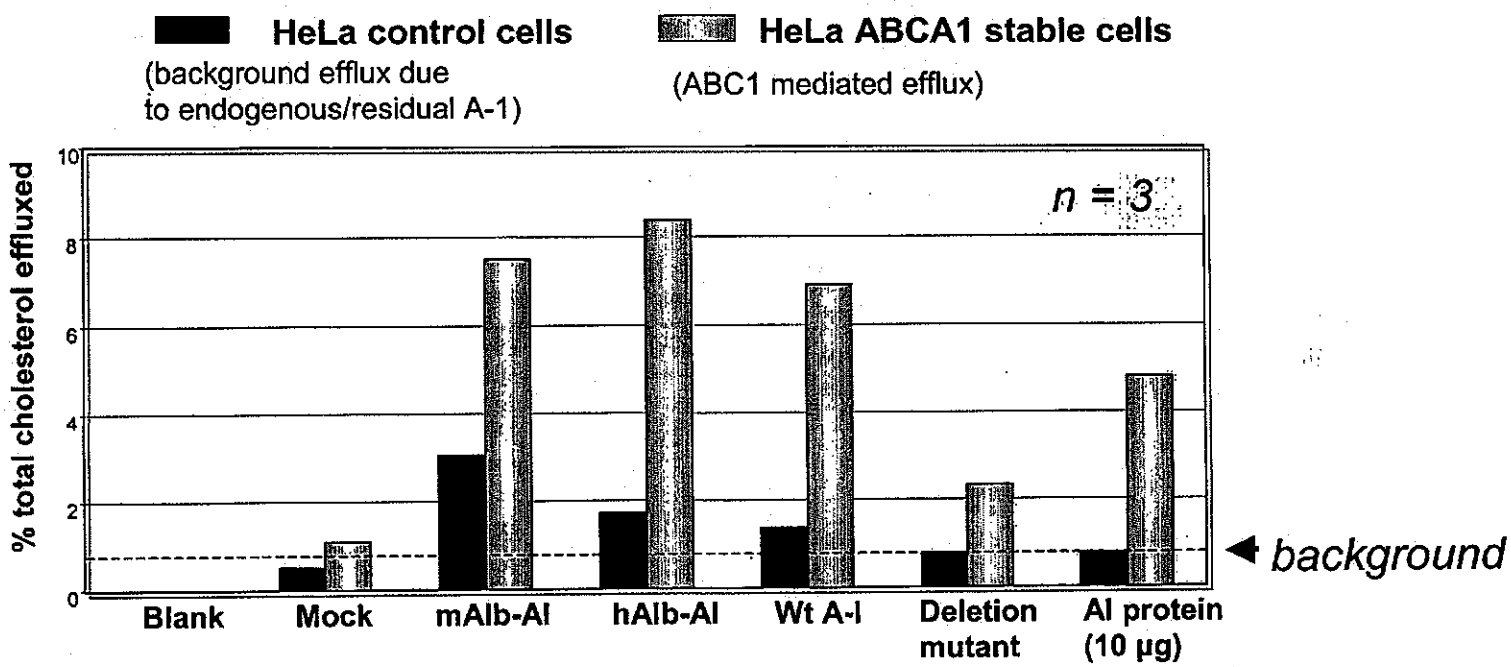
- IP and Western conditions same as above.



PTM injected sample

Figure 43

Albumin-hu ApoA-I Protein Functionally Active: Efflux Results



Conclusion:

- **Both mouse and human Alb-hApoA-I proteins are functionally active.**

Figure 44

In Vivo Dyslipidemia Studies

Goal: Demonstrate use of plasmid-based delivery system for long-term production of human HDL in mice.

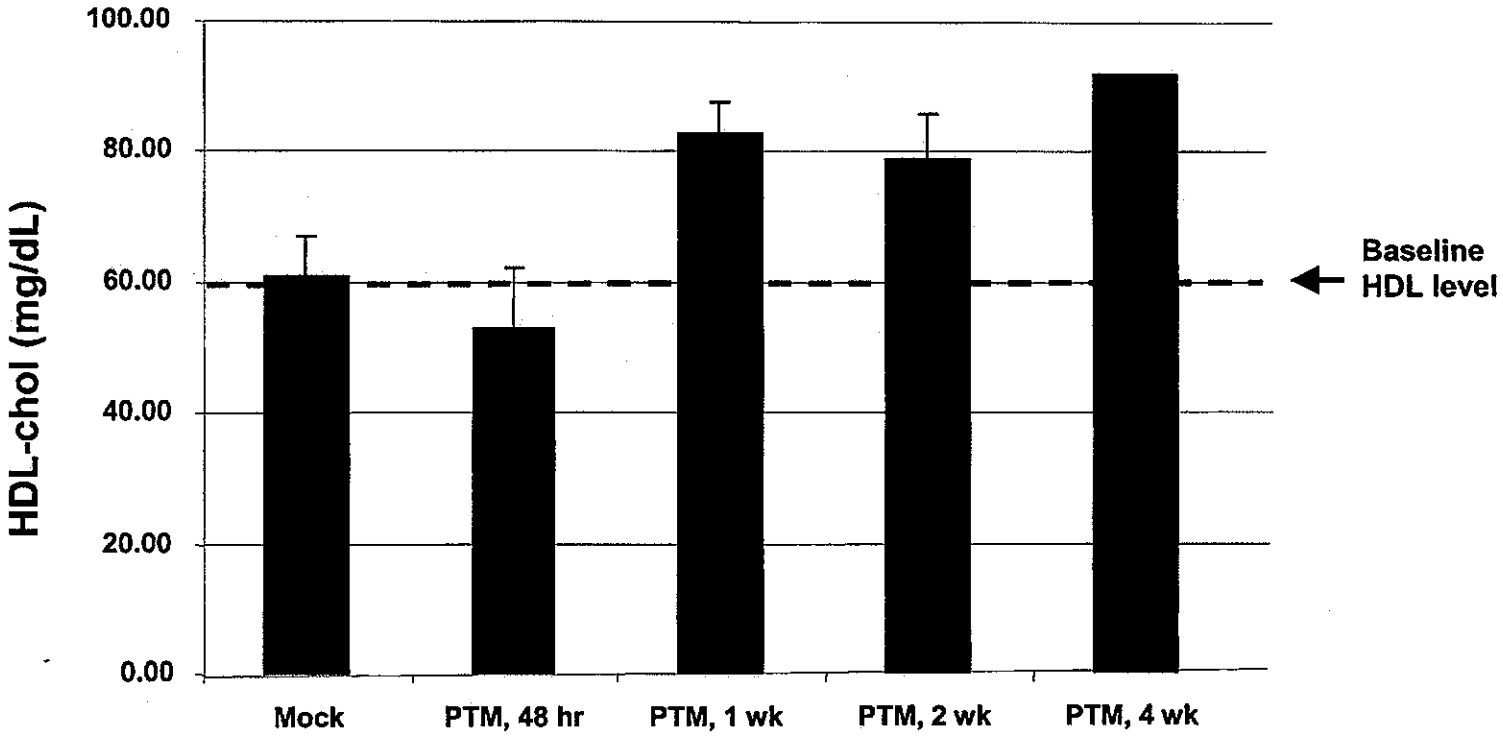


Figure 45

Generation of secreted FVIII through trans-splicing to exon 1 of albumin

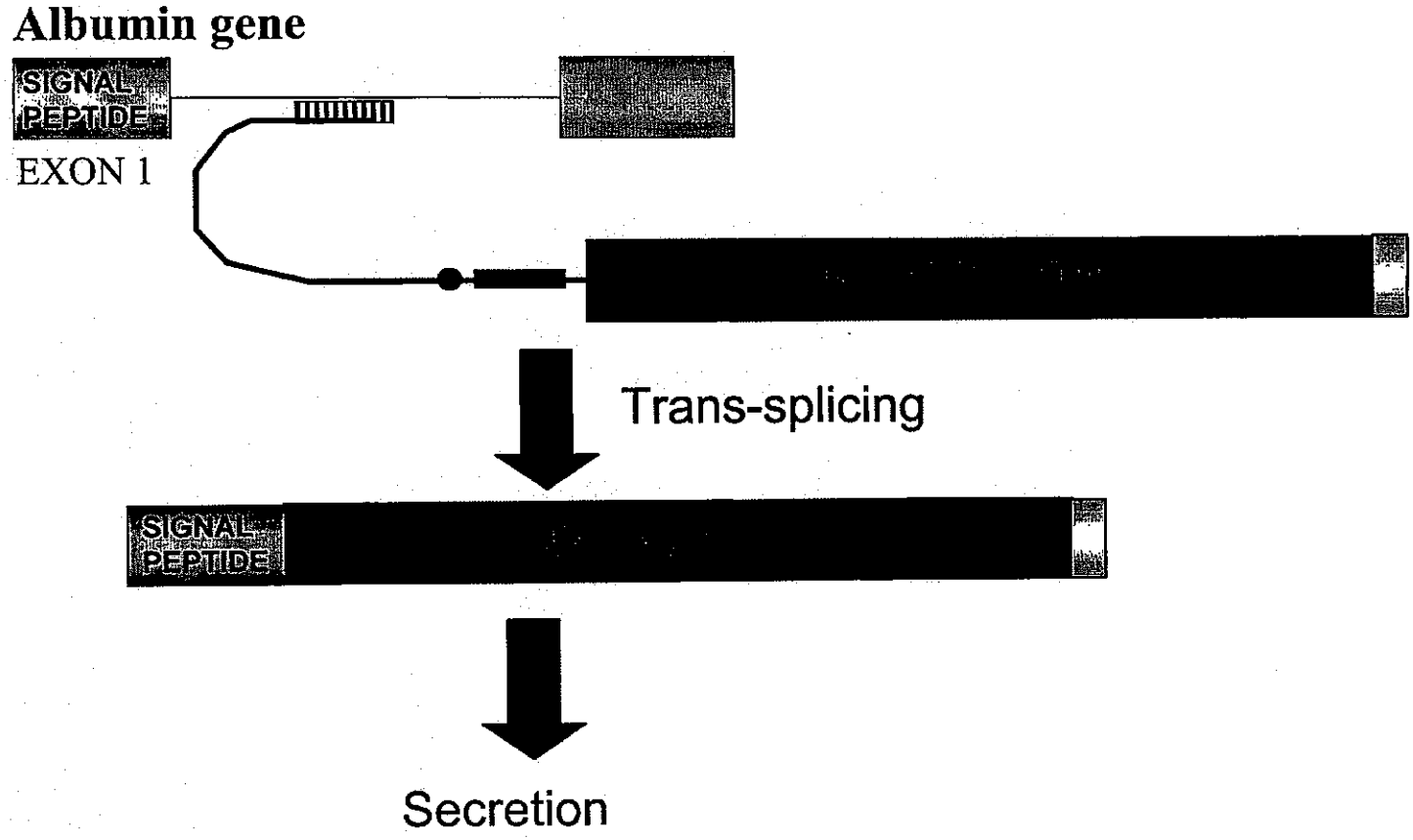


Figure 46

Study E8: Testing of an albumin targeted PTM

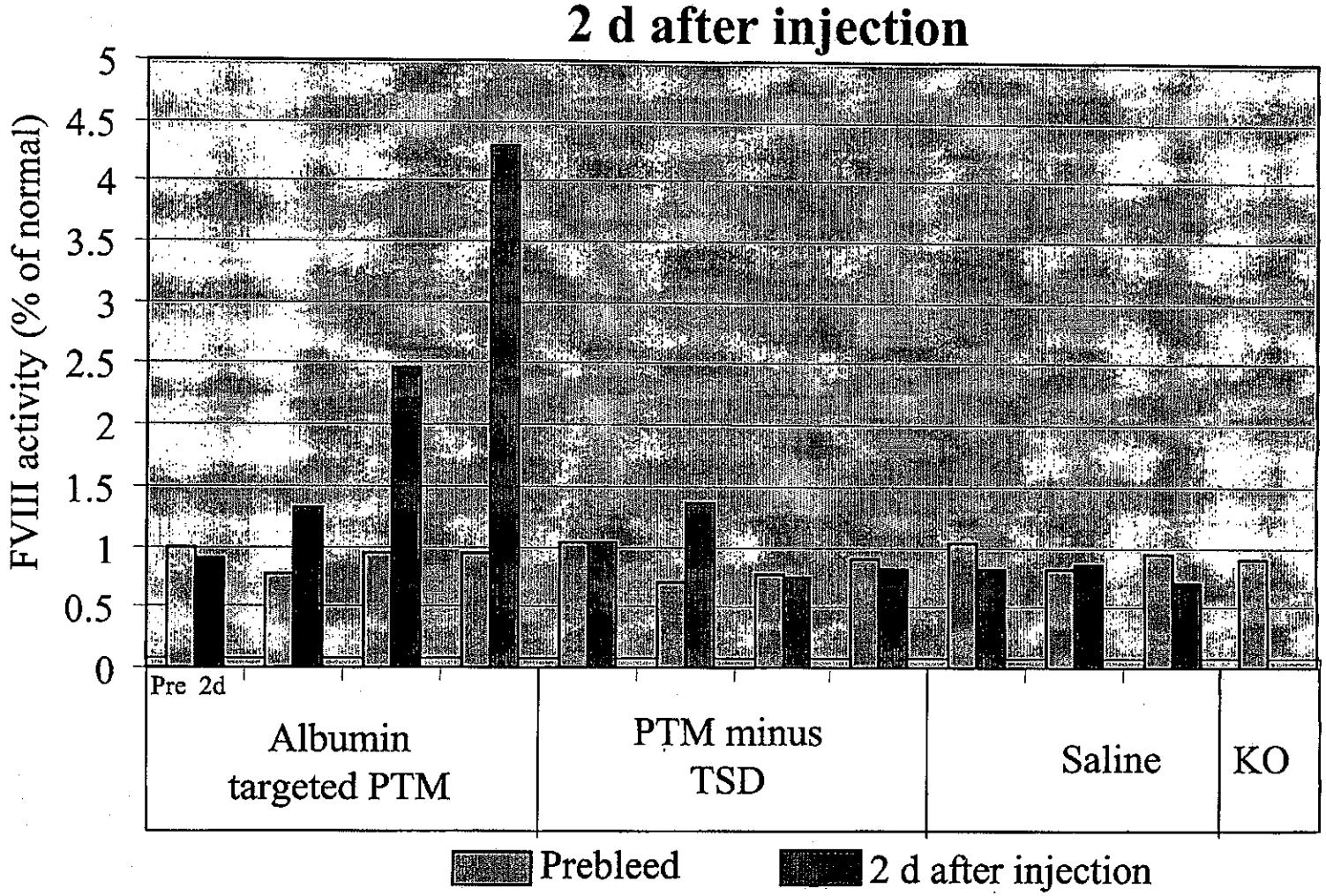


Figure 47

Study E9: Testing of an albumin targeted PTM in KO mice

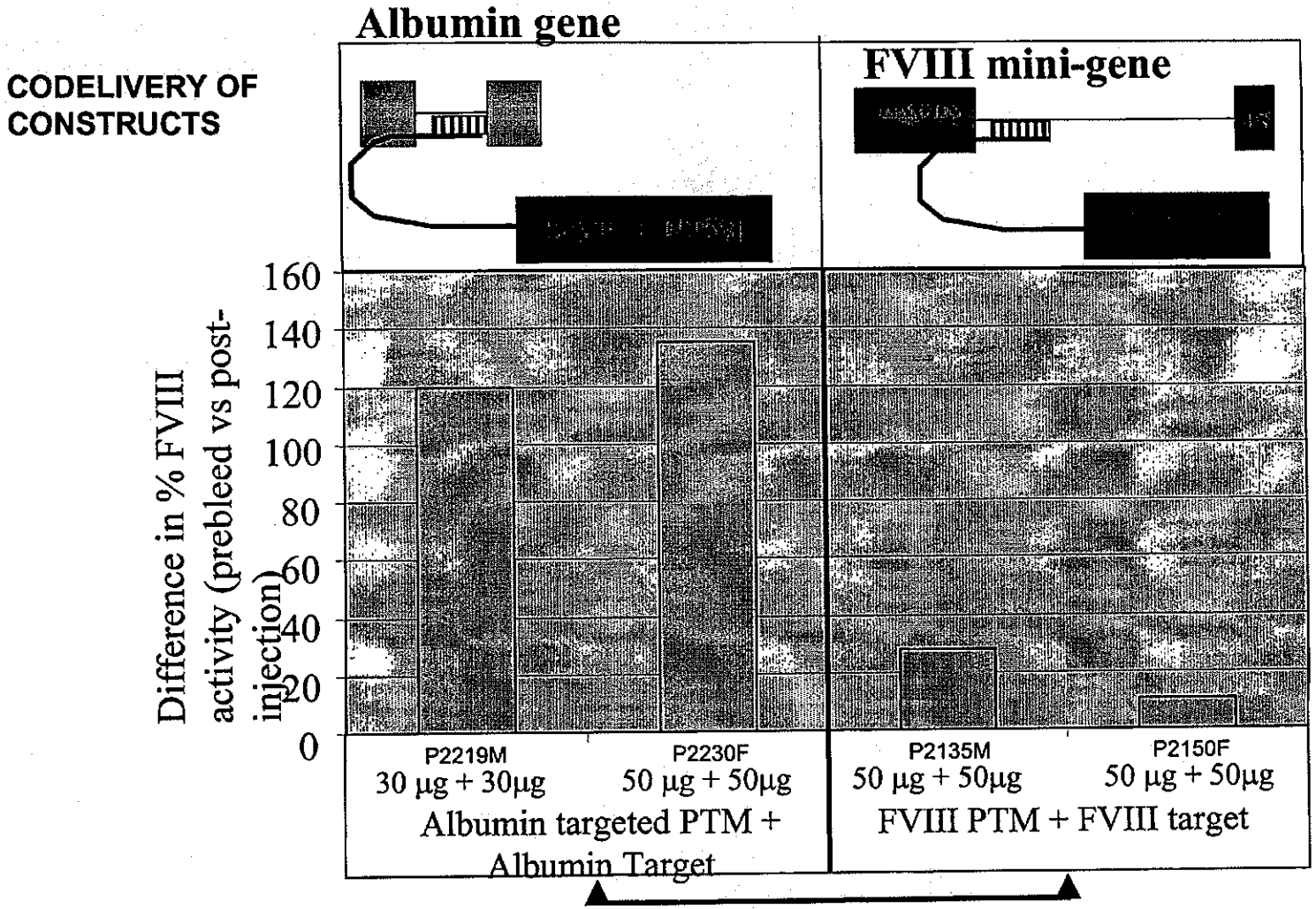
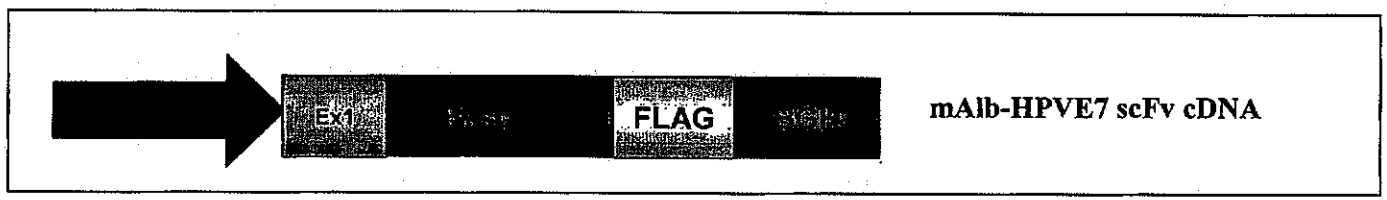
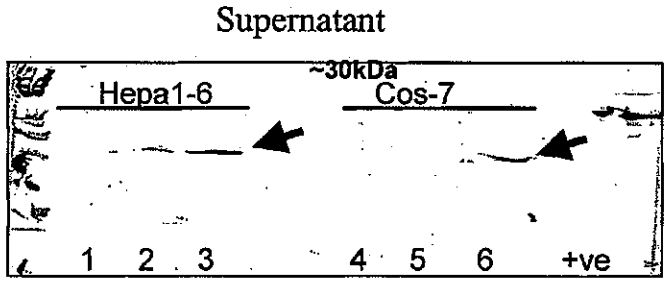


Figure 48

Trans-spliced mAlb-E7scFv is translated and secreted



The mAlbEx1-E7scFv protein is expressed, processed and secreted properly in both hepatoma and COS-7 cells.



Lane 1, 4: Mock
Lane 2, 5: E7scFv (-FLAG)
Lane 3,6: mAlbHPVE7scFv-FLAG
+ve: Positive control for FLAG (sigma)

Figure 49

Trans-spliced Antibody Product is Functional

- **Trans-spliced scFv protein is functional:**
  - Cervical cancer cells, SiHa, or the matching control cells that do not express E7 oncoprotein was transfected with mAlb-E7scFv cDNA plasmid. Cells were grown for 5 days and assayed for cell survival by MTT assay.

- Results: ~70% cell killing was observed with SiHa cervical cancer cells compared to control cells which do not express E7 oncoprotein.

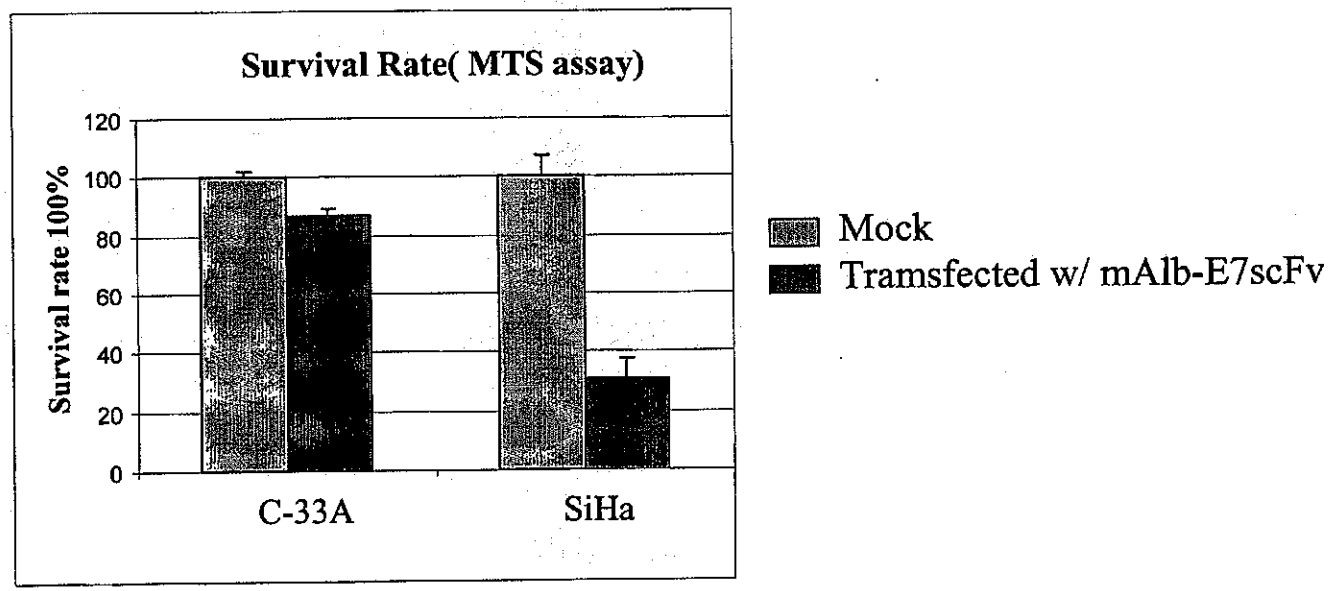


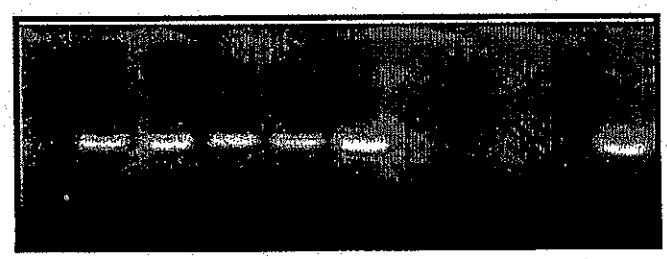
Figure 50

Levels of pre-mRNA in Mouse Liver: albumin is 270x Higher Than FVIII

Target	**Pre-mRNA**	**mRNA**
	#molecules/50ng RNA	# molecules/50ng RNA
Albumin	8.1×10^4	4.7×10^7
Factor VIII	3.0×10^2	ND

Figure 51

Trans-splicing to albumin pre-mRNA target is accurate



Trans-spliced (404 bp)

Lane 1: Target only
Lane 2: Target+ scFvPTM1 (97C2)
Lane 3: Target+scFv-SM-PTM1
Lane 4: Target+scFvPTM2 (158ΔGTs)
Lane 5: Target +scFv-SMPTM2
Lane 6: mAlb-E7scFv cDNA control
Lane 7: RT- (T+P, same as lane 2)
Lane 8: RT- (T+P, same as lane 4)
Lane 9: Primer control
Lane 10: Plasmid control

RT-PCR product gel purified and sequenced

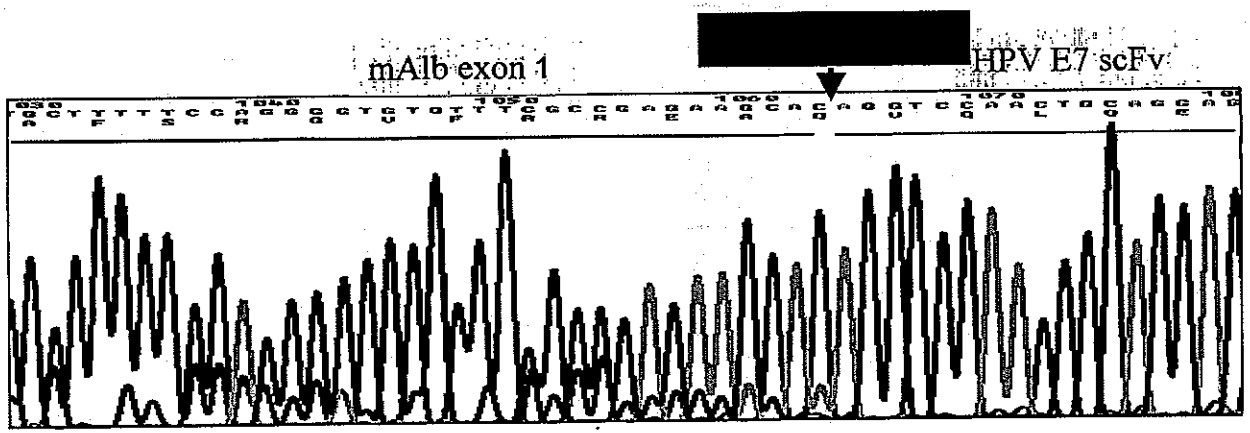


Figure 52

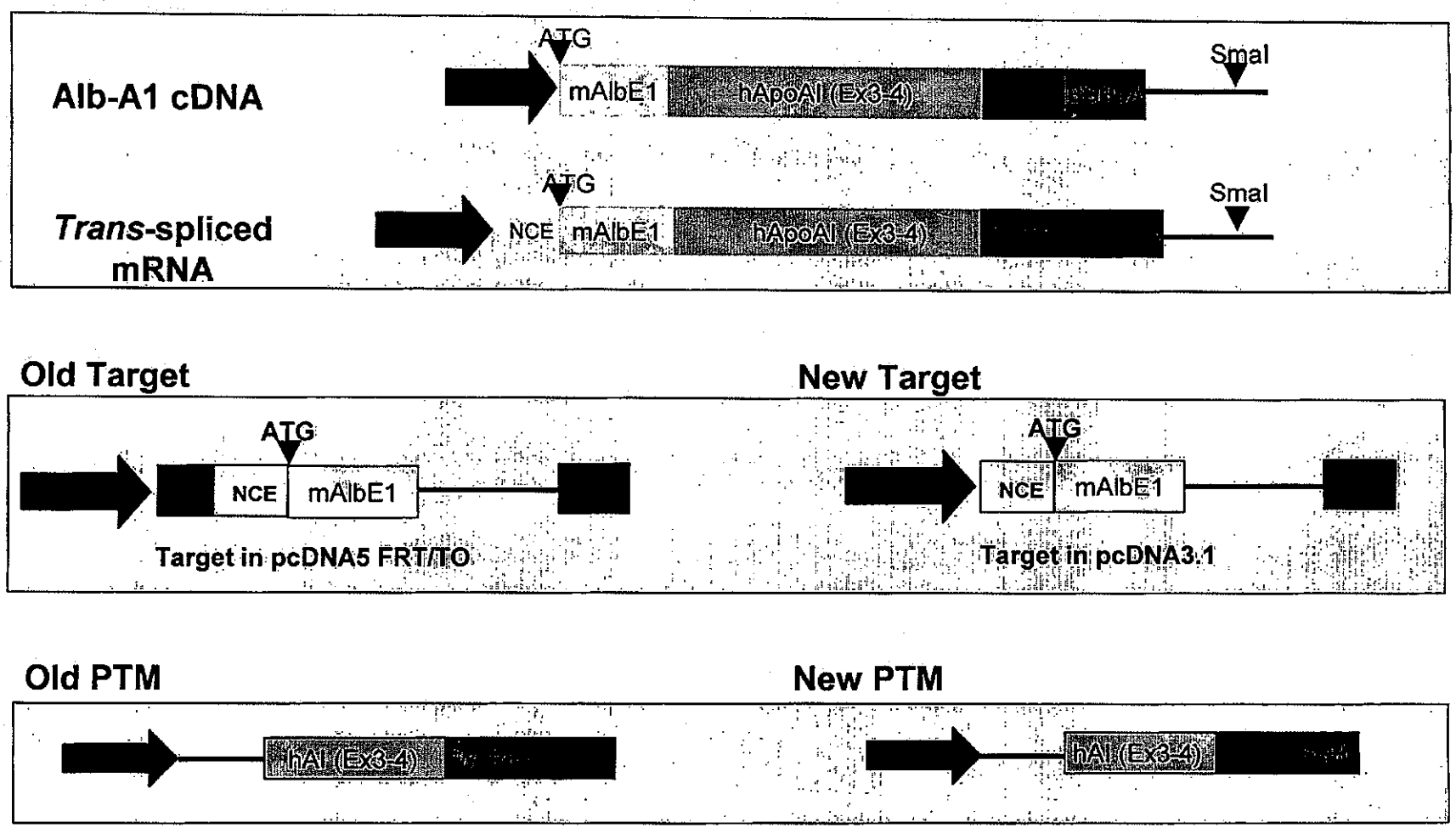


Fig. 53. Schematic drawings of albumin-human Apo A-I cDNA, trans-spliced mRNA, old and new PTM and targets used in the current study. NCE, non-coding exon; hAI, human Apo A-I and Ex, exon.

Trans-spliced mAlb-hAI Protein is Functional: Efflux results

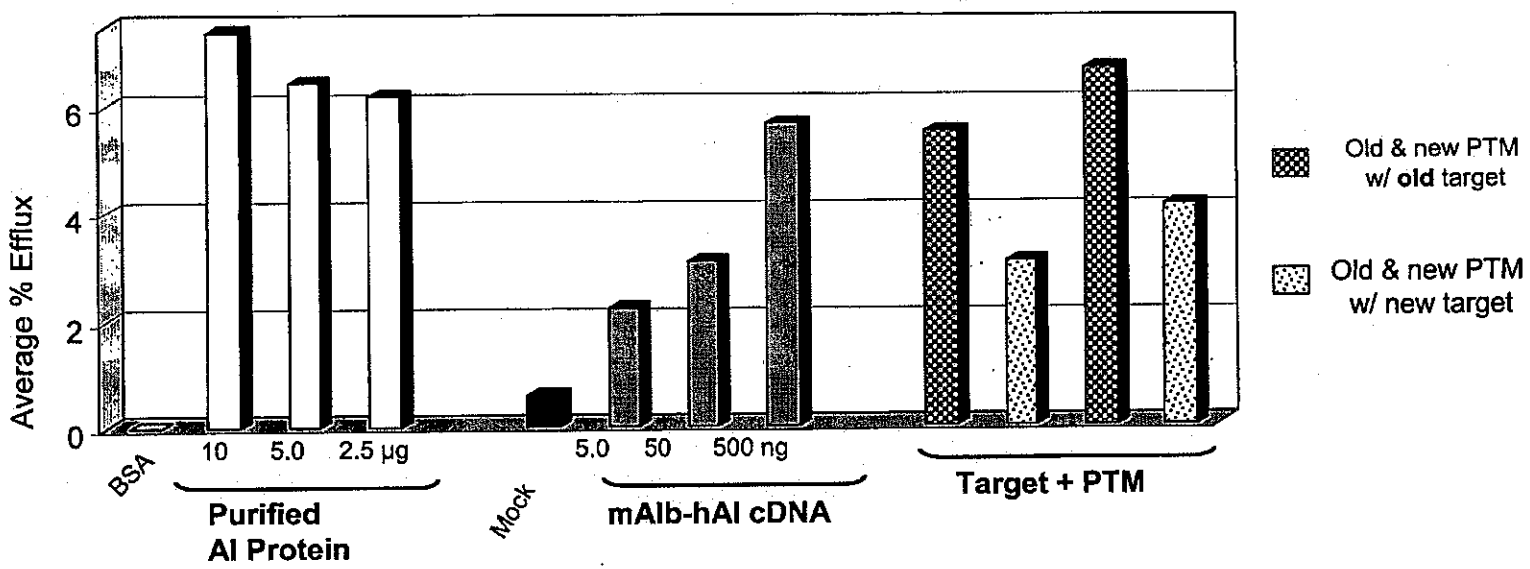


Fig. 54. *Trans*-splicing between target and PTM plasmids produces functional protein in 293 cells. 293 cells transfected with different concentrations of mAlb-hAI cDNA or PTM + target plasmids. 48 hrs post-transfection, media was collected, processed and assayed (efflux potential) for activity as described before.

Trans-splicing efficiency in 293 cells: qRT-PCR results

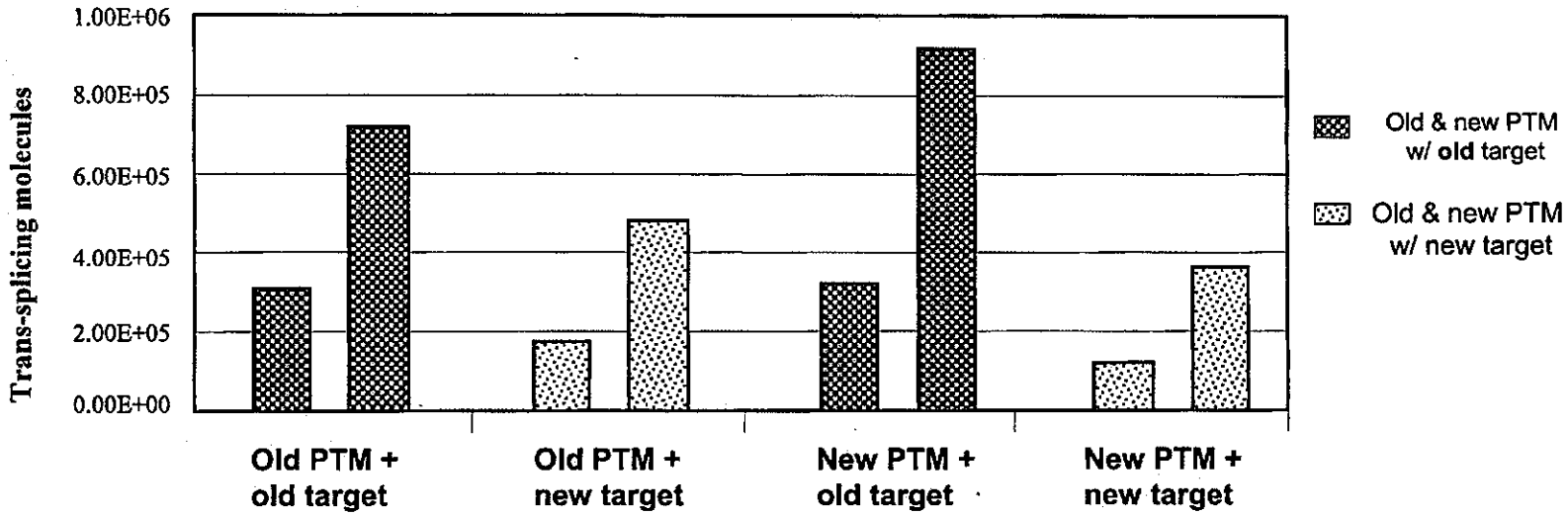
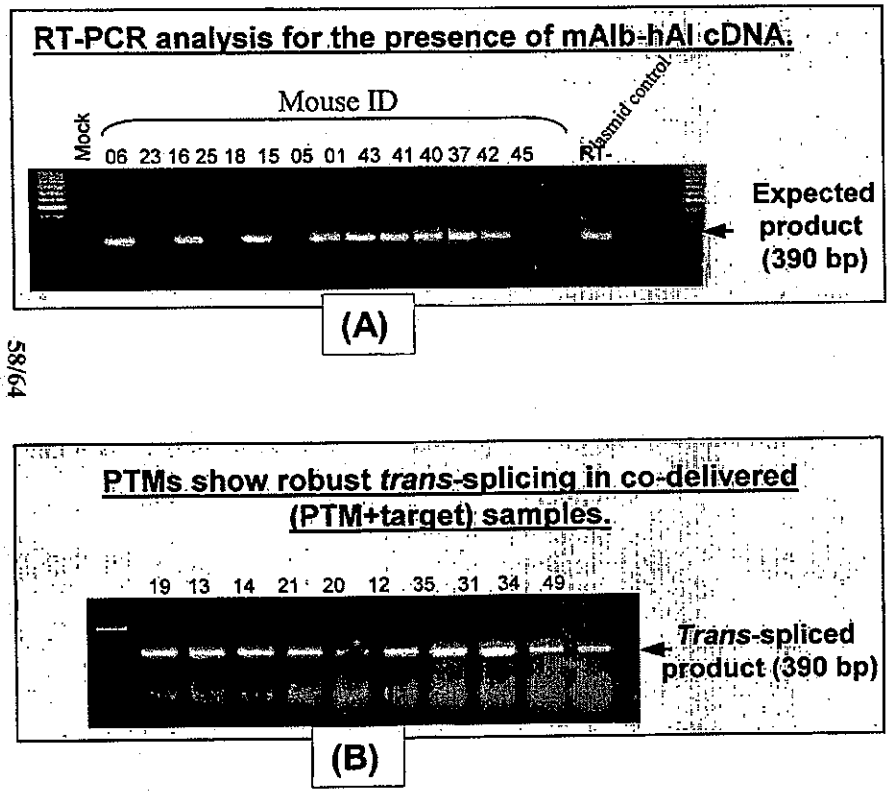


Fig. 55. *Trans*-splicing efficiency of the new and old PTMs in 293 cells. 293 cells transfected with different concentrations of PTM + target plasmids. 48 hrs post-transfection, total RNA isolated and trans-splicing efficiency was quantified by qRT-PCR using specific primers.

In Vivo POP Studies in Normal Mice - PCR Results



Experimental Details:

- Total RNA from liver tissue isolated and analyzed by end-point PCR using mouse albumin exon 1 and human ApoAI specific primers.
  - RNA per reaction = 1 μg
  - PCR cycles = 25

Results:

mAlb-hAI cDNA:

- 11 out of 14 positives
  - 3 that were negative (#23, 42 & 45): questionable injections.

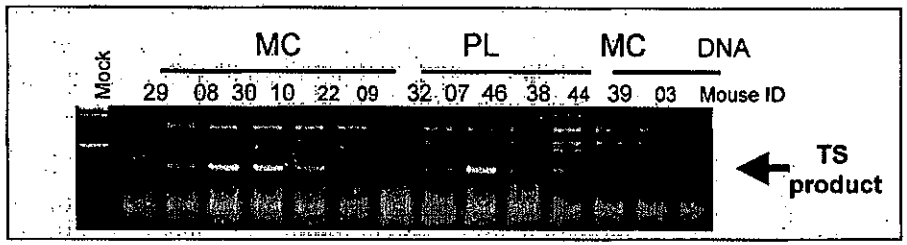
Co-delivered samples:

- 100% positive for *trans*-splicing.

Fig. 56. RT-PCR results showing the presence of (A) mouse albumin-human Apo A-I mRNA and (B) *trans*-spliced mRNA in mice.

In Vivo POP Studies in Normal Mice - PCR Results

- **PTM only samples analyzed for trans-splicing into endogenous albumin target pre-mRNA**
- **10/13 mice were positive for trans-splicing.**
  - 03, 09, 22; questionable injection



Re-analysis of positive samples along with RT(-) controls

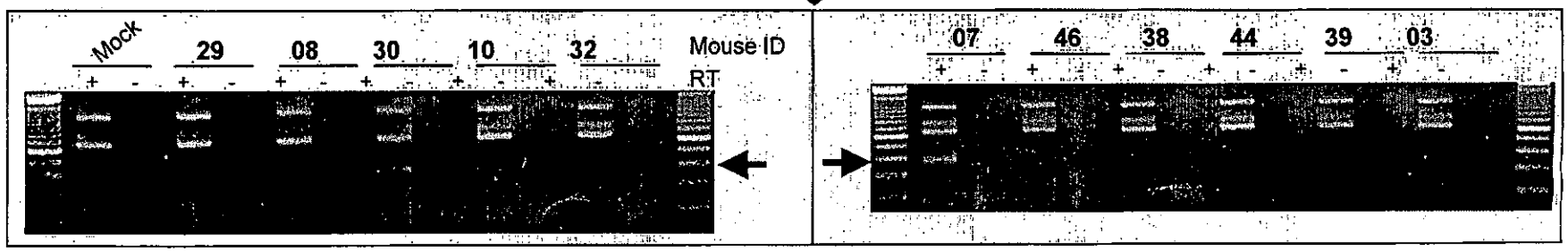


Fig. 56C. RT-PCR results showing *trans*-splicing of human Apo A-I PTM into endogenous mouse albumin pre-mRNA in mice. MC, minicircles, PL, plasmid DNA; RT, reverse transcription and +/- indicate RT+ and RT- reactions.

Production of Human ApoAI Protein in Mice

Western Results

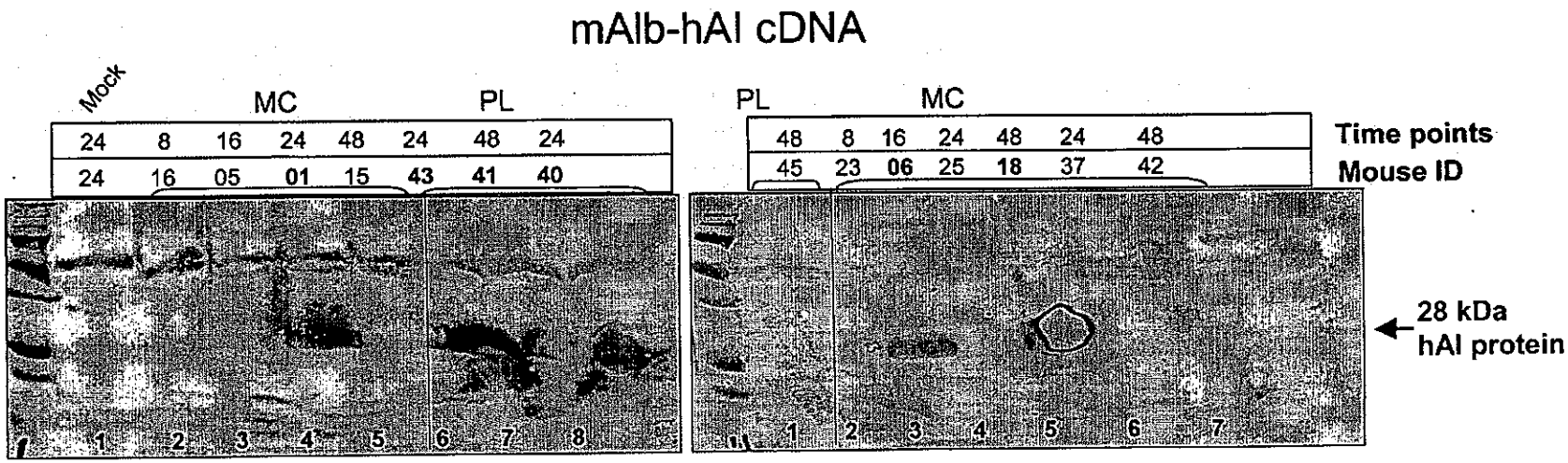
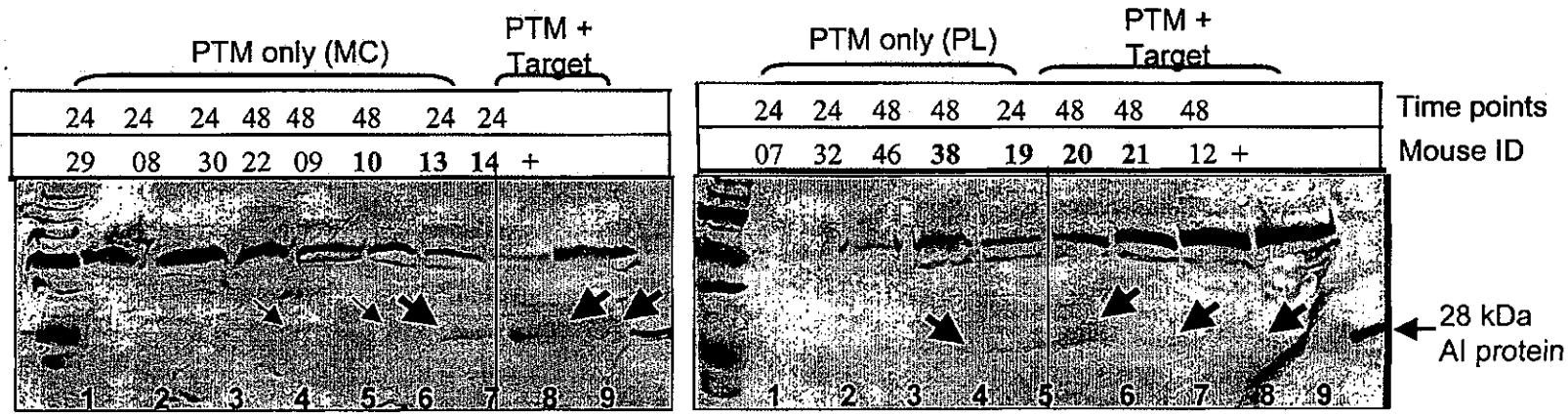


Fig. 57A. Western blot analysis of serum samples from mice injected with mAlb-hAI cDNA. 20 µl serum passed through Proto-Blue column (to deplete albumin + IgG) and analyzed by Western blot using human ApoAI specific antibody. MC, minicircles and PL, plasmid DNA RT.

Production of Human ApoAI Protein in Mice

Western Results



Lane 9: AI marker protein

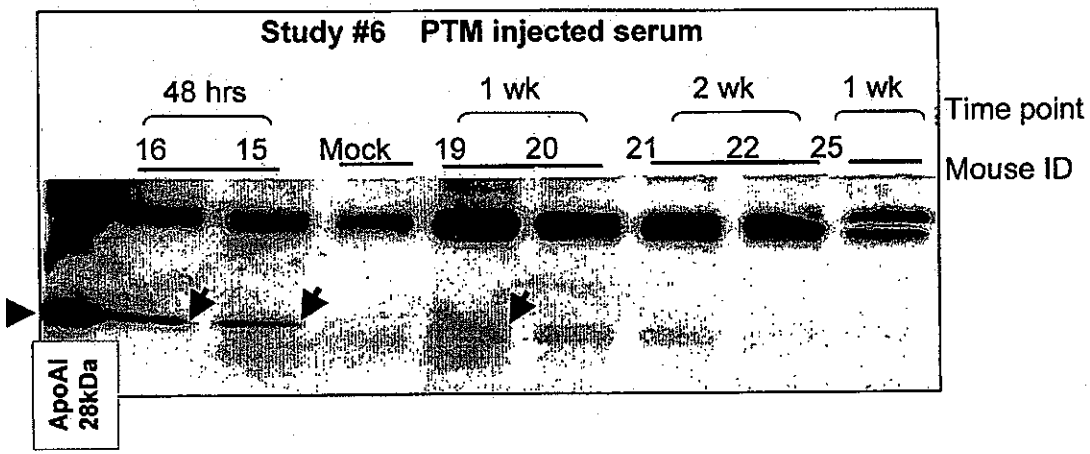
Fig. 57B. Western blot analysis of serum samples from mice injected with PTM only and PTM + Target plasmids. 20-50 µl serum passed through Proto-Blue column (to deplete albumin + IgG) and analyzed by Western blot using human ApoAI specific antibody. MC, minicircles and PL, plasmid DNA.

hApoAI protein produced through *trans*-splicing in mice

Study #6

- Minicircle-PTM (75 µg/mouse)
- Time point: 48-2 wks
- Mice: C57BL/6 males

- 50 µl serum immuno-precipitated using monoclonal antibody to human ApoAI followed by Western w/ the same antibody.



Study #7

- Minicircle-PTM (75 µg/mouse)
- Time point: 48 hrs
- Mice: C57BL/6 females

- IP and Western conditions same as above.

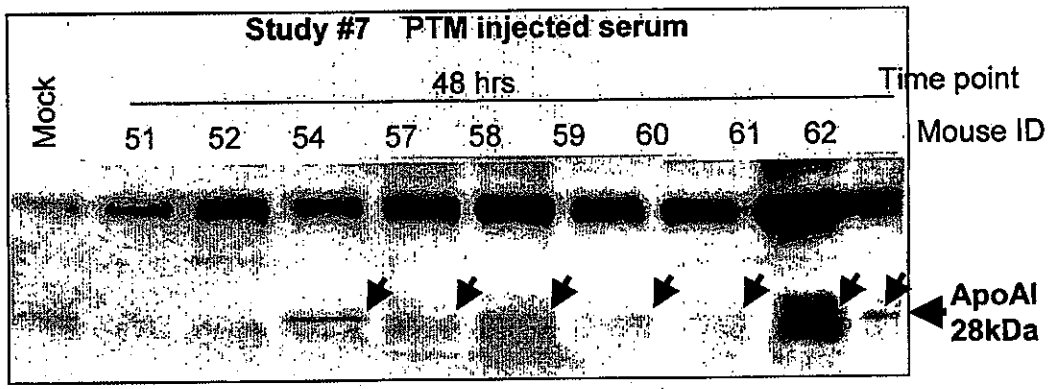


Fig. 58A. Western blot analysis of serum samples from mice injected with PTM plasmid. 50 µl serum was immunoprcipitated and analyzed by Western blot using human ApoAI specific antibody. Arrows indicate 28 kDa human Apo A-I protein.

Production of hApoA-I protein in mice: cDNA samples

Study #6

- Minicircle-cDNA (75 µg/mouse)
- Time point: 48 hrs - 4 wks
- Mice: C57BL/6 males
- 10 µl serum used for IP. IP and Western conditions same as previously described.

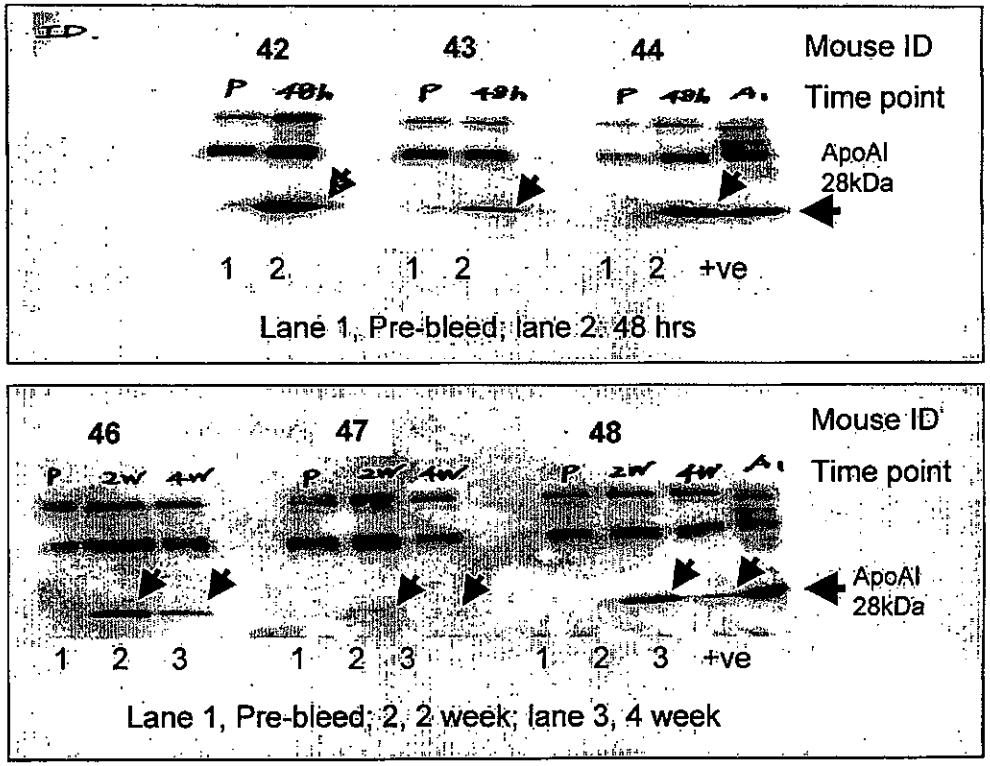


Fig. 58B. Western blot analysis of serum samples from mice injected with cDNA plasmid. 10 µl serum was immunoprcipitated and analyzed by Western blot using human Apo A-I specific antibody.

Production of human Apo A-I protein through *trans*-splicing results in HDL increase in mice

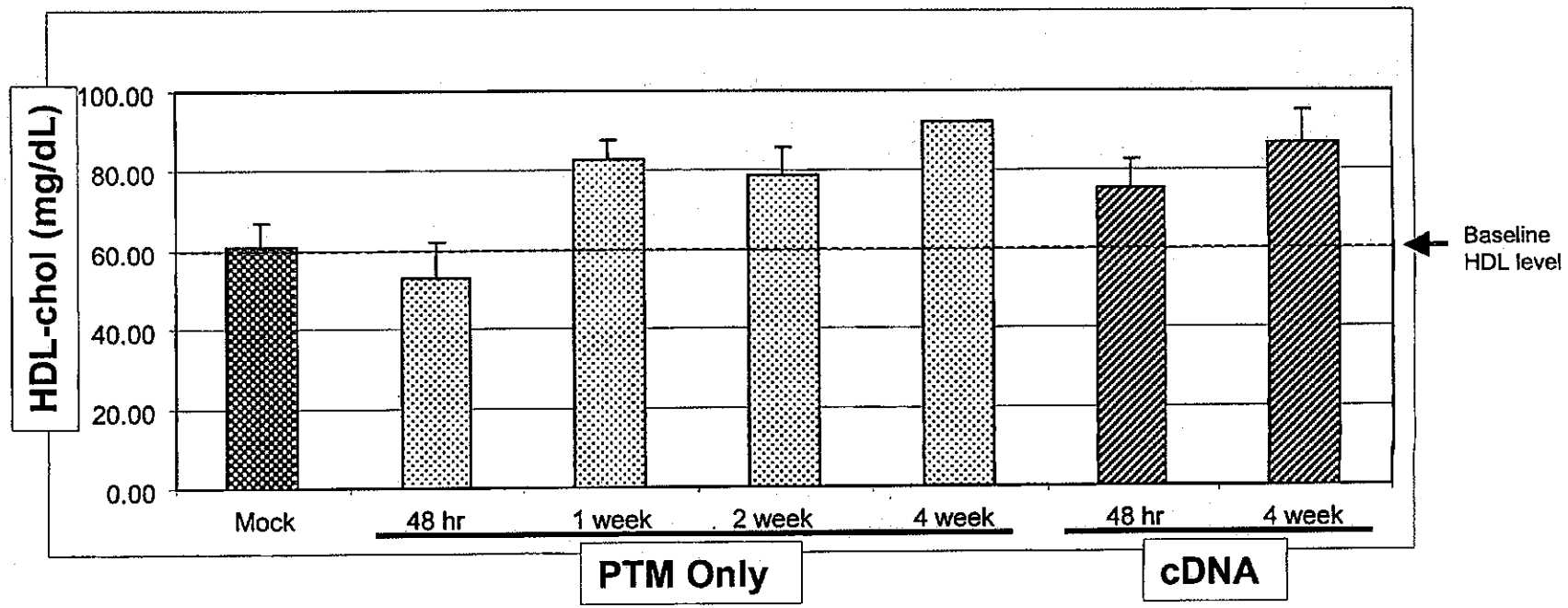


Fig. 59. HDL analysis of serum samples from mice injected with PTM and cDNA plasmids.