

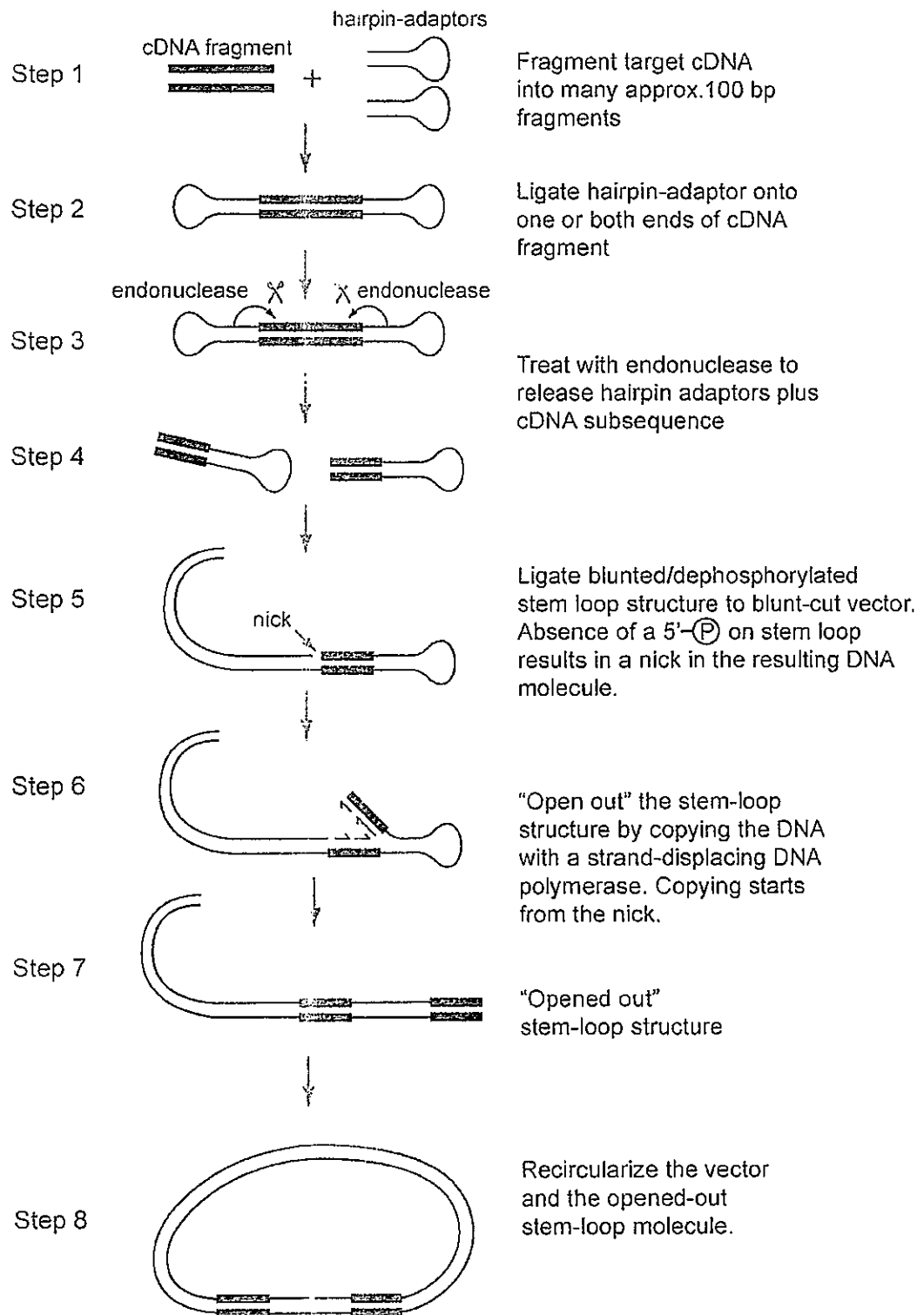


Fig. 1

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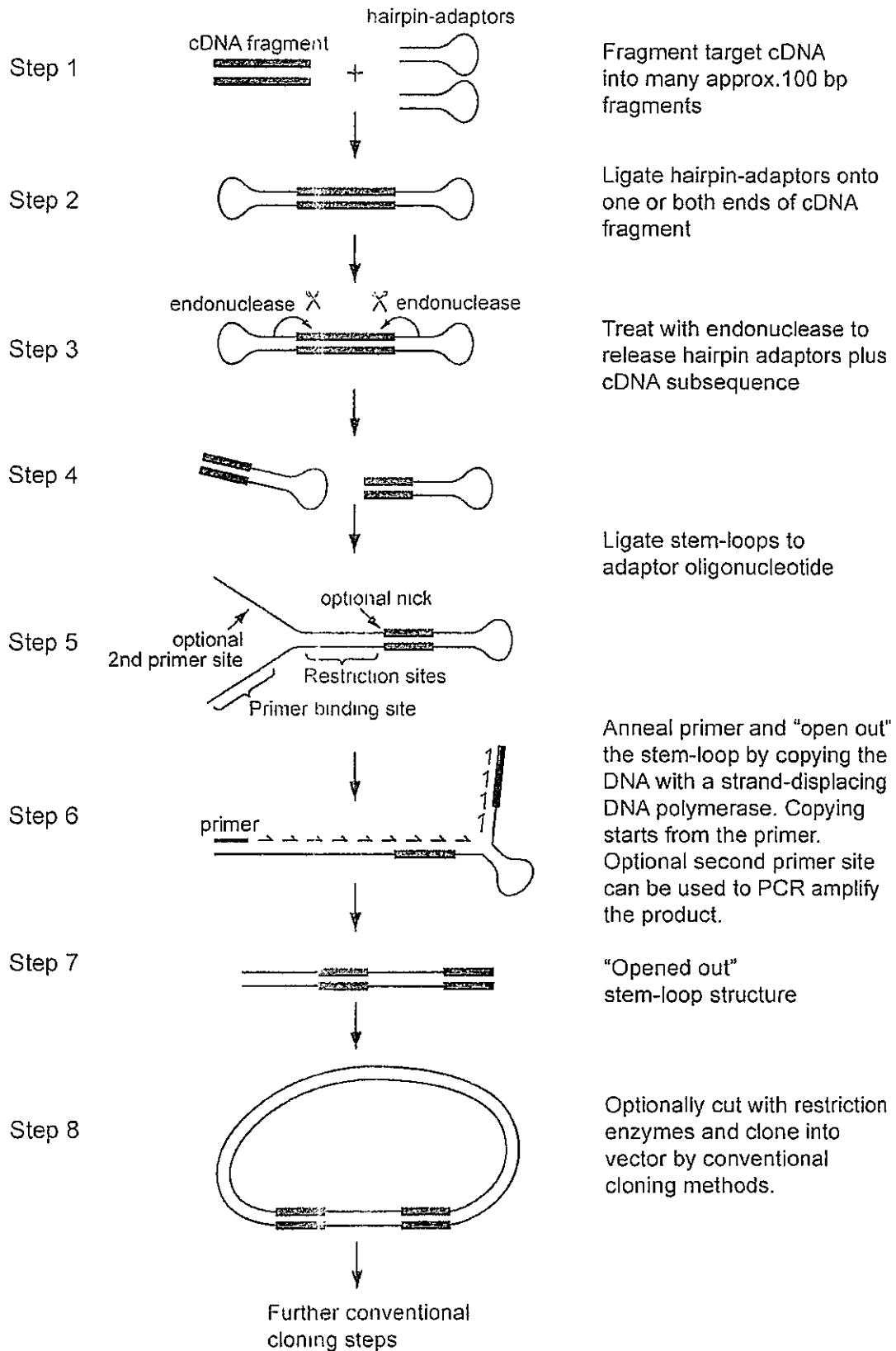


Fig. 2

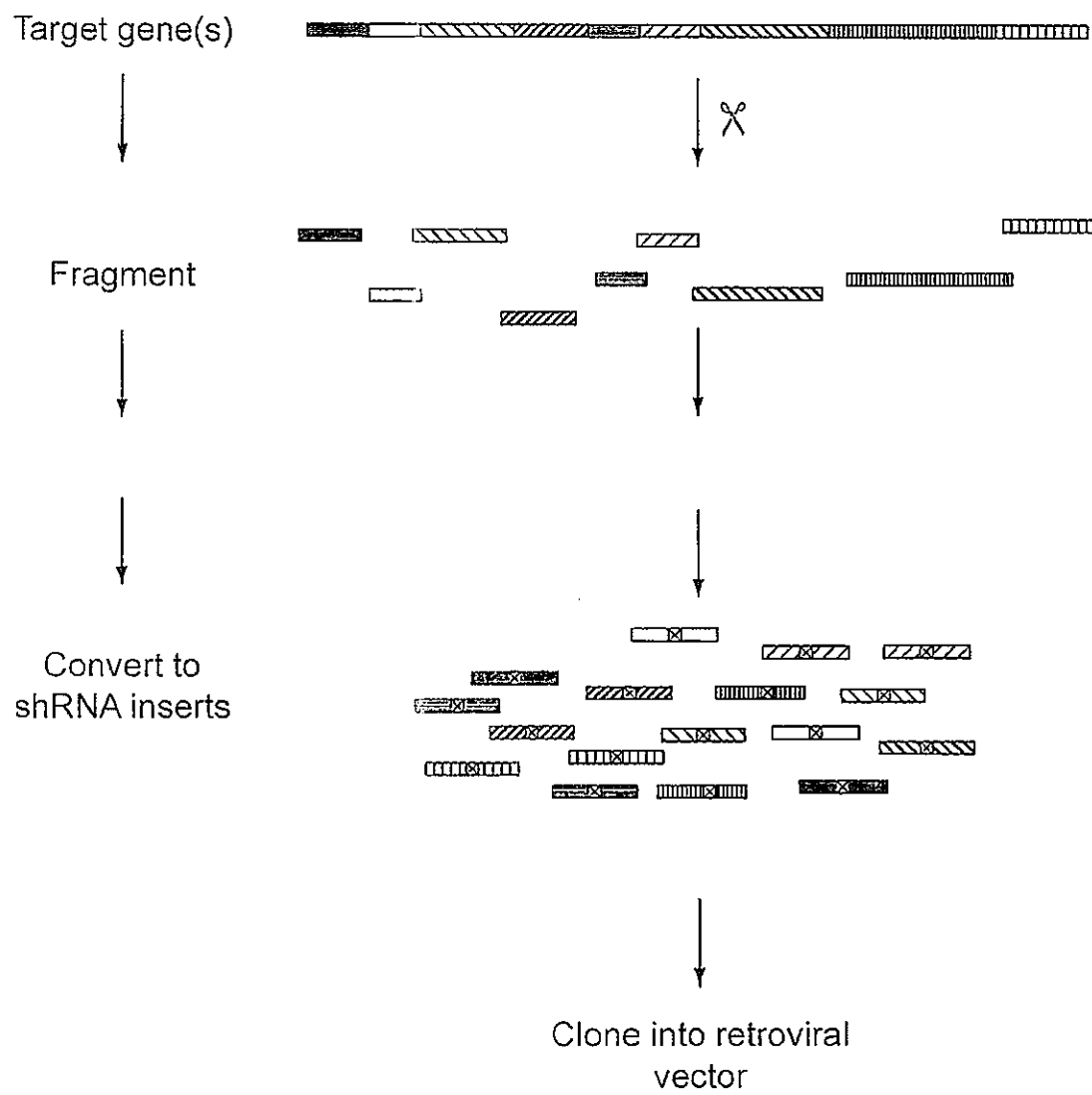


Fig. 3

Generating shRNA expression cassette from
hairpin tags

- A) Ligate on cloning adaptor loop
B) Anneal primer and extend (or extend from a nick between
vector and stem-loop) Bst DNA Polymerase can efficiently copy
the whole loop because it can displace the base-paired DNA.
No amplification avoids bias.

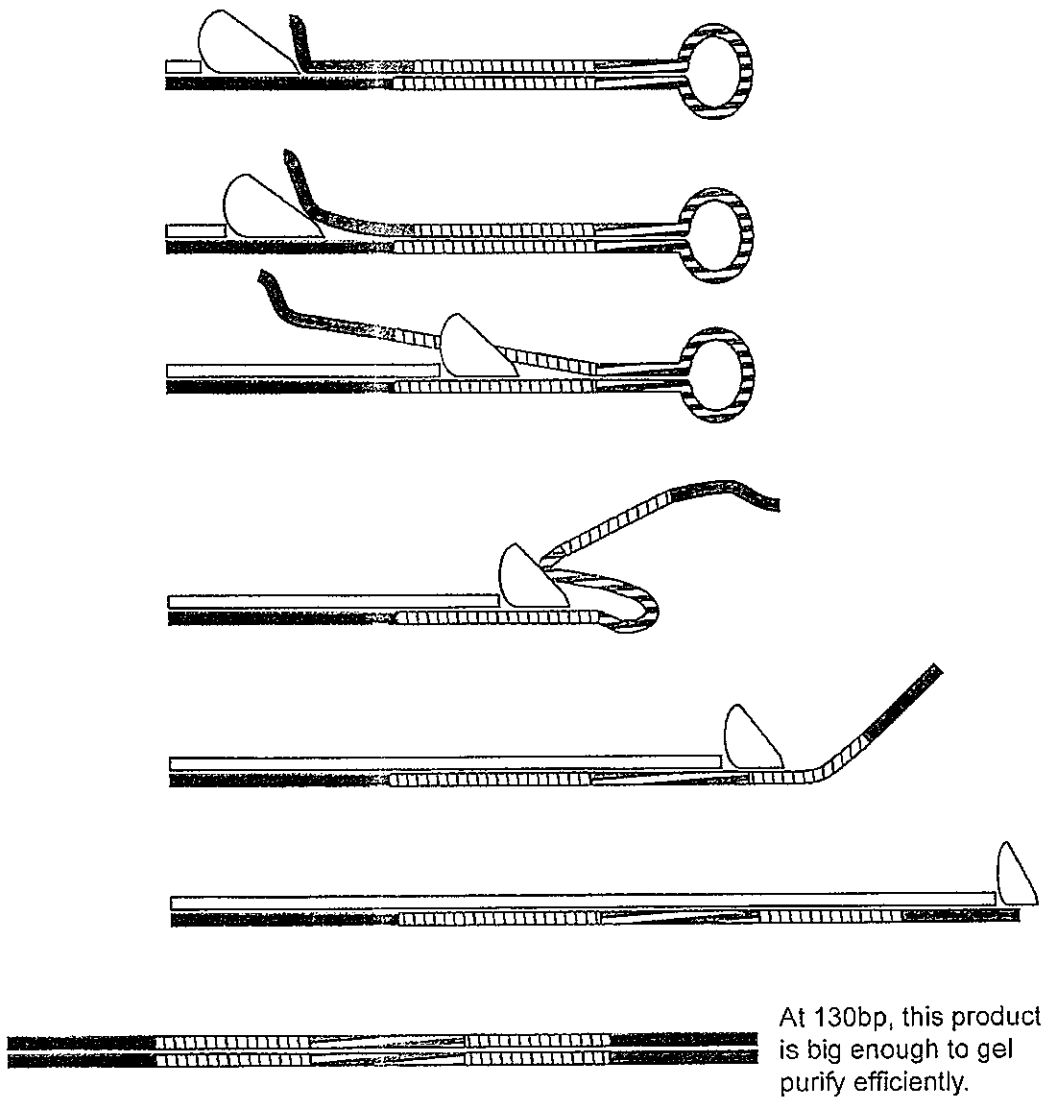


Fig. 4A

Generating shRNA expression cassette from
hairpin tags

- C) Gel purify.
- D) Subclone into a suitable expression cassette by digesting with cloning enzymes. In a preferred method, this isn't required as the opened out loop is already attached to the vector. All that is required is to circularize the vector by ligation.
- E) If necessary, subclone the expression cassette into an appropriate vector, e.g., a retroviral expression vector.
- F) Transcribe the expression cassette in a host cell to produce a candidate shRNA.

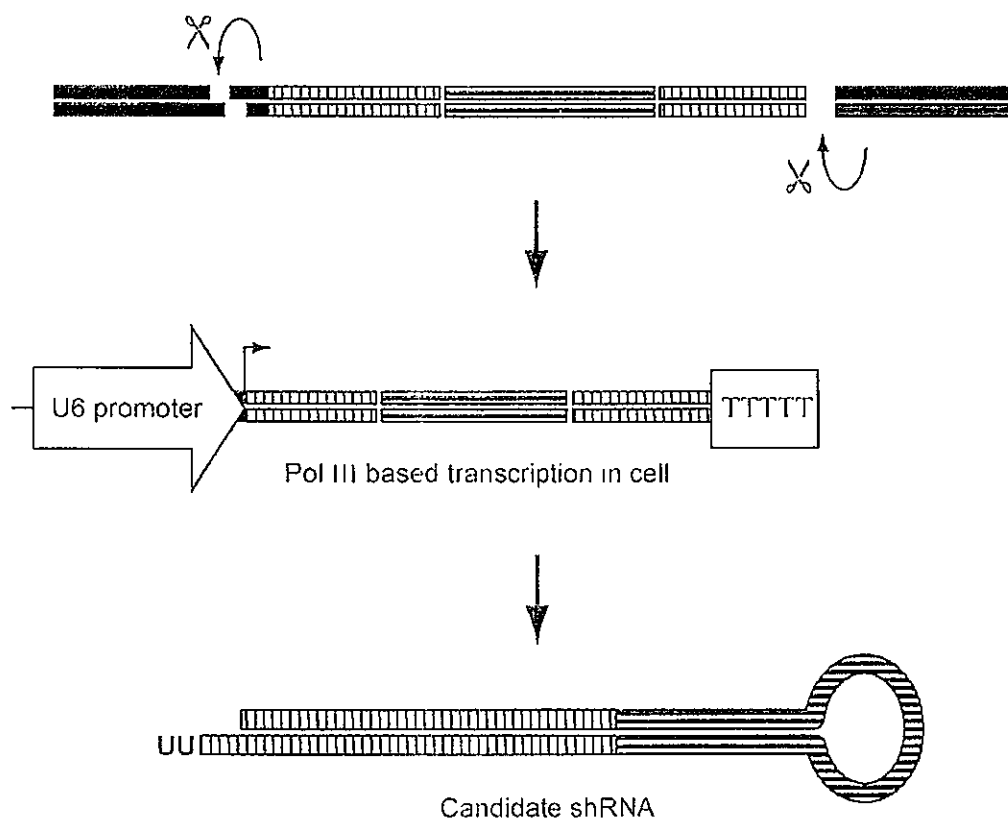


Fig. 4B

Fig. 5A

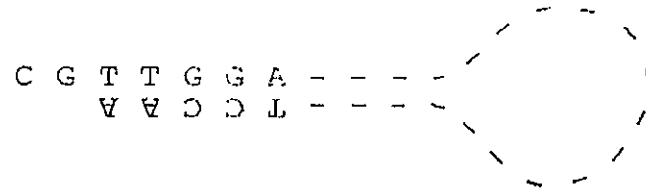


Fig. 5B

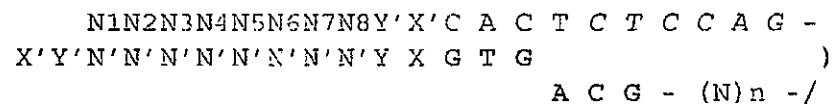
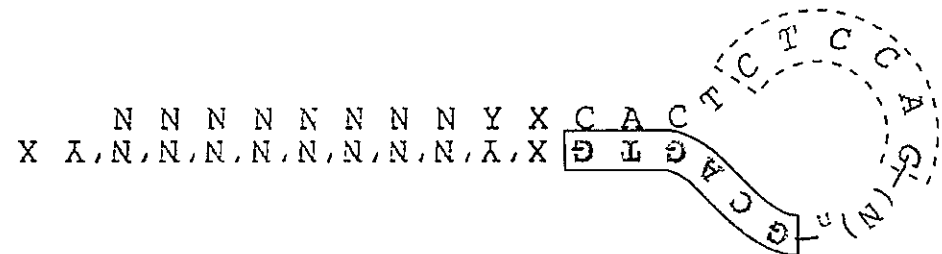
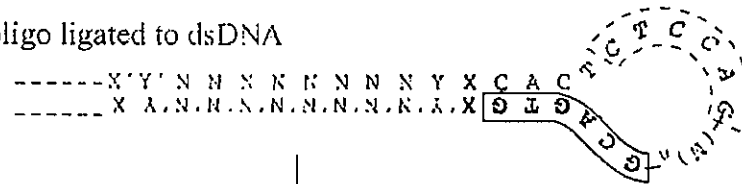


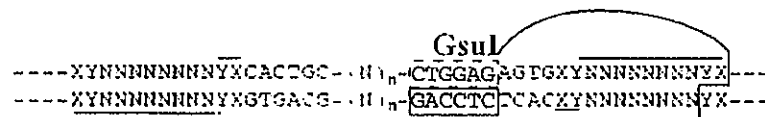
Fig. 5C



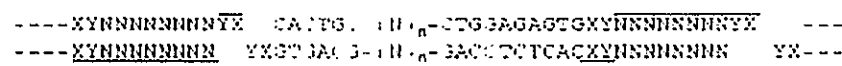
Hairpin oligo ligated to dsDNA



'Open-out' and
clone into circular
vector



Cut with BtsI, GsuI



Recircularise

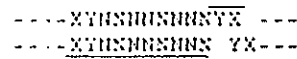


Fig. 5D

Use of restriction enzymes that cut outside their recognition sequence to seamlessly juxtapose sequence elements

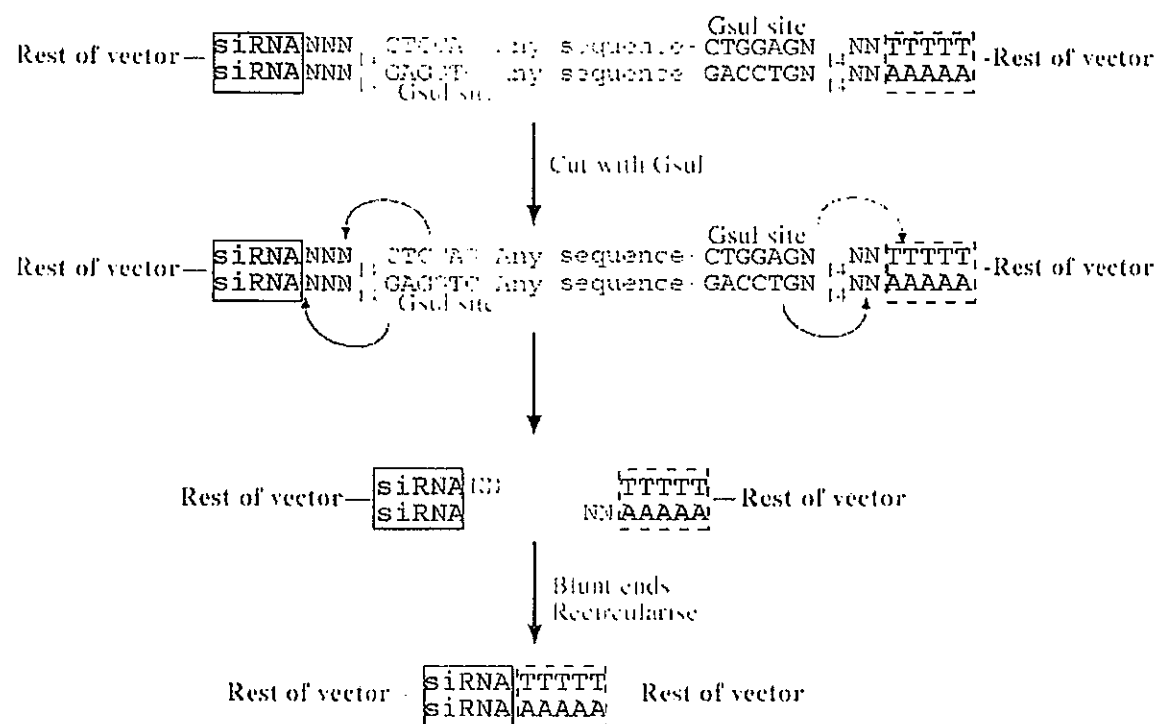


Fig. 6

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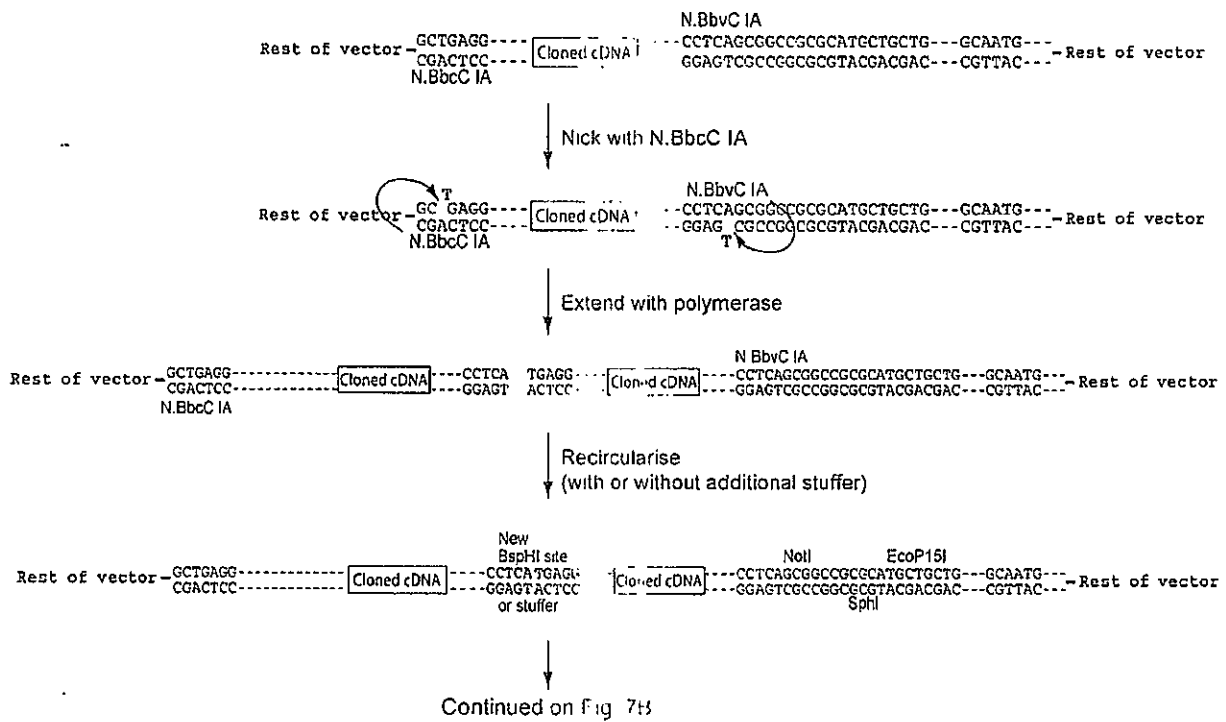


Fig 7A

Continued from Fig 7A

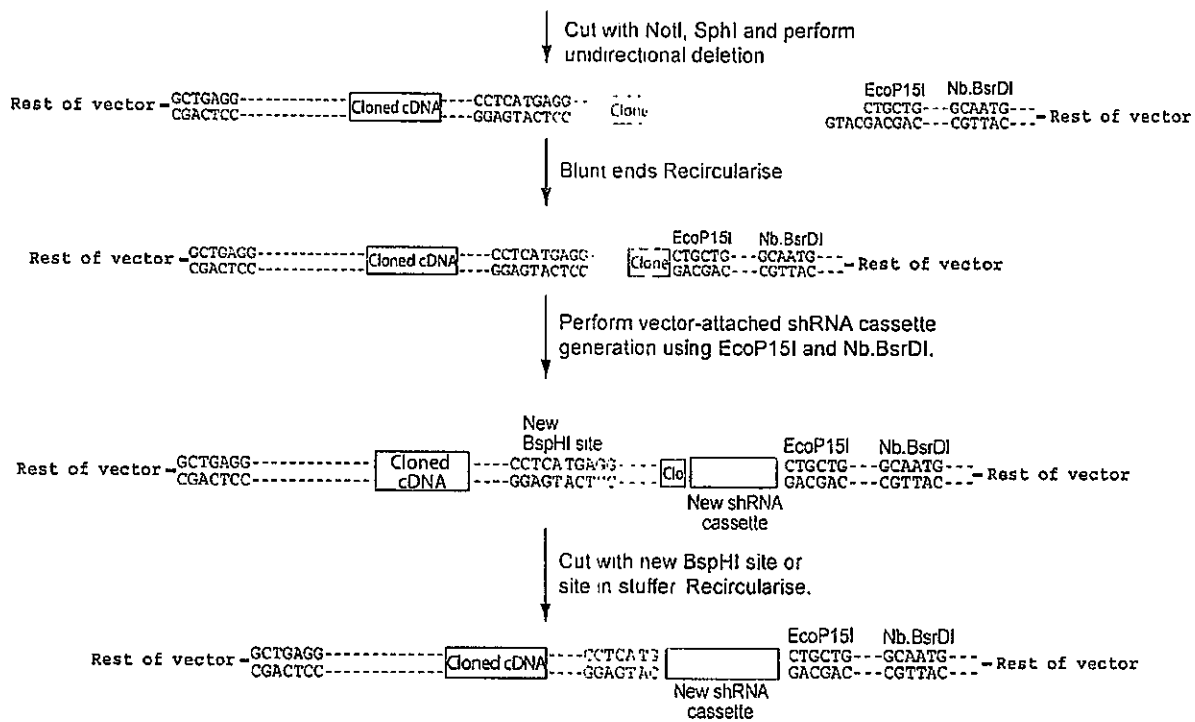


Fig 7B

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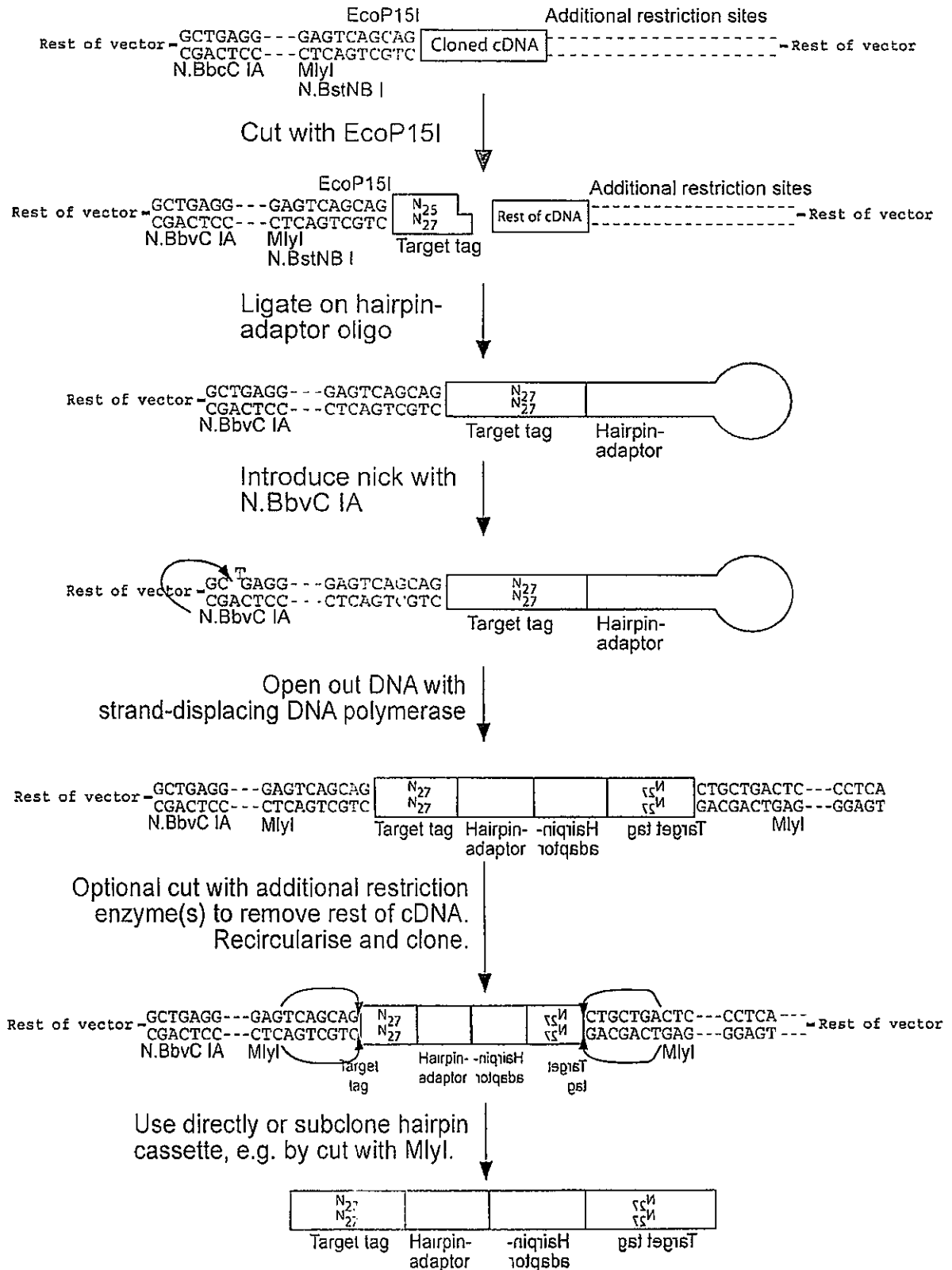


Fig. 8

Fig. 9

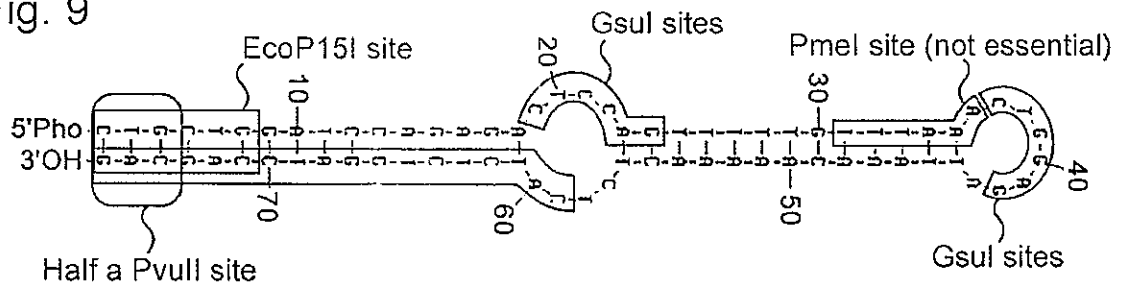


Fig. 10

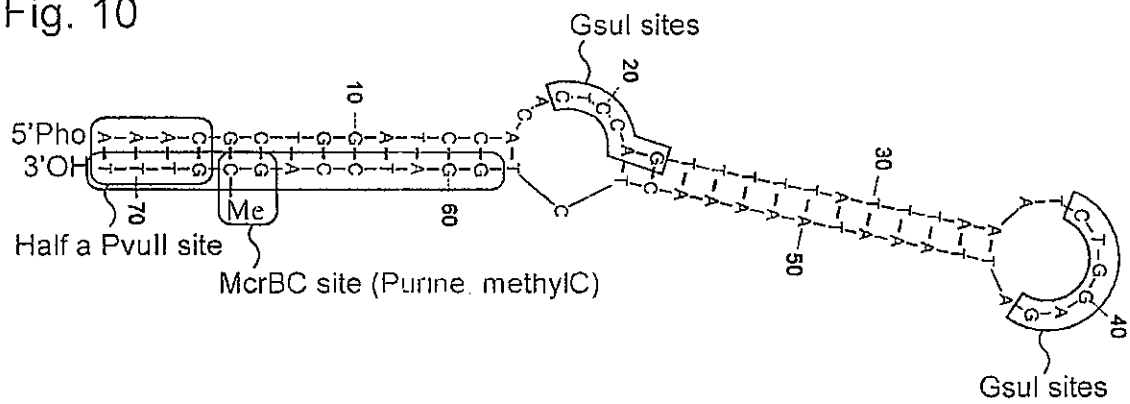
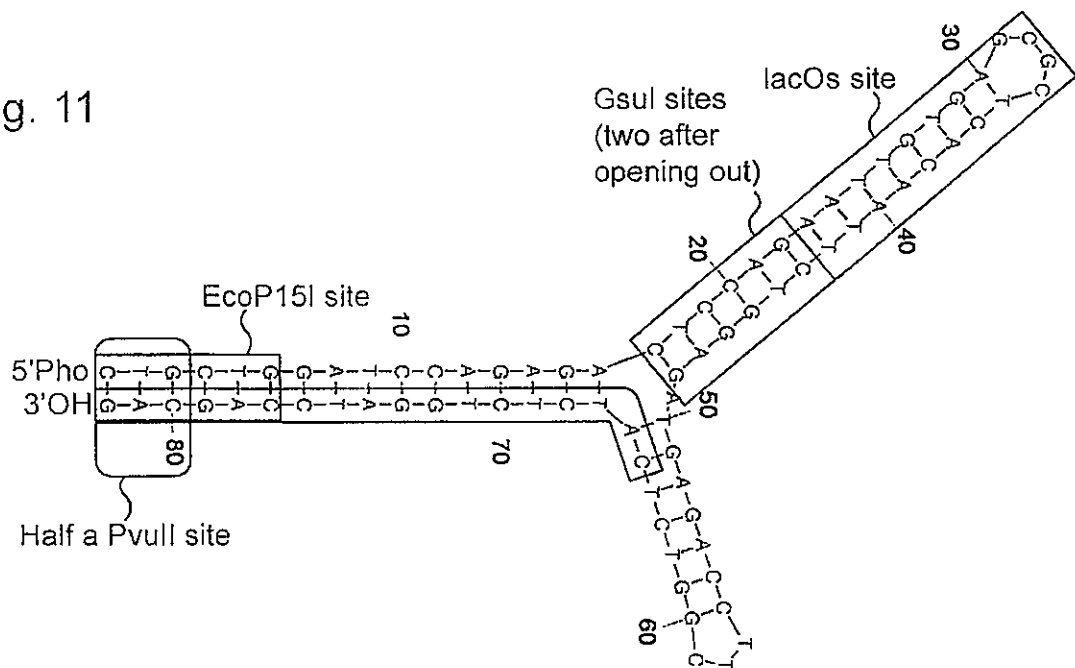


Fig. 11



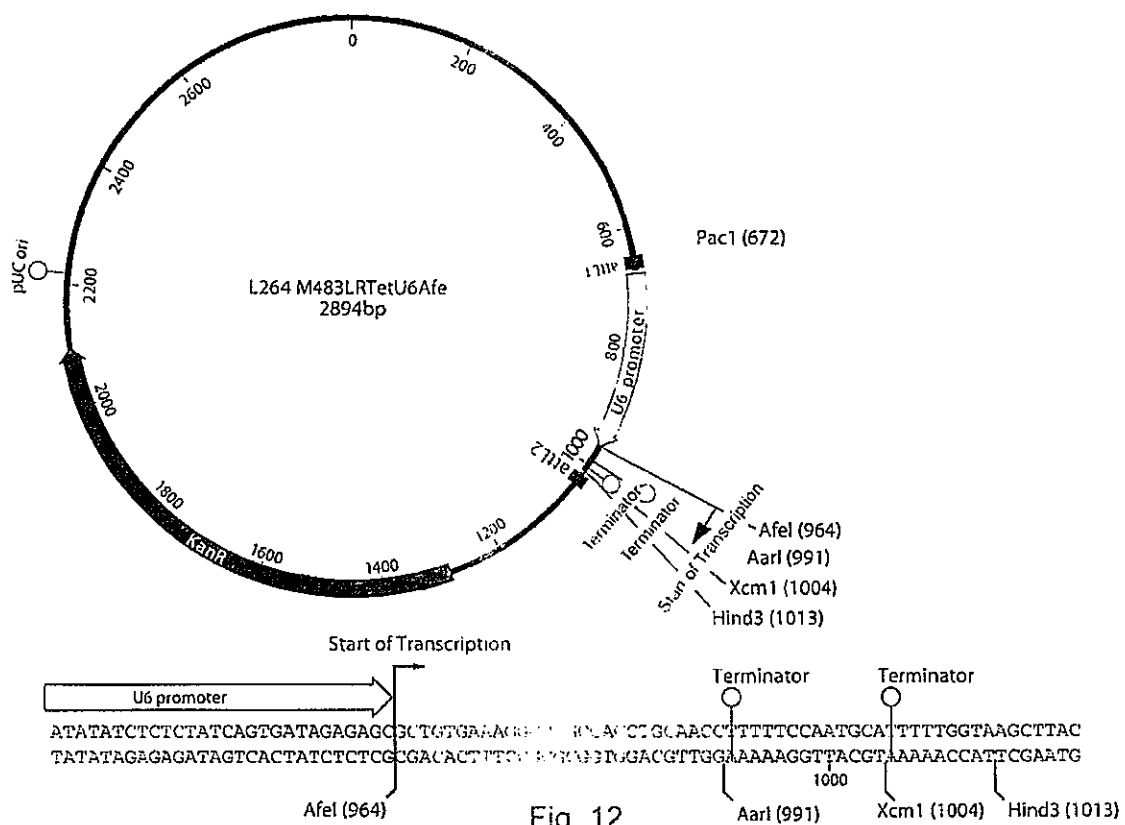
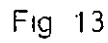


Fig. 12



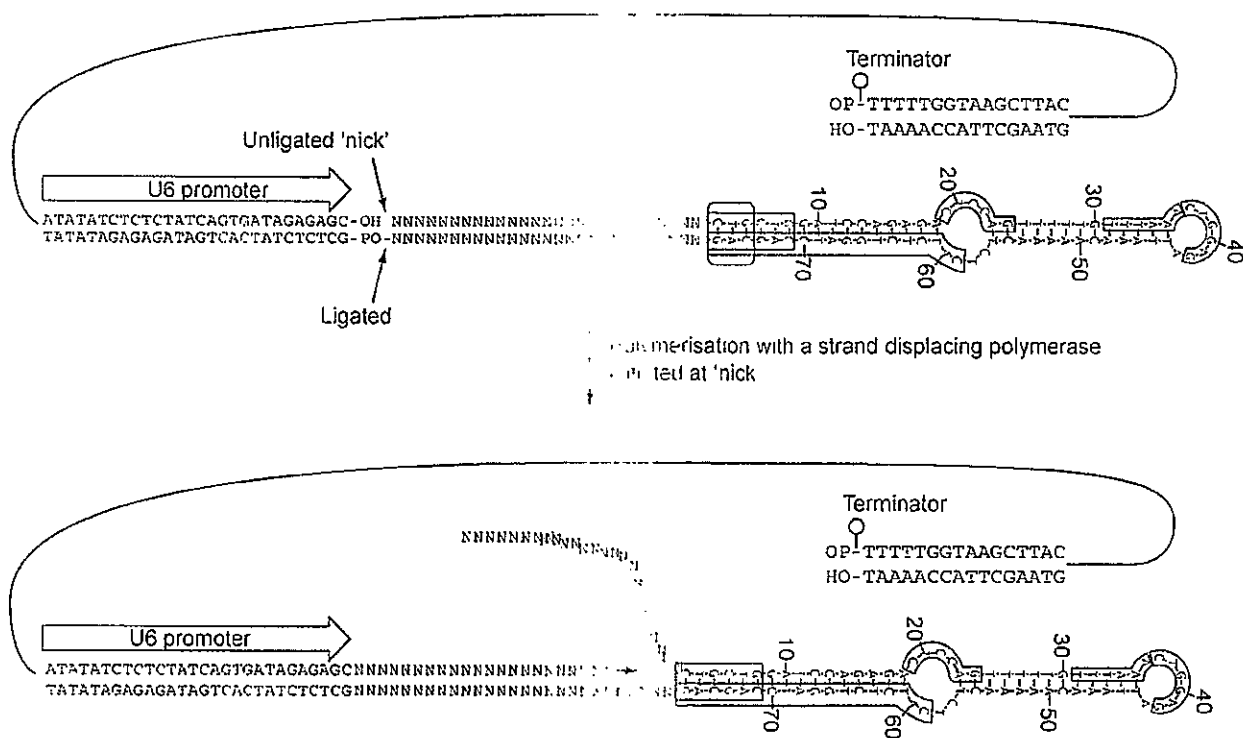


Fig 14

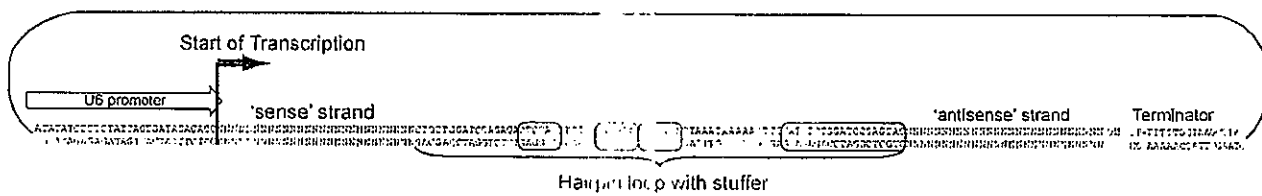


Fig. 15

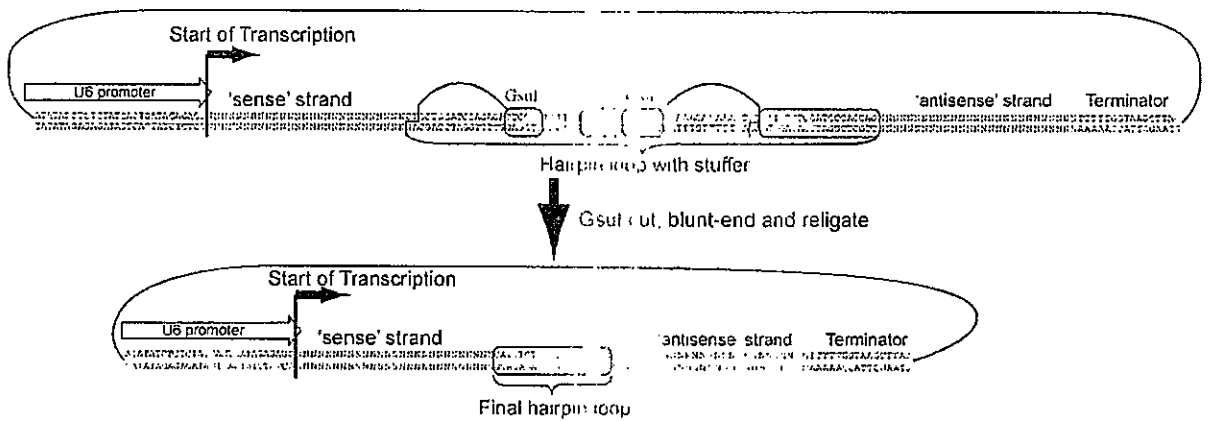


Fig. 16

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Fig. 17

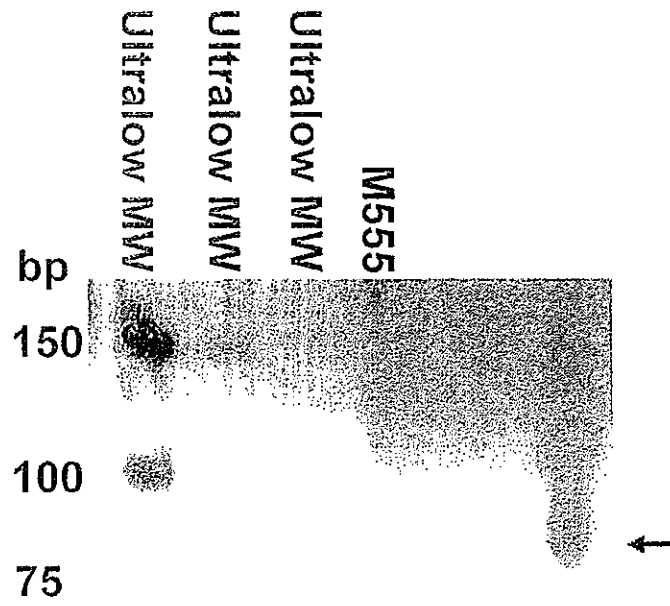
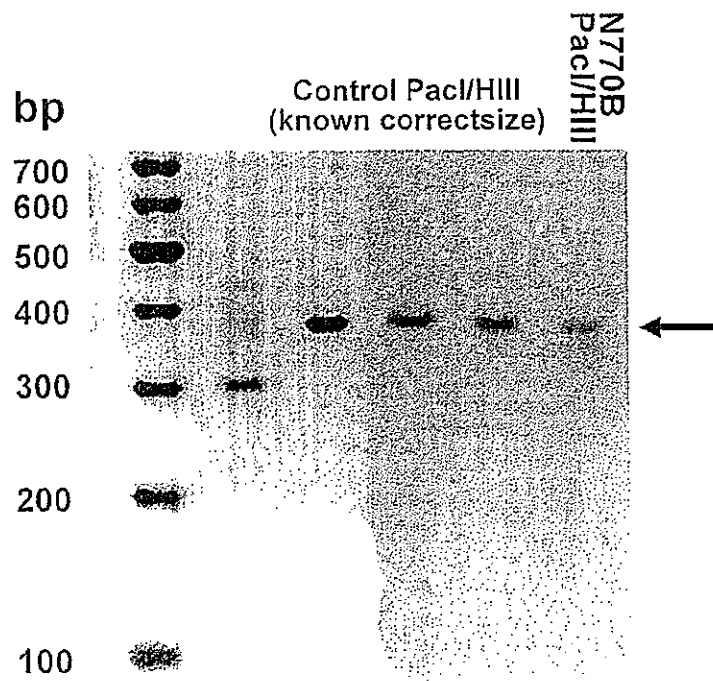


Fig. 18



N783
PacI/HIII
100bp
ladder

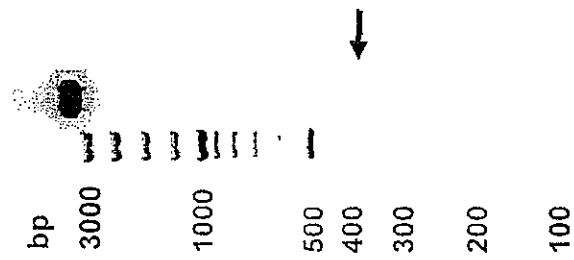


Fig. 19

TCAGTGATGTAGAAGAAAGAGGAAG	CTGCTGGATCCAGAGATG	CTTCCTCTTCTTCTACATCACTGA	GG	TTTTT	Matches	KDR	NM002253.1	3251-3274
TTCACCTCACTTCCATAATCGTCAGT	CTGCTGGATCCAGAGATG	ACTGACGATTATGGAAGTGACTGAA	AG	TTTTT	Matches	KDR	NM002253.1	1473-1449
ATTTATGTCTATGTTCAGATTACAG A	CTGCTGGATCCAGAGATG	CTGTAATCTTGAACATAGACATATAAT		TTTTT	Matches	KDR	NM002253.1	643-669
TTATGATGCCAGCAAATGGGAATT	CTGCTGGATCCAGAGATG	AATCCCAATTTCTGGCATCATAA	GG	TTTTT	Matches	KDR	NM002253.1	2766-2789
TCAGTGATGTAGAAGAAAGAGGAAG	CTGCTGGATCCAGAGATG	CTTCCTCTTCTTCTACATCACTGA	GG	TTTTT	Matches	KDR	NM002253.1	3251-3274
CCGCTCGGCCACCCAGCTTCTTGT	CTGCTGGATCCAGAGAT	ACRAGAAAGCTGGGTGCCGAGGCGG C		TTTTT	Matches	vector	L304	
TCCACGCAGAGGCCACAGGGCGACGG	CTGCTGGATCCAGAGAT	CCGTGCCCCCTGTTGGCTCTGGGTGGA GA		TTTTT	Matches	KDR	NM002253.1	350-326
TCCCTCAGTGATGTAGAAGAGAGGAAG	CTGCTGGATCCAGAGAT	CTTCCTCTTCTTCTACATCACTGAGGGA		TTTTT	Matches	KDR	NM002253.1	3247-3274

Fig. 20

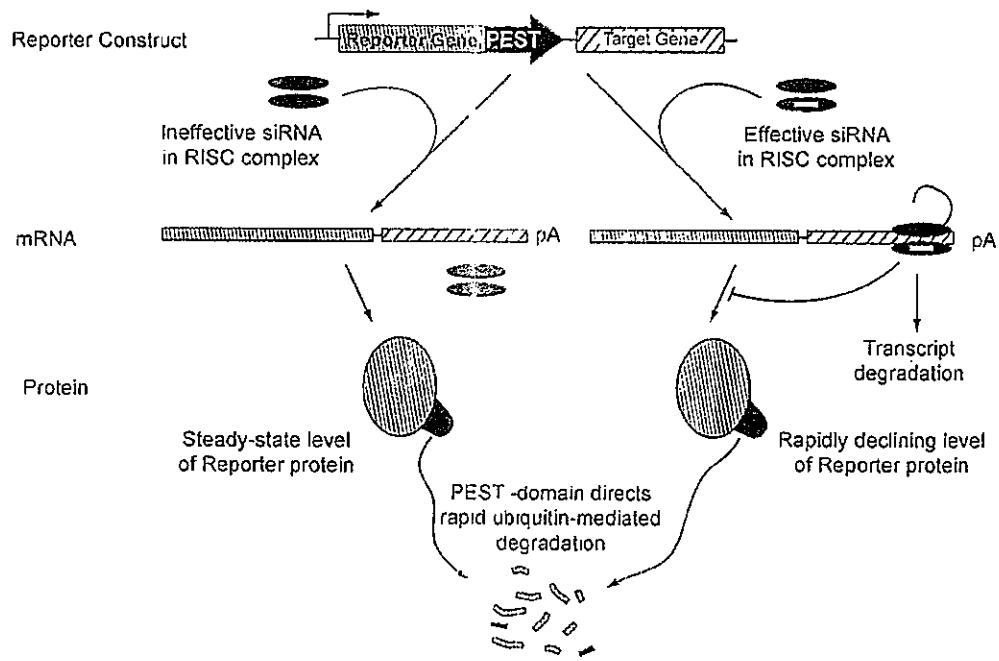


Fig. 21

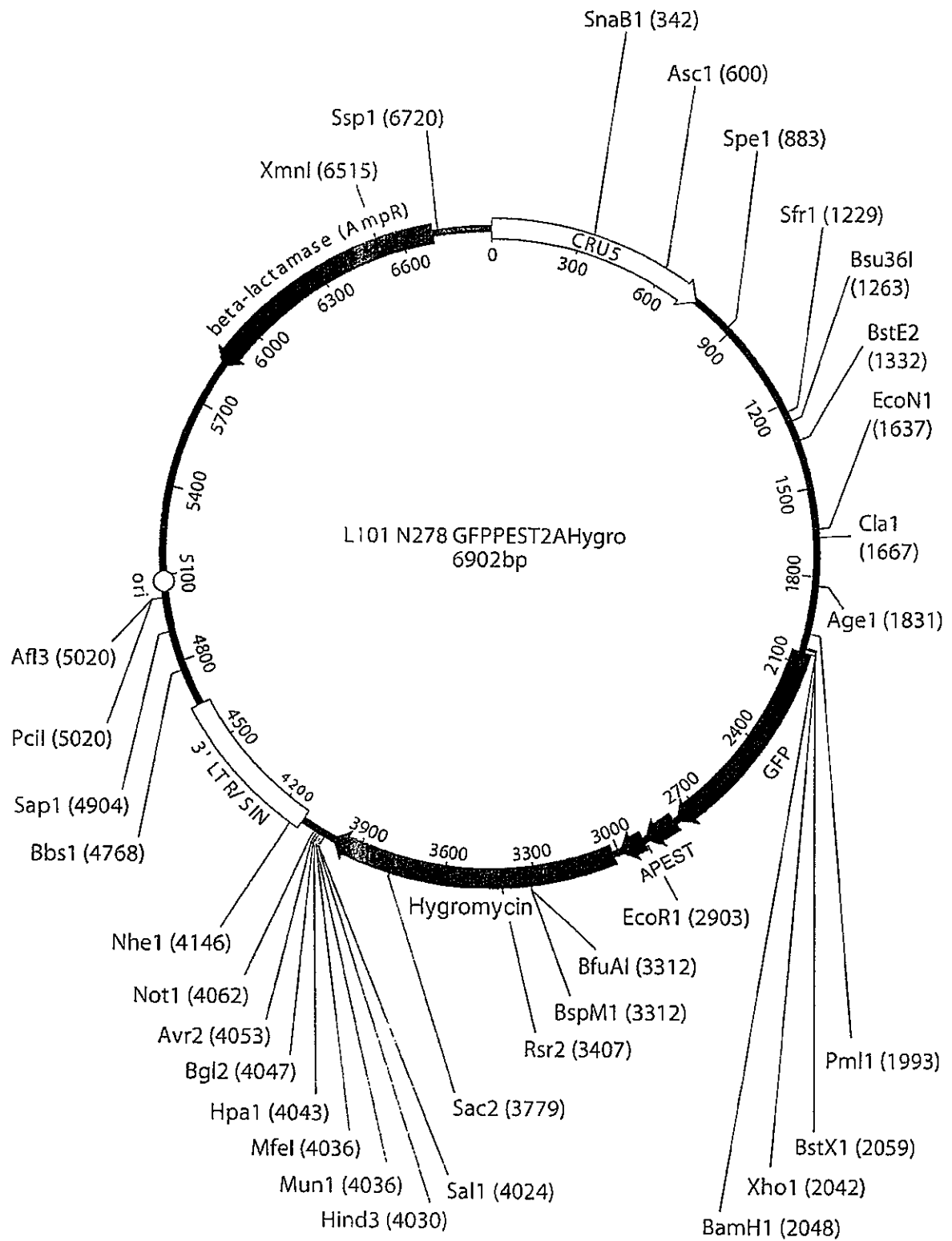
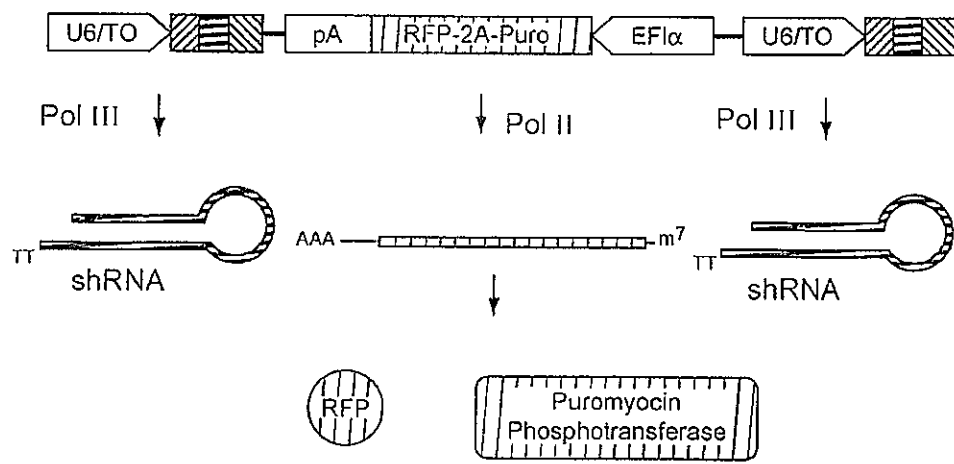
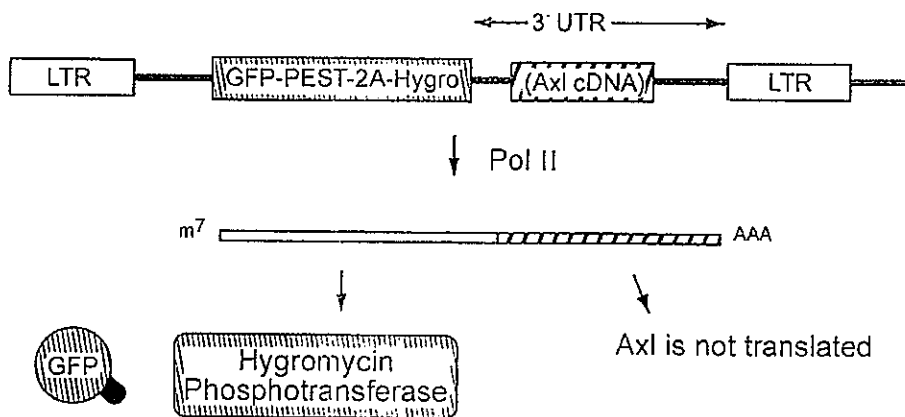


Fig. 22



shRNA targeting Axl or Luciferase (control) in an RRI vector

Fig. 23A



GFP-PEST reporter with or without Axl cDNA

Fig. 23B

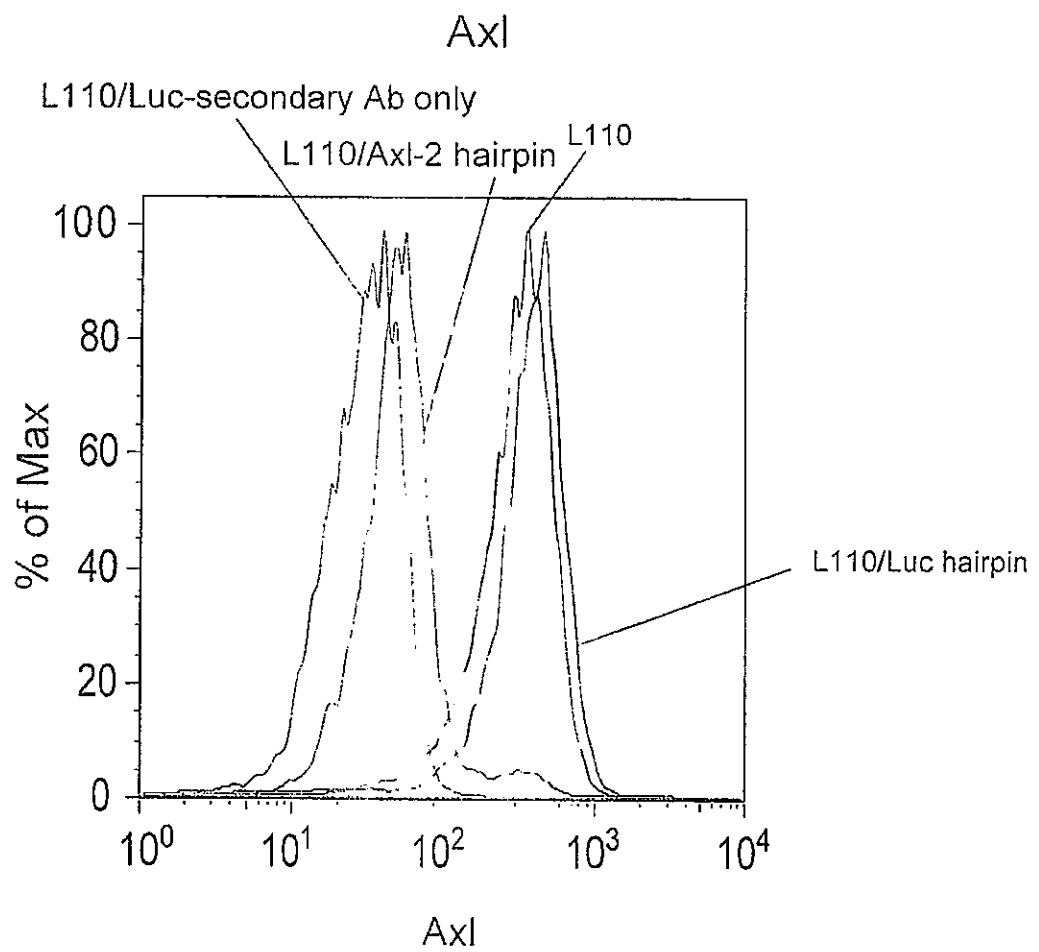


Fig. 24

Fig. 25A

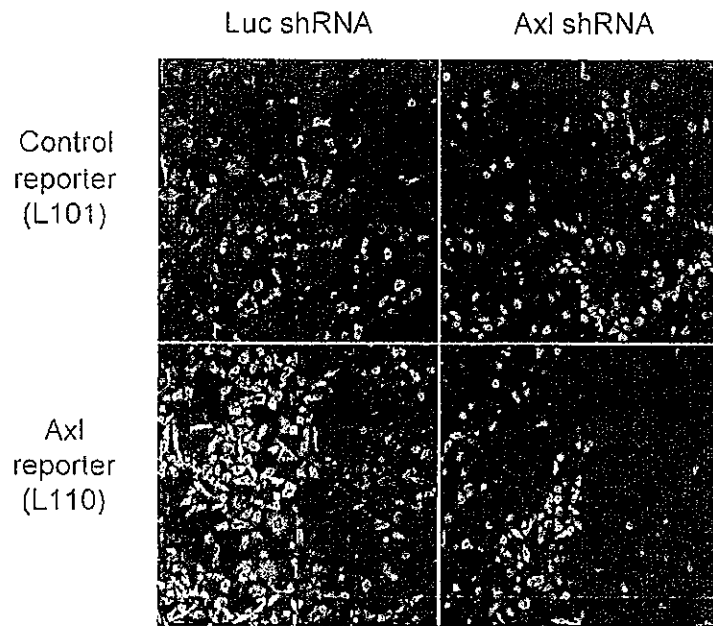
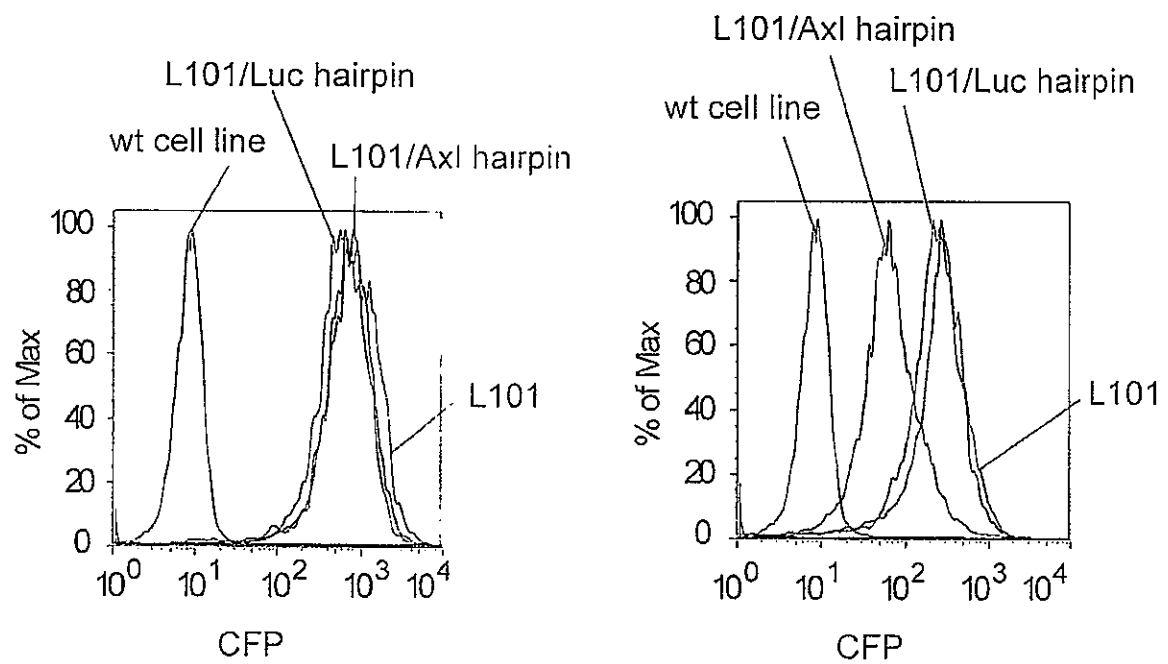


Fig. 25B

Fig. 26A

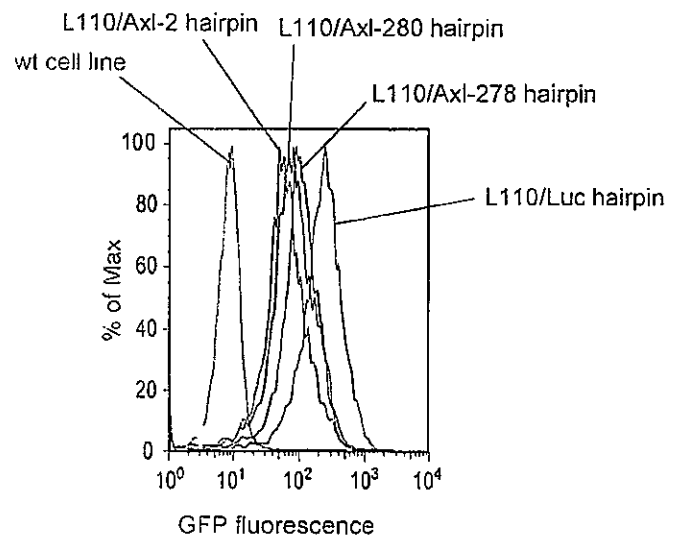


Fig. 26B

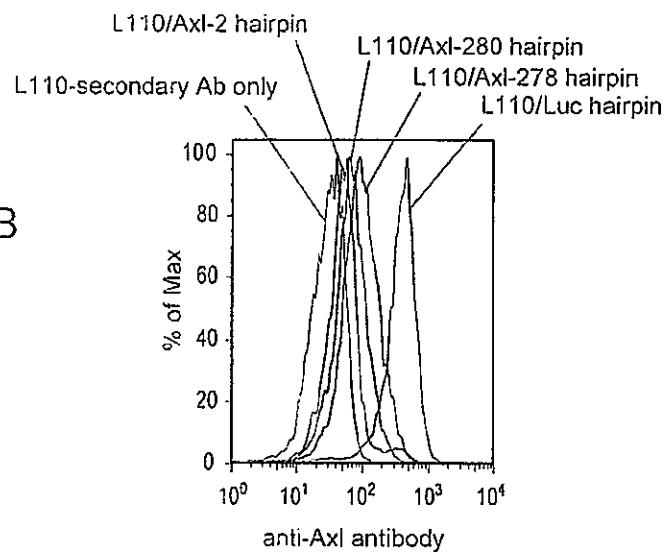
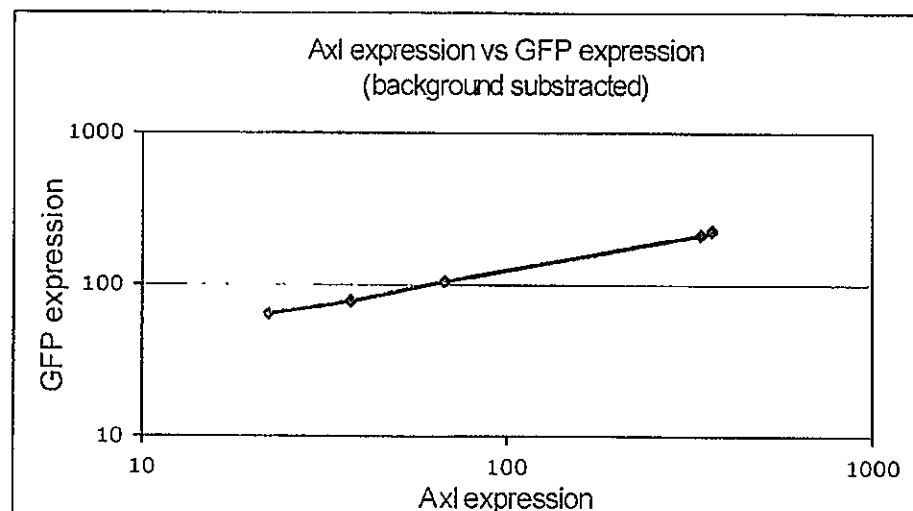


Fig. 26C



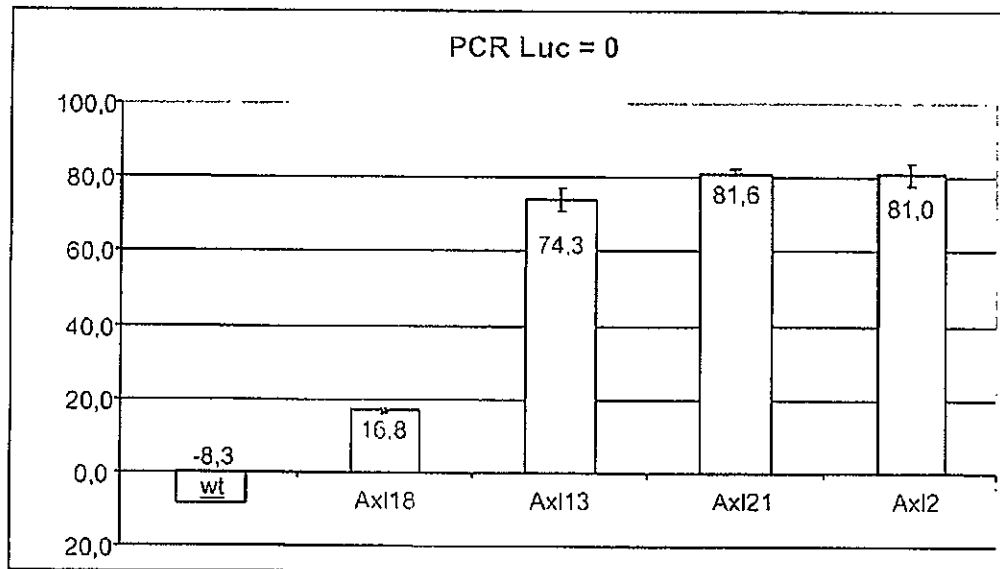


Fig. 27A

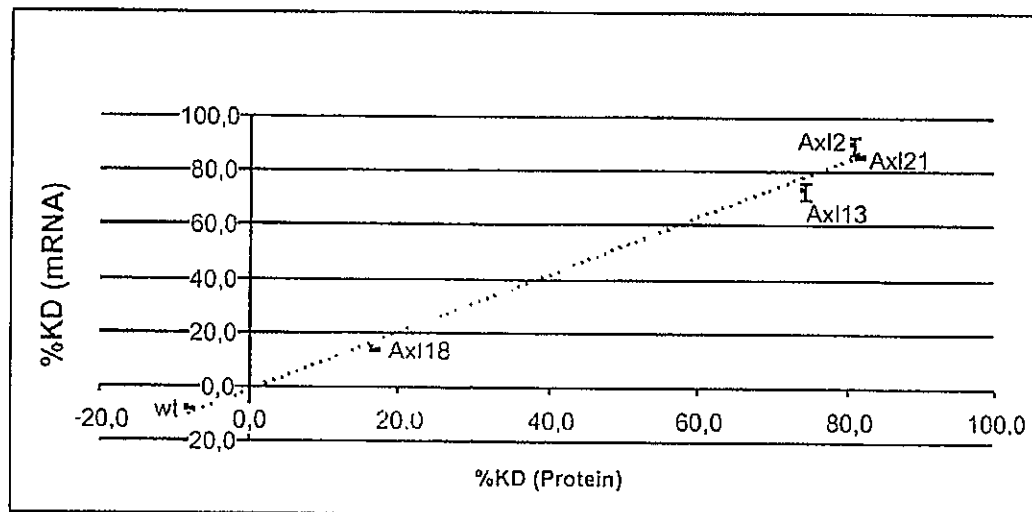


Fig. 27B

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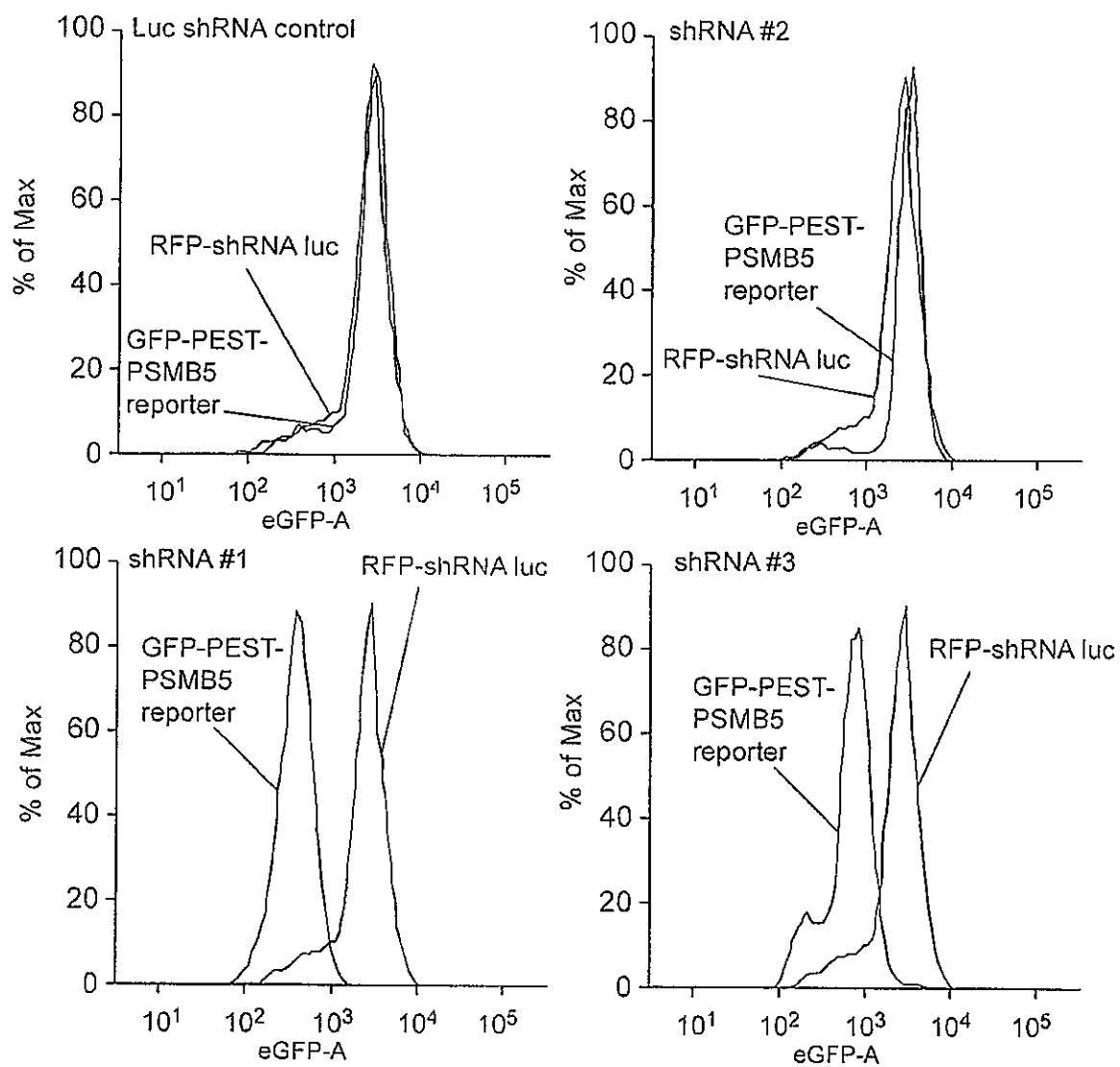
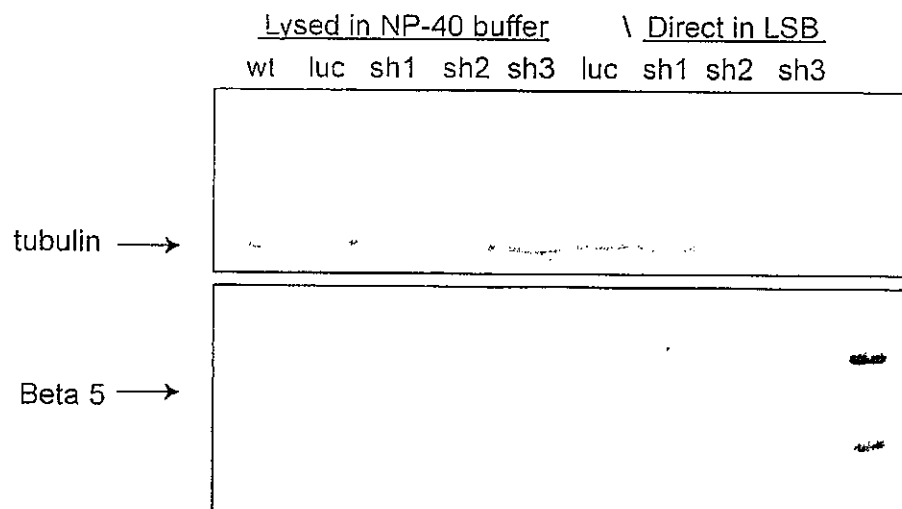


Fig. 28A

Fig. 28B



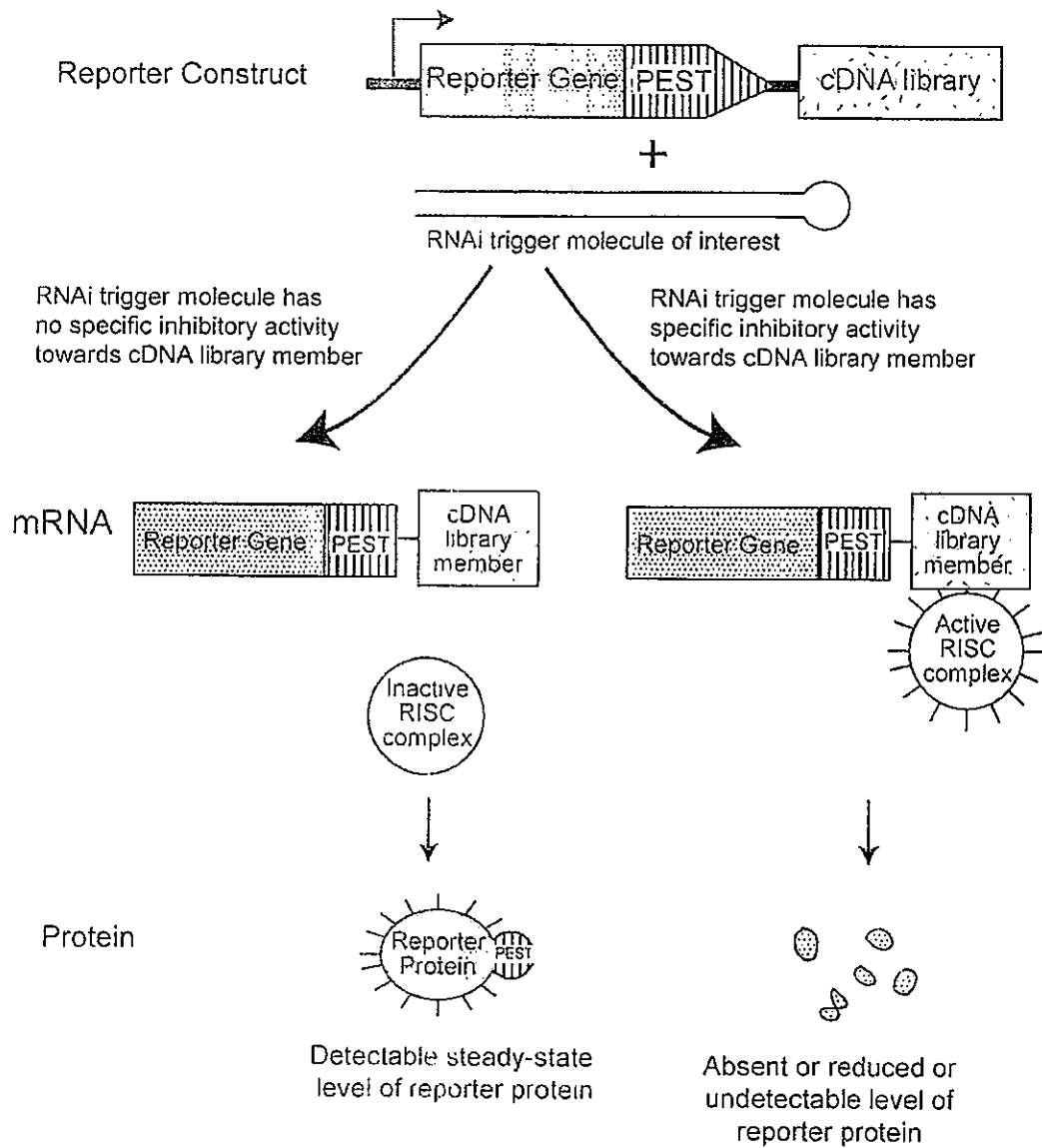


Fig. 29

Fig. 30

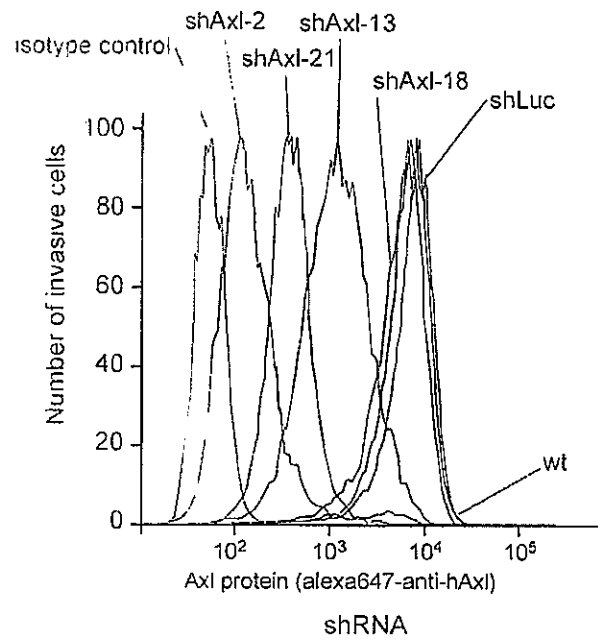


Fig. 31

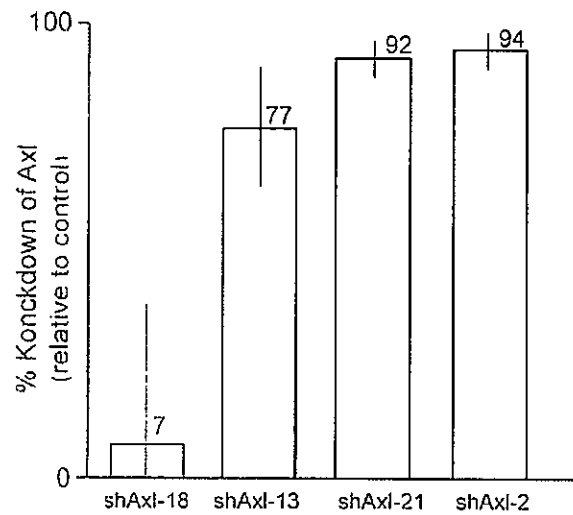
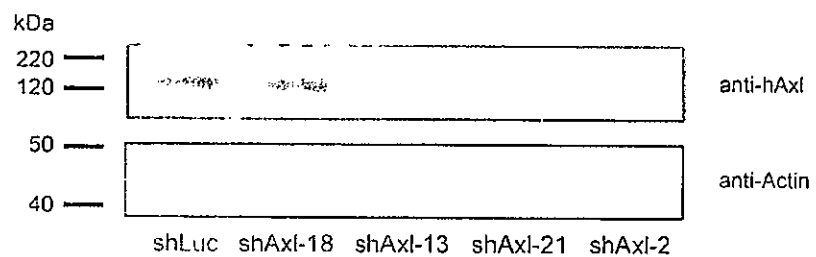
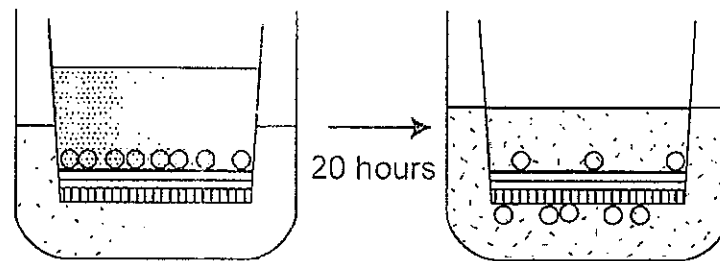


Fig. 32



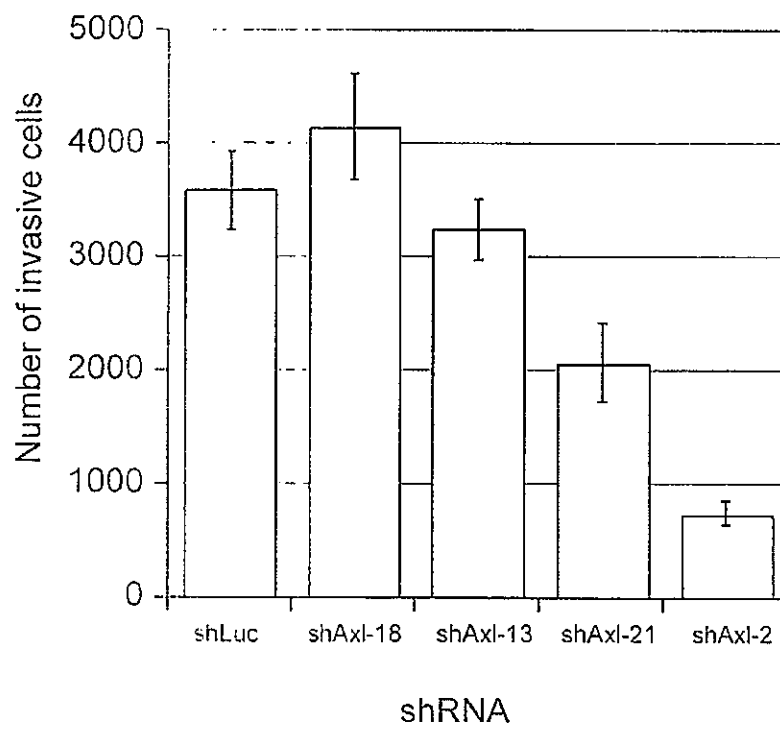
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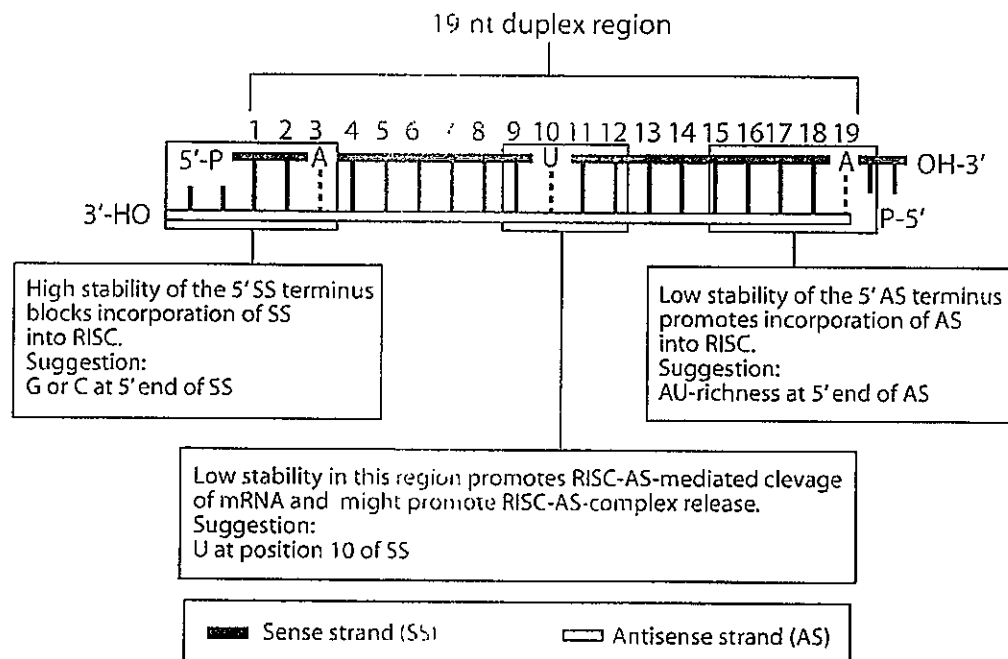
Fig. 33



-  Medium with FBS
-  Growth Factor Reduced Matrigel™ Matrix
-  Membrane with 8 mm pores
-  Serum-free medium
-  Cells

Fig. 34





Sequence of RNAi trigger molecule identified by the invention

Algorithm prediction

Actual efficiency

CGCTGTGCGGCTGGGCGTGCA

Score: 1/20

GGCGAAAGAAGGAGACCCGTT

Score: 0/20

>95%
knockdown

Fig. 35

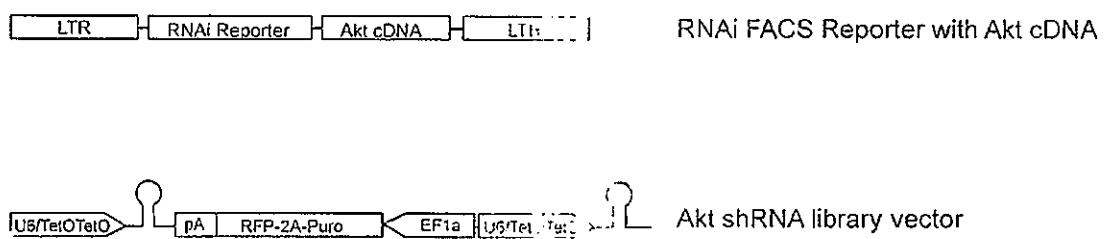


Fig. 36

Proteasomal subunit beta 5		
shRNA Hairpin Sequence	Corresponding mRNA target sequence	Relative Activity
gacctctatgtgttaccagtcattaatgaCTGGTCtcattaatg actggtaacacatagaggtc (SEQ ID NO: 1) Orientation: sense-LOOP-antisense	1: ATATGTGTTACCAGTCATTAATGA (SEQ ID NO: 16)	Strong
gaatctatgagcttcgaaataaggaacgcCTGGTCgcgttcctt atttcgaagtcatagattc (SEQ ID NO: 2) Orientation: sense-LOOP-antisense	2: ATGAGCTTCGAAATAAGGAACGC (SEQ ID NO: 17)	Strong
gatctacatgagaagtatagtggctctacCTGGTCgtagagcca ctatacttctcatgtagatc (SEQ ID NO: 3) Orientation: sense-LOOP-antisense	3: ATGAGAAGTATAGTGGCTCTAC (SEQ ID NO: 18)	Intermediate

Fig. 37

Axl Receptor Tyrosine Kinase			
Name	shRNA Hairpin Sequence	Corresponding mRNA target sequence	Relative Activity
shAxl21	atgcacgcccagccgcacagcgTTGGATCCCTGGTGGGATCCAA cgctgtgcggctgggctgaat (SEQ ID NO: 4) Orientation: antisense-LOOP-sense	CGCTGTGCGGCTGGGCGTGCAT (SEQ ID NO: 19)	Strong
shAxl13	acgggtctccttctttccgctTTGGATCCCTGGTGGGATCCAA ggcgaagaaggagaccgt (SEQ ID NO: 5) Orientation: antisense-LOOP-sense	CGGCGAAAGAAGGAGACCCGT (SEQ ID NO: 20)	Intermediate
shAxl18	gcttcaggcgatttcccgccgTTGGATCCCTGGTGGGATCCAA cgccggggaaatcgctgaagc (SEQ ID NO: 6) Orientation: antisense-LOOP-sense	CGCGGGGGAATCGCCTGAAGC (SEQ ID NO: 21)	Weak

Fig. 37 cont'd

Akt serine/threonine-specific protein kinase	
shRNA Hairpin Sequence	Corresponding mRNA target sequence
atcctcatggaggagatccgcttcccgcTGCTGGATCCAGAGATccctggatgc ggatctcctccatgaggat (SEQ ID NO: 7) Orientation: sense-LOOP-antisense	ATCCTCATGGAGGAGATCCGCTTCCCCG (SEQ ID NO: 22)
cccagccccaccagtcactgcacggcTGCTGGATCCAGAGATccctggatgc eggactggtgggggctggg (SEQ ID NO: 8) Orientation: antisense-LOOP-sense	CCCAGCCCCACCAGTGGTGGGGGCTGGG (SEQ ID NO: 23)
gtcacgcggtgcttgggcttggcTGCTGGATCCAGAGATgcctgacccaaagca ccgcgtgac (SEQ ID NO: 9) Orientation: antisense-LOOP-sense	GCCAAGCCCAAGCACCGCGTGAC (SEQ ID NO: 24)
cttcattggtgtagtagcggcTGCTGGATCCAGAGATccgctactatggcatga ag (SEQ ID NO: 10) Orientation: antisense-LOOP-sense	CCGCTACTACGCCATGAAG (SEQ ID NO: 25)
gtggcccacacactcaccgagaaccgcTGCTGGATCCAGAGATccctggatgc gagtggtggggccac (SEQ ID NO: 11) Orientation: sense-LOOP-antisense	GTGGCCCACACACTCACCAGAAACCGC (SEQ ID NO: 26)
ccttttgcgccacactgagtagcggcTGCTGGATCCAGAGATccctggatgc tcagggtggtggcgaagg (SEQ ID NO: 12) Orientation: sense-LOOP-antisense	CCTTTTGCGGCACACCTGAGTACCTGGC (SEQ ID NO: 27)

Fig 37 cont'd

Akt serine/threonine-specific protein kinase	
shRNA Hairpin Sequence	Corresponding mRNA target sequence
ccatgaacgagttttgagtacctgaagcTGCTGGATCCAGAG (SEQ ID NO: 13) Orientation: sense-LOOP-antisense	CCATGAACGAGTTTGAGTACCTGAAGC (SEQ ID NO: 28)
aagatcctcaagaaggaagtcacgtggtgctTGCTGGATCCAGAG (SEQ ID NO: 14) Orientation: sense-LOOP-antisense	AAGATCCTCAAGAAGGAAGTCATCGTGGC (SEQ ID NO: 29)
cctgcactcggagaagaacgtggtgtCTGCTGGATCCAGAGAT (SEQ ID NO: 15) Orientation: sense-LOOP-antisense	CCTGCACTCGGAGAAGAACGTGGTGT (SEQ ID NO: 30)

Fig 37 cont'd

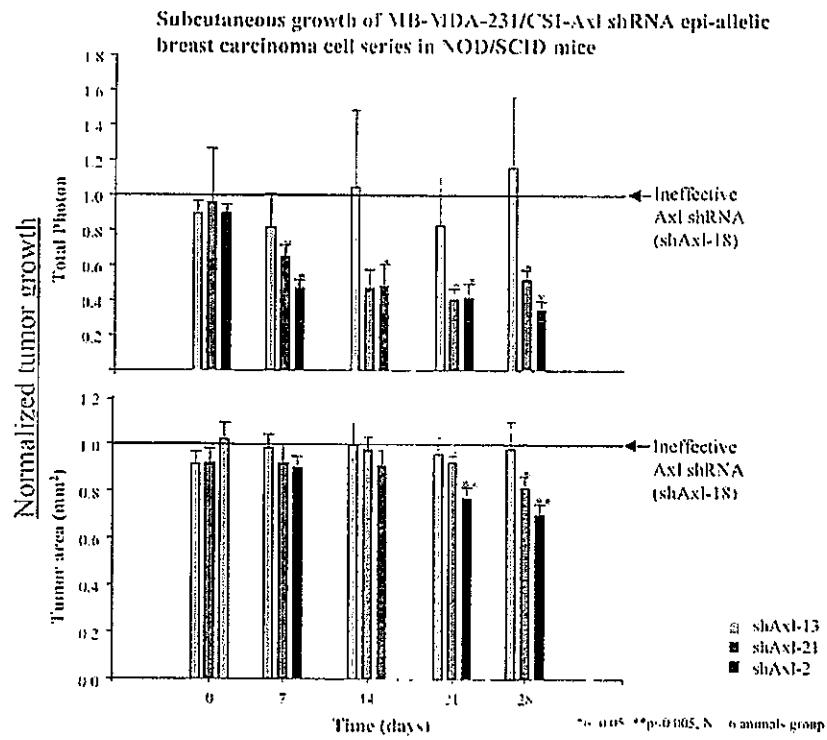


Fig 38