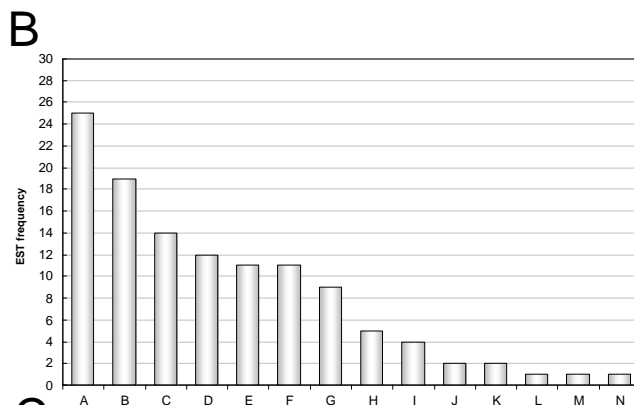
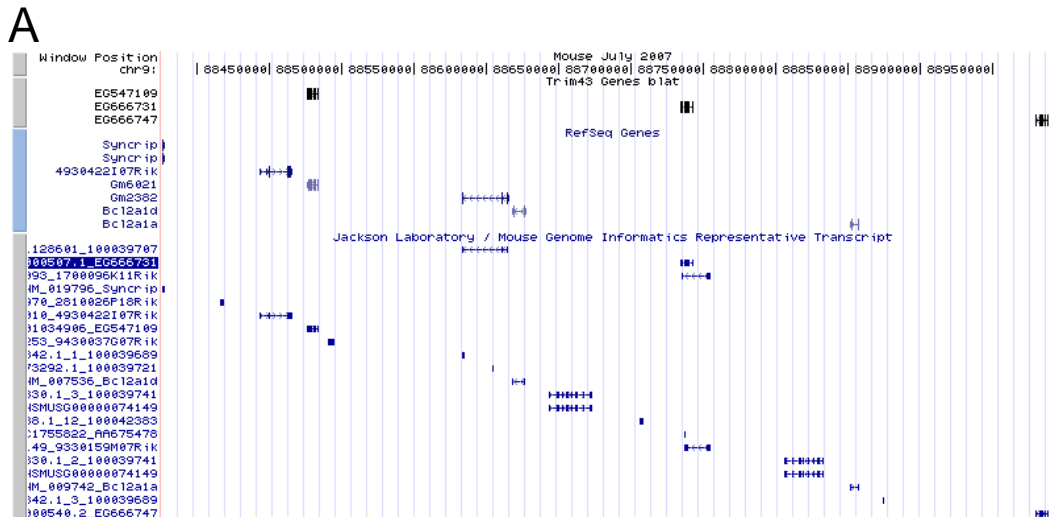


Supplemental Figures

Trim43a, Trim43b, and Trim43c: novel mouse genes expressed specifically in mouse preimplantation embryos

Ilaria Stanghellini, Geppino Falco, Sung-Lim Lee, Manuela Monti and Minoru S. H. Ko



A: NIA Mouse eight-cell-Embryo cDNA library (Long)
 B: RIKEN full-length enriched, 8 cells embryo
 C: RIKEN full-length enriched, blastocyst
 D: NIA Mouse Blastocyst cDNA Library (Long)
 E: eight-cell stage embryo cDNA
 F: NIA Mouse four-cell-Embryo cDNA library (Long)
 G: embryo, Knowles Solter 2 cell
 H: NIA Mouse 15K cDNA Clone Set
 I: RIKEN full-length enriched, 2 cell egg
 J: sixteen-cell-embryo cDNA
 K: NIA Mouse Unique gene Set Version 2
 L: blastocyst cDNA
 M: RIKEN full-length enriched, adult male testis
 N: RIKEN full-enriched, pooled tissues, 1 cell embryo..

C

Expression Report for TC1513598

Total ESTs found in TC1513598: 117

Cat#	Library	# of ESTs	% of library total
#FAS	NIA Mouse eight-cell-Embryo cDNA library (Long)	25	<> 0.26
#9TL	RIKEN full-length enriched, 8 cells embryo	19	<> 0.18
#BUL	RIKEN full-length enriched, blastocyst	14	<> 0.13
#9PA	NIA Mouse Blastocyst cDNA Library (Long)	12	<> 0.05
MB36	eight-cell stage embryo cDNA	11	<> 0.32
#FAT	NIA Mouse four-cell-Embryo cDNA library (Long)	11	<> 0.11
MA49	embryo, Knowles Solter 2 cell	9	<> 0.06
#8DE	NIA Mouse 15K cDNA Clone Set	5	<> 0.02
5391	RIKEN full-length enriched, 2 cells egg	4	<> 0.03
MB52	sixteen-cell-embryo cDNA	2	<> 0.06
#EN3	NIA Mouse Unique Gene Set Version 2	2	<> 0.04
MB08	blastocyst cDNA	1	<> 0.02
2511	RIKEN full-length enriched, adult male testis	1	<> 0.01
#BU9	RIKEN full-length enriched, pooled tissues, 1 cell embryo, etc.	1	<> 0.06

Supplemental Figure S1. A. Genome coordinates of EG547109 (Trim43a), EG666747 (Trim43b), EG666731 (Trim43c) in the UCSC Mouse Genome Browser (July 2007). **B.** Frequency of expressed sequence tags (ESTs) in the public EST database (NCBI/NIH). **C.** Frequency of expressed sequence tags (ESTs) in the public EST database (TIGR Gene Index).

```

Trim43a -----
Trim43b AGATTGGCCAAGGTGTTAGTTCATCTCTCATCCATTAGAAATAATCCCACTTAAGGGAG 60
Trim43c AGATTGGCCAAGGTGTTAGTTCATCTCTCATCCATTAGAAATAATCCCACTTAAGAGAG 60

Trim43a -----
Trim43b CTCATCTATTGGAAGCATTAGTAAAAGGGAGGAAGTGGGTCTTTAGAGACTCTAA 120
Trim43c CTCATCTATTGGAAGCATTAGTAAAAGGGAGGAATGGGTCTTTAGAGACTCTAA 120

Trim43a -----CATTCTTCTCCAGACCAGAGGTAGAGTGTTCCTAA 35
Trim43b GTACATCCCTGGGGCCACCAGGTTCACTTCTCTCCAGACCAGAGGTAGAGTGTTCCTAA 180
Trim43c GTACATCCCTGGGGCCACCAGGTTCACTTCTCTCCAGACCAGAGGTAGAGTGTTCCTAA 180
***** ** *****

Trim43a CCTTTTGCTCCAGACACTGCTAGATCTATCACCTCACTCTCTGAGGATCTGATCTCAGAG 95
Trim43b CCTTTTGCTCCAGACACTGCTAGATCTATCACCTCACTCTCTGAGGATCTGATCTCAGAG 240
Trim43c CCTTTTGCTCCAGACACTGCTAGATCTATCACCTCACTCTCTGAGGATCTGATCTCAGAG 240
*****

Trim43a CTGAGCGAGTATCGCATTGTCTACCAACATTGTCTAAGCAGGGACGGGATAATTGCTTGG 155
Trim43b CTGAGCTAGTATCGCATTGTCTACCAAGCATTGTCTAAGCAGGGACCGGAATAATTGCTTGG 300
Trim43c CTGAGCTAGTATCGCATTGTCTACCAAGCATTGTCTAAGCAGGGACGGGATAATTGCTTGG 300
***** * *****

Trim43a AAAAAATGGAATCAGACAATTTACAAGACCCTCAGGAGGAAACACTCACCTGCTCCATCTG 215
Trim43b AAAAAATGGAATCAGACAATTTACAAGACCCTCAGGAGGAAACACTCACCTGCTCCATCTG 360
Trim43c AAAAAATGGAATCAGACAATTTACAAGACCCTCAGGAGGAAACACTCACCTGCTCCATCTG 360
*****

Trim43a CCAGAGTATCTTTATGAATCCAGTTTATTTAAGGTGTGGCCATAAGTTCTGCGAGGCATG 275
Trim43b CCAGGGTATCTTTATGAATCCAGTTTATTTAAGGTGTGGCCATAAGTTCTGCGAGGCATG 420
Trim43c CCAGGGTATCTTTATGAATCCAGTTTATTTAAGGTGTGGCCATAAGTTCTGCGAGGCATG 420
**** *****

Trim43a TCTCTTACTTTCTCAAGAAGACATCAAATTCCTGCCTACTGCCCACTGTGTATGCAACC 335
Trim43b TCTCTTACTTTCTCAAGAAGACATCAAATTCCTGCCTACTGCCCACTGTGTATGCAACC 480
Trim43c TCTCTTACTTTCTCAAGAAGACATCAAATTCCTGCCTACTGCCCACTGTGTATGCAACC 480
***** *****

Trim43a ATTTAACCAGGAATATATAAATGACATTTCTCTGAAGAAGCAGGTGTCCATTGTCAGAAA 395
Trim43b ATTTAACCAGGAATATATAAATGACATTTCTCTGAAGAAGCAGGTGTCCATTGTCAGAAA 540
Trim43c ATGCAACCAGAGATATATAAATGACATTTCTCTGAAGAAGCAGGTGTCCATTGTCAGAAA 540
** *****

Trim43a GAAAGGCTCATGAATATTTGAATTTAGGAGCACAAGTGTGTGACCCACAAGGCAAA 455
Trim43b GAAAGGCTCATGAATATTTGAATTTAGGAGCACAAGTGTGTGACCCACAAGGCAAA 600
Trim43c GAAAGGCTCATGAATATTTGAATTTAGGAGCACAAGTGTGTGACCCACAAGGCAAA 600
***** *****

Trim43a AAAGATGATCTTCTGTGATAAGAGCAAGATCCCTCTGTACCTGTGTTCTGACTCCCA 515
Trim43b AAAGATGATCTTCTGTGATAAGAGCAAGATCCCTCTGTACCTGTGTTCTGACTCCCA 660
Trim43c AAAGATGATCTTCTGTGATAAGAGCAAGATCCCTCTGTACCTGTGTTCTGACTCCCA 660
*****

Trim43a GGAGCACAGTGGTCACACACTGTTCCATTGATGTAGCTGTTTCAGGAGAAAATGGAGGA 575
Trim43b GGAGCACAGCGGTCACACACTGTTCCATTGATGTAGCTGTTTCAGGAGAAAATGGAGGA 720
Trim43c GGAGCACAGCGGTCACACACTGTTCCATTGATGTAGCTGTTTCAGGAGAAAATGGAGGA 720
*****

Trim43a ACTTCTAAAGCACATGGACTCATTATGGCGGAGGCTCAAATCCAGCAGAATTATGTAGA 635
Trim43b ACTTCTAAAGCACATGGACTCATTATGGCGGAGGCTCAAATCCAGCAGAATTATGTAGA 780
Trim43c ACTTCTAAAGCACATGGACTCATTATGGCGAGGCTCAAATCCAGCAGAATTATGTAGA 780
*****

Trim43a AATAGAGAGGAGAACGACCTTGTGGTGGTTG---TCCGTGAAGCTACGGGAGGAAGTGAT 692
Trim43b AAAGAGAGGAGAACGACCTTGTGGTGGTTGAAGTCCATGAAGCTACGGGAGGAAGTGAT 840
Trim43c AAAGAGAGGAGAAATGACCTTGTGGTGGTTGAAGTCCGTGAAGCTACGGGAGGAAGTGAT 840
** *****

Trim43a CAAGAGAGTGTATGAAAAACAATGTCCACCCCTCTGTGAAGAAAGGGATCAACACATAGA 752
Trim43b CAAGAGAGTGTATGAAAAACAATGTCCACCCCTCTGTGAAGAAAGGGATCAACACATAGA 900
Trim43c CAAGAGAGTGTATGAAAAACAATGTCCACCCCTCTGTGAAGAAAGGGATCAACACATAGA 900
*****

Trim43a GTGTTTGAGACATCAAAGCAACTACTTTAGAGGAGCTCAGGAAAAGTGAAGCTACGAT 812
Trim43b GTGTTTGAGACATCAAAGCAACTACTTTAGAGGAGCTCAGGAAAAGTGAAGCTACGAT 960
Trim43c GTGTTTGAGACATCAAAGCAACTACTTTAGAGGAGCTCAGGAAAAGTGAAGCTACGAT 960
*****

Trim43a AGTCCACGAGAGAAATCAACTAATAGAGGTTTATCGGGAGCTGATGACAATGTCCAGAG 872
Trim43b AGTCCACGAGAGAAATCAACTAATAGAGGTTTATCGGGAGCTGATGACAATGTCCAGAG 1020
Trim43c AGTCCACGAGAGAAATCAACTAATAGAGGTTTATCGGGAGCTGATGACAATGTCCAGAG 1020
*****

Trim43a GCCATACCAGGAGCTGCTGGTGCAGGACTTGGATGACTTGTTCAGAAGGAGTAAGCTAGC 932
Trim43b GCCATACCAGGAGCTGCTGGTGCAGGACTTGGATGACTTGTTCAGAAGGAGTAAGCTAGC 1080
Trim43c GCCATACCAGGAGCTGCTGGTGCAGGACTTGGATGACTTGTTCAGAAGGAGTAAGCTAGC 1080
*****

Trim43a GGCAAAGCTGGACATGCCACAGGGTATGATACCAAGACTCCATGCCCATTCCTCTCGG 992
Trim43b GGCAAAGCTGGACATGCCACAGGGTATGATACCAAGACTCCATGCCCATTCCTCTCGG 1140
Trim43c GGCAAAGCTGGACATGCCACAGGGTATGATACCAAGACTCCATGCCCATTCCTCTCGG 1140
*****

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Supplemental
Figure S2.

CLUSTAL 2.0.10 multiple sequence alignment of Trim43 genes

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Trim43a    MESDNLQDPQEETLTCSICQSIFMNPVYLRCGHKFCEACLLLSQEDIKFPAYCPMCMQPF 60
Trim43b    MESDNLQDPQEETLTCSICQGIFMNPVYLKCGHKFCEACLLLFQEDIKFPAYCPMCMQPF 60
Trim43c    MESDNLQDPQEETLTCSICQGIFMDPVYLRCGHKFCETCLLLFQEDIKFPAYCPTCRQPC 60
*****.***:***:***:***:*** ***** * **

Trim43a    NQEYINDISLKKQVSIVRKKRLMKYLNSKEHKCVTHKAKMIFCDKSKILLCHLCSDSQE 120
Trim43b    NQEYINDISLKKQVSIVRKKRLMEYLNSEEHKCVTHKAKMIFCDKSKILLCHLCSDSQE 120
Trim43c    NQRYINDISLKKQVFIVRKKRLMEYLNSEEHKCVTHKAKMIFCDKSKILLCHLCSDSQE 120
**.*:***** *****:***:*****

Trim43a    HSGHTHCSIDVAVQEKMEELLKHMDSLRRLKIQQNYVEIERRTTLWWLS-VKLREEVIK 179
Trim43b    HSGHTHCSIDVAVQEKMEELLKHMDSLRRLKIQQNYVEKERRTTLWWLKSMKLREEVIK 180
Trim43c    HSGHTHCSIDVAVQEKMEELLKHMDSLQRLKIQQNYVEKERRMTLWWLKSVKLREEVIK 180
*****:***** ** *****. :*****

Trim43a    RVYGKQCPPLCEERDQHIECLRHQSNTTLEELRKSEATIVHERNQLIEVYRELMTSQRP 239
Trim43b    RVYGKQCPPLSEERDQHIECLRHQSNTTLEELRKSEATIVHERNQLTEVYRELMTSQRP 240
Trim43c    RVYGKQCPPLSEERDQHIECLRHQSNTTLEELRKSEATIVHERNQLTEVYQELMTSQRP 240
*****.*:***** *****:*****

Trim43a    YQELLVQDLDDLFRRSKLAAKLDMPQGMIPRLHAHSIPGLTARLNSFRVKISFKHSIMFG 299
Trim43b    YQELLVQDLDDLFRRSKLAAKLDMPQGMIPRLRAHSIPGLTARLNSFRVKISFKHSIMFG 300
Trim43c    YQELLVQDLDDLFRRSKLAAKVDMPQGMIPRLRAHSIPGLTARLNSFRVKISFKHSIMFG 300
*****:*****:*****

Trim43a    YTSVRPFDIRLLHESTSLDSAETHRVSWGKKSFSRGKYYWEVDLKDHEQWTVGVRKDPWL 359
Trim43b    YTSLRPFDIRLLHESTSLDSAETHRVSWGKKSFSRGKYYWEVDLKDYRRWTVGVCKDPWL 360
Trim43c    YNSVRPFDIRLLHESTSLDSAKTHRVSWGKKSFSRGKYYWEVDLKDYRRWTVGVCKDPWL 360
**.*:*****:*****:*****:*****

Trim43a    RGRSYAATPTDLFLECLRKDHYILITRIGEHYIEKPVGQVGVFLDCEGGYVSFVDVA 419
Trim43b    RGRSYVATPTDLF-LECLRKDHYILITRIGEHYIEKPVGQVGVFLDCEGGYVSFVDVA 419
Trim43c    RGRSYVVTPTDIFLECLRNKDHYILITRIGREHYIEKPVGQVGVFLDCEGGYVSFVDVA 420
*****.*:*****.* *****:*****

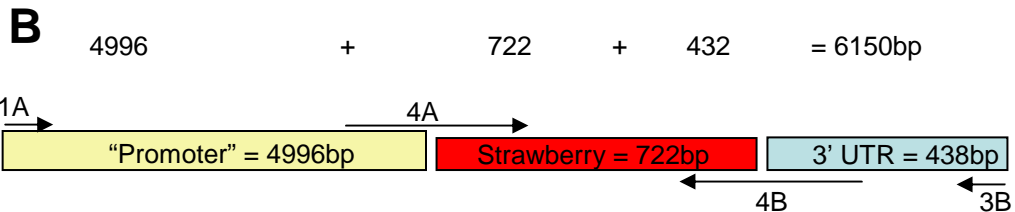
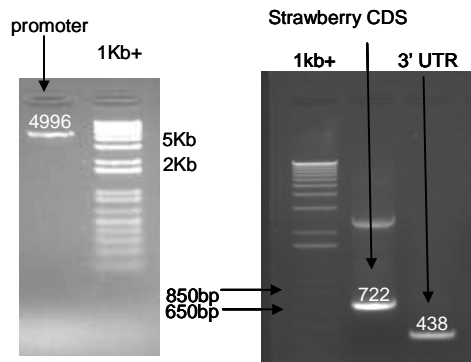
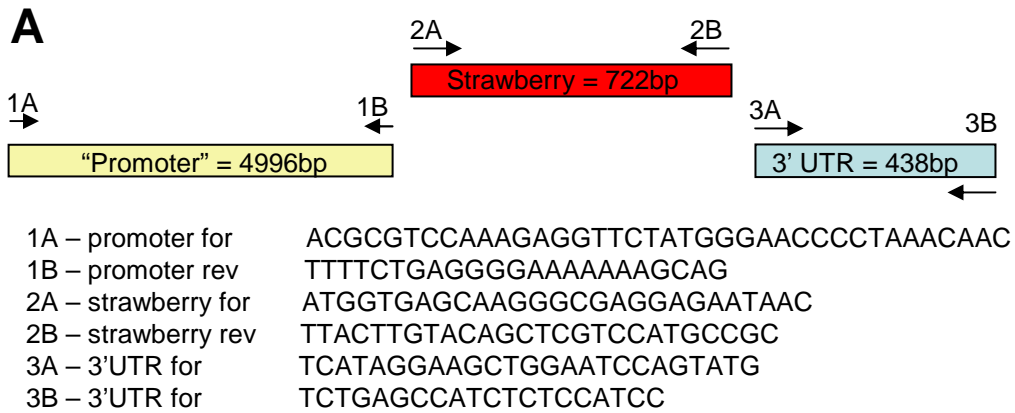
Trim43a    KSSLILSYSPGTFHCAVRPFFSAVYT 445
Trim43b    KSSLILSYSPGTFHCAVRPFFSAVYT 445
Trim43c    KSSLILSYSPGTFHCAVRPFFFAAYT 446
*****.*:***** **

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Supplemental Figure S3. Multiple alignment of Trim43a, Trim43b, and Trim43c.

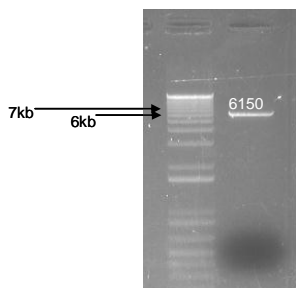
Primer	Oligo Sequence
<i>Southern blot</i>	
SB probe	fwr TTGAAGGGCACCTCTGGTCATTAC
SB probe	rev TACCCTGTCTCCTGATCATGGG
<i>CDS amplify</i>	
CDS	fwr ATGGAATCAGACAATTTACAAGACCC
CDS	rev TTATGTGTAGACAGCAGAGAAGAAAGGCC
<i>qPCR primers</i>	
qPCR	fwr CCATGCCATTCCATTCTGG
qPCR	rev TCTGACTGAGGTGTAGCCGAACATG
qPCR nested	fwr ATTCCATTCTGGGCTGACT
qPCR nested	rev CTGACTGAGGTGTAGCCGAAC
Pou5f1	fwr CGGTGTGAGGTGGAGTCTGGAGAC
Pou5f1	rev CGCCGGTTACAGAACCATACTCG
Chuk nested	fwr AGGACCGTGTCTCAAGGAGCTGT
Chuk nested	rev CTCTGGTCTCATTTGCTTCACGA
<i>Antigen for antibody production</i>	
Ab	fwr CCGGAATTCGATGTAGCTGTTCCAGGAGAAAATG
Ab	rev CCCAAGCTTCACTCCCGATAAACCTCTATTAGTTG
<i>Promoter study</i>	
Mlul 5kb up 1st exon	fwr ACGCGTGCAAGTATAGGGATGAAAAGTGAAC
Mlul 5kb up ATG	fwr ACGCGTCCAAAGAGGTTCTATGGGAACCCCTAAACAAC
3 UTR	fwr TCATAGGAAGCTGGAATCCAATGATG
3 UTR	rev TCTGAGCCATCTCTCCATCC
strawberry	fwr ATGGTGAGCAAGGCGAGGAGAATAAC
strawberry	rev TTAAGTTGTACAGCTCGTCCATGCCGC
emerald	fwr ATGGTGAGCAAGGCGAGGAGCTGTTTAC
emerald	rev TTAAGTTGTACAGCTCGTCCATGC
<i>Assymmetric PCR</i>	
5kb upATG promoter-strawberry overlapping for	fwr CTGCTTTTTTCCCTCAGAAAA ATGGTGAGCAAGGCGAGGAGAATAAC
strawberry-3'UTR overlapping rev	rev CATACTGGATTCCAGCTTCTATGA TTAAGTTGTACAGCTCGTCCATGCCGC
5kb upATG promoter-emerald overlapping for	fwr CTGCTTTTTTCCCTCAGAAAAATGGTGAGCAAGGCGAGGAGCTGTTT
emerald-3'UTR overlapping rev	rev CATACTGGATTCCAGCTTCTATGATTACTTGTACAGCTCGTCC
<i>Transfection experiment-Flag tag</i>	
HindIII_kozac_flag	fwr CCCAAGCTTGCCACCATGGATTACAAGGATGACGACGATAAGATGGAATCAGACAATTTACAAGACCCCTCA
NotI_Trim43	rev ATAAGAATGCGGCGCTCATGTGTAGACAGCAGAGAAAGAA
HindIII_kozac_emerald	fwr CCCAAGCTTGCCACCATGGTGAGCAAGGCGAGGAGCTGTTTAC
emerald_half P2A peptide	rev GAAGGTCAAATTCAAAGTCTGTTTACCAGTCCGATGCCTTGTACAGCTCGTCCATGC
half P2A BamHI flag trim43	fwr TCAAGTTGGCGGGAGAGCTCGAGTCCAACCTGGCCCGGATCCATGGATTACAAGGATGACGACGATAAG
P2 overlapping	fwr TGAACAGACTTTGAATTTTACCTTCTCAAGTTGGCGGGAGAGCTCGAGTCCAA

Supplemental Figure S4. Primer sequences used in the current study.



4A – promoter-strawberry overlapping for
 CTGCTTTTTTCCCCTCAGAAAA ATGGTGAGCAAGGGCGAGGAGAATAAC

4B – strawberry-3'UTR overlapping rev
 CATACTGGATTCCAGCTTCCTATGA TTACTTGTACAGCTCGTCCATGCCGC



Supplemental Figure S5. A The promoter sequence and the 3'UTR of *Trim43a* were PCR amplified from RP23-46B12-BAC DNA giving a band of 4996 bp and 438 bp respectively. The CDS of strawberry fluorescent molecule was amplified from pRSET-B plasmid (722 bp). **B** The three fragments obtained were fused together by the use of two overlapping primers (4A, 4B).