AVIDD ASAP: A71EV3CPROA & D68EV3CPROA & A71EV2A Avitag insertion and C2A mutations

PAGE23-00333

Author: **Fairhead, Michael**
Date Started: **2023-Feb-14**
Experiment Started:
Projects: **Cloning;ASAP**
Related Pages:
Referenced by:
Tags:

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A71EV3CPROA-c001

```
ATGggtcctagcctggattttgcgttgtcattgctgcgccgtaatatcagacaggttcagaccgatcagggccatttcacca
tgctcggagttcgcgatcgtttggcggtgctcccgcgtcactctcagccaggtaaaacgatttgggttgaacataaattaat
aaacatattggacgccgttgagctggttgatgaacagggtgtaaacttagaactgactttagtgaccctggataccaatgaa
aagtttcgcgacattaccaaatttatccccgaaaatatctcagccgcctcagatgccacactggttatcaacactgagcaca
tgcccagcatgtttgtcccggttggagatgtagttcagtacggttttcttaacctgagcggtaagcctacacatcgtaccat
gatgtataactttccgacgaaggctggtcagtgtggggggtgtggttacgagtgttggtaaagtgatcgggatacatatagga
gggaatggtcgtcagggcttttgcgctggtctgaaacgttcgtattttgcatcagaacagcttgagcaccatcatcaccacc
atTAATGACTCGAGCACCcgtcgacaagcttgcggccgc
```

```
MGPSLDFALSLLRRNIRQVQTDQGHFTMLGVRDRLAVLPRHSQPGKTIWVEHKLINILDAVELVDEQGVNLELTLVTLDTNE
KFRDITKFIPENISAASDATLVINTEHMPSMFVPVGDVVQYGFLNLSGKPTHRTMMYNFPTKAGQCGGVVTSVGKVIGIHIG
GNGRQGFCAGLKRSYFASEQLEHHHHHH
```

Result

```
 H   H   H   H   H   H   G   L   N   D   I   F   E   A   Q   K   I   E   W   H   E   *   *
CACCATCATCACCACCATggcctgaacgatatttttgaagcgcagaaaattgaatggcatgaaTAATGACTCGAGCACCCG
GTGGTAGTAGTGGTGGTAccggacttgctataaaaacttcgcgtcttttaacttaccgtacttATTACTGAGCTCGTGGGC
```

Top of Form

Bottom of Form

Required Primers

Name (F/R)	Oligo (Uppercase = target-specific primer)	Len	% GC	Tm	Ta *
A71EV3CPROA_AVI_F	gcagaaaattgaatggcatgaaTAATGACTCGAGCACCCG	40	45	64°C	62°C
A71EV3CPROA_AVI_R	gcttcaaaaatatcgttcaggccATGGTGGTGATGATGGTG	41	46	61°C	

* Ta (recommended annealing temperature)

A71EV3CPROA-c002

```
atgggtcctagcctggattttgcgttgtcattgctgcgccgtaatatcagacaggttcagaccgatcagggccatttcacca
tgctcggagttcgcgatcgtttggcggtgctcccgcgtcactctcagccaggtaaaacgatttgggttgaacataaattaat
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aagtttcgcgacattaccaaatttatccccgaaaatatctcagccgcctcagatgccacactggttatcaacactgagcaca
tgcccagcatgtttgtcccggttggagatgtagttcagtacggttttcttaacctgagcggtaagcctacacatcgtaccat
gatgtataactttccgacgaaggctggtcagtgtggggggtgtggttacgagtgttggtaaagtgatcgggatacatatagga
gggaatggtcgtcagggcttttgcgctggtctgaaacgttcgtattttgcatcagaacagcttgagCACCATCATCACCACC
ATGGCCTGAACGATATTTTTGAAGCGCAGAAAATTGAATGGCATGAATAATGACTCGAGCACCCGtcgacaagcttgcggcc
gc
```

```
MGPSLDFALSLLRRNIRQVQTDQGHFTMLGVRDRLAVLPRHSQPGKTIWVEHKLINILDAVELVDEQGVNLELTLVTLDTNE
KFRDITKFIPENISAASDATLVINTEHMPSMFVPVGDVVQYGFLNLSGKPTHRTMMYNFPTKAGQCGGVVTSVGKVIGIHIG
GNGRQGFCAGLKRSYFASEQLEHHHHHHGLNDIFEAQKIEWHE
```

Sequencing

600154701_1B_pLICR_B11.seq

NNNNNNNNNNNNNNNNNNNNCTCNGNGGTGGTGGTGGTGGTGCTCGAGTGCGGCCGCAAGCTTGTCGACGGGTGCTCGCGTCAT
TATTCATGCCATTCAATTTTCTGCGCTTCAAAAATATCGTTCAGGCCATGGTGGTGATGATGGTGCTCAAGCTGTTCTGATG
CAAAATACGAACGTTTCAGACCAGCGCAAAAGCCCTGACGACCATTCCCTCCTATATGTATCCCGATCACTTTACCAACACT
CGTAACCACACCCCCACACTGACCAGCCTTCGTCGGAAAGTTATACATCATGGTACGATGTGTAGGCTTACCGCTCAGGTTA
AGAAAACCGTACTGAACTACATCTCCAACCGGGACAAACATGCTGGGCATGTGCTCAGTGTTGATAACCAGTGTGGCATCTG
AGGCGGCTGAGATATTTTCGGGGATAAATTTGGTAATGTCGCGAAACTTTTCATTGGTATCCAGGGTCACTAAAGTCAGTTC
TAAGTTTACACCCTGTTCATCAACCAGCTCAACGGCGTCCAATATGTTTATTAATTTATGTTCAACCCAAATCGTTTTACCT
GGCTGAGAGTGACGCGGGAGCACCGCCAAACGATCGCGAACTCCGAGCATGGTGAAATGGCCCTGATCGGTCTGAACCTGTC
TGATATTACGGCGCAGCAATGACAACGCAGAATCCAGGCTAGGACCCATGTGTATATCTCCTTCTTAAGGTTAAACAAAATT
ATTTCTAGAGGGGAATTGTTATCCGCTCACAATTCCCCTATAGTGAGTCGTATTAATTTCGCGGGATCGAGATCTCGATCCT
CTACGCCGGACGCATCGTGGCCGGCATCACCGGCGCCACAGGTGCGGTTGCTGGCGCCTATATCGCCGACATCACCGATGGG
GAAGATCGGGCTCGCCACTTCGGGCTCATGAGCGCTTGTTTCGGCGTGGGTATGGTGGCAGGCCCCGTGGCCGGGGGACTGT
TGGGCGCCATCTCCTTGCATGCACCATTCCTTGCGGCGGCGGTGCTCAACGGCCTCAACCTACTACTGGGCTGCTTCCTAAT
GCAGGAGTCGCATAAGGGAGAGCGTCGAGATCCCGGAACCCATCGAATGGCGCAAAACCTTTTCGGGGTATGGCATGATAGC
GCCCNGAANNNNAGTCAATTCAGGGTGGTGAATGTNAAACCNGTAACGTTANACGATGTCGAAAAGTANNCCGGTGTCN

MGPSLDSALSLLRRNIRQVQTDQGHFTMLGVRDRLAVLPRHSQPGKTIWVEHKLINILDAVELVDEQGVNLELTLVTLDTNE
KFRDITKFIPENISAASDATLVINTEHMPSMFVPVGDVVQYGFLNLSGKPTHRTMMYNFPTKAGQCGGVVTSVGKVIGIHIG
GNGRQGFCAGLKRSYFASEQLEHHHHHH<mark>GLNDIFEAQKIEWHE</mark>

	Score	Expect	Identities	Gaps	Strand
	1205 bits(652)	0.0	656/658(99%)	0/658(0%)	Plus/Minus

```
Query  1    ATGGGTCCTAGCCTGGATTTTGCGTTGTCATTGCTGCGCCGTAATATCAGACAGGTTCAG  60
Sbjct  705  ...................C........................................  646


Query  61   ACCGATCAGGGCCATTTCACCATGCTCGGAGTTCGCGATCGTTTGGCGGTGCTCCCGCGT  120
Sbjct  645  ............................................................  586


Query  121  CACTCTCAGCCAGGTAAAACGATTTGGGTTGAACATAAATTAATAAACATATTGGACGCC  180
Sbjct  585  ............................................................  526


Query  181  GTTGAGCTGGTTGATGAACAGGGTGTAAACTTAGAACTGACTTTAGTGACCCTGGATACC  240
Sbjct  525  ............................................................  466


Query  241  AATGAAAAGTTTCGCGACATTACCAAATTTATCCCCGAAAATATCTCAGCCGCCTCAGAT  300
Sbjct  465  ............................................................  406


Query  301  GCCACACTGGTTATCAACACTGAGCACATGCCCAGCATGTTTGTCCCGGTTGGAGATGTA  360
Sbjct  405  ............................................................  346


Query  361  GTTCAGTACGGTTTTTCTTAACCTGAGCGGTAAGCCTACACATCGTACCATGATGTATAAC  420
Sbjct  345  ............................................................  286
```

```
Query  421   TTTCCGACGAAGGCTGGTCAGTGTGGGGGTGTGGTTACGAGTGTTGGTAAAGTGATCGGG   480

Sbjct  285   ............................................................   226


Query  481   ATACATATAGGAGGGAATGGTCGTCAGGGCTTTTGCGCTGGTCTGAAACGTTCGTATTTT   540

Sbjct  225   ............................................................   166


Query  541   GCATCAGAACAGCTTGAGCACCATCATCACCACCATGGCCTGAACGATATTTTTGAAGCG   600

Sbjct  165   ............................................................   106


Query  601   CAGAAAATTGAATGGCATGAATAATGACTCGAGCACCCGTCGACAAGCTTGCGGCCGC    658

Sbjct  105   .............................G............................    48
```

	Score	Expect	Method	Identities	Positives	Gaps
	428 bits(1100)	3e-160	Compositional matrix adjust.	206/207(99%)	206/207(99%)	0/207(0%)

```
Query  1     MGPSLDFALSLLRRNIRQVQTDQGHFTMLGVRDRLAVLPRHSQPGKTIWVEHKLINILDA   60

Sbjct  1     ......S.....................................................   60


Query  61    VELVDEQGVNLELTLVTLDTNEKFRDITKFIPENISAASDATLVINTEHMPSMFVPVGDV   120

Sbjct  61    ............................................................   120


Query  121   VQYGFLNLSGKPTHRTMMYNFPTKAGQCGGVVTSVGKVIGIHIGGNGRQGFCAGLKRSYF   180

Sbjct  121   ............................................................   180


Query  181   ASEQLEHHHHHHGLNDIFEAQKIEWHE    207

Sbjct  181   ..........................    207
```

Genuine S mutation? Seems T from Chromatogram

Title missing - double click to edit

A71EV3CPROA-c001

ATGggtcctagcctggattttgcgttgtcattgctgcgccgtaatatcagacaggttcagaccgatcagggccatttcacca
tgctcggagttcgcgatcgtttggcggtgctcccgcgtcactctcagccaggtaaaacgatttgggttgaacataaattaat
aaacatattggacgccgttgagctggttgatgaacagggtgtaaacttagaactgactttagtgaccctggataccaatgaa
aagtttcgcgacattaccaaatttatccccgaaaatatctcagccgcctcagatgccacactggttatcaacactgagcaca
tgcccagcatgtttgtcccggttggagatgtagttcagtacggttttcttaacctgagcggtaagcctacacatcgtaccat

3
```

```
gatgtataactttccgacgaaggctggtcagtgtgggggtgtggttacgagtgttggtaaagtgatcgggatacatatagga
gggaatggtcgtcagggcttttgcgctggtctgaaacgttcgtattttgcatcagaacagcttgagcaccatcatcaccacc
atTAATGACTCGAGCACCcgtcgacaagcttgcggccgc
```


```
  M  Y  N  F  P  T  K  A  G  Q  A  G  G  V
GATGTATAACTTTCCGACGAAGGCTGGTCAGgctGGGGGTGTGG
CTACATATTGAAAGGCTGCTTCCGACCAGTCCGACCCCCACACC
```

Top of Form

Bottom of Form

Required Primers

Name (F/R)	Oligo (Uppercase = target-specific primer)	Len	% GC	Tm	Ta *
A71EV3CPROA_C2A_F	GGCTGGTCAGgctGGGGGTGTGG	23	74	62°C	61°C
A71EV3CPROA_C2A_R	TTCGTCGGAAAGTTATACATC	21	38	60°C	

* Ta (recommended annealing temperature)

A71EV3CPROA-c003

```
Atgggtcctagcctggattttgcgttgtcattgctgcgccgtaatatcagacaggttcagaccgatcagggccatttcacca
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aaacatattggacgccgttgagctggttgatgaacagggtgtaaacttagaactgactttagtgaccctggataccaatgaa
aagtttcgcgacattaccaaatttatccccgaaaatatctcagccgcctcagatgccacactggttatcaacactgagcaca
tgcccagcatgtttgtcccggttggagatgtagttcagtacggttttcttaacctgagcggtaagcctacacatcgtaccat
GATGTATAACTTTCCGACGAAGGCTGGTCAGGCTGGGGGTGTGGttacgagtgttggtaaagtgatcgggatacatatagga
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```

```
MGPSLDFALSLLRRNIRQVQTDQGHFTMLGVRDRLAVLPRHSQPGKTIWVEHKLINILDAVELVDEQGVNLELTLVTLDTNE
KFRDITKFIPENISAASDATLVINTEHMPSMFVPVGDVVQYGFLNLSGKPTHRTMMYNFPTKAGQAGGVVTSVGKVIGIHIG
GNGRQGFCAGLKRSYFASEQLEHHHHHH
```


600154701_2B_pLICR_D11.seq

```
NNNNNNNNNNNCCGNNNNTNNGTGGTNNGTGGTGGTGGTGCTCGAGTGCGGCCGCAANGCTTGTCGACGGGTGCTCGAGNTCATTAATGGTGGTGATGATGGTGCTCAAGCTG
TTCTGATGCAAAATACGAACGTTTCAGACCAGCGCAAAAGCCCTGACGACCATTCCCTCCTATATGTATCCCGATCACTTTACCAACACTCGTAACCACACCCCCAGCCTGAC
CAGCCTTCGTCGGAAAGTTATACATCATGGTACGATGTGTAGGCTTACCGCTCAGGTTAAGAAAACCGTACTGAACTACATCTCCAACCGGGACAAACATGCTGGGCATGTGC
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GTTTACACCCTGTTCATCAACCAGCTCAACGGCGTCCAATATGTTTATTAATTTATGTTCAACCCAAATCGTTTTACCTGGCTGAGAGTGACGCGGGAGCACCGCCAAACGAT
CGCGAACTCCGAGCATGGTGAAATGGCCCTGATCGGTCTGAACCTGTCTGATATTACGGCGCAGCAATGACAACGCAAAATCCAGGCTAGGACCCATGTGTATATCTCCTTCT
TAAGGTTAAACAAAATTATTTCTAGAGGGGAATTGTTATCCGCTCACAATTCCCCTATAGTGAGTCGTATTAATTTCGCGGGATCGAGATCTCGATCCTCTACGCCGGACGCA
TCGTGGCCGGCATCACCGGCGCCACAGGTGCGGTTGCTGGCGCCTATATCGCCGACATCACCGATGGGGAAGATCGGGCTCGCCACTTCGGGCTCATGAGCGCTTGTTTCGGC
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CCTAATGCAGGAGTCGCATAAGGGAGAGCGGTCGAGATCCCGGACACCATCGAATGGCGCAAAACCTTTCGCGGTATGGCATGATAGCGCCCGGAAAGAGAGTCAATTCAGGGN
NGGTGAATGTGAACCAGTAACGTTAAACGATGTCGCAAGAGAATGCCGGGTGTCTCTTATCAAACCGTTNCCCGGGGGGGNGAACCAGGCNNGCCANGTTTTCTGNNAAAAA
CGCGGGAAAAANNNGGNAAGCGGNGAATGGGCGGANNTNNAATTACATTTCCCAACCNNGNNGGAANCAACAACTGGNNGGNNNAANCNNTTCTTNNCTGAATTGGNGTTGCC
NNCNNCCAGTTNGGNCGCTG
```

```
MGPSLDFALSLLRRNIRQVQTDQGHFTMLGVRDRLAVLPRHSQPGKTIWVEHKLINILDAVELVDEQGVNLELTLVTLDTNEKFRDITKFIPENISAASDATLVINTEHMPSM
FVPVGDVVQYGFLNLSGKPTHRTMMYNFPTKAGQAGGVVTSVGKVIGIHIGGNGRQGFCAGLKRSYFASEQLEHHHHHH
```

	Score	Expect	Identities	Gaps	Strand
	1123 bits(608)	0.0	613/615(99%)	2/615(0%)	Plus/Minus

```
Query  1    ATGGGTCCTAGCCTGGATTTTGCGTTGTCATTGCTGCGCCGTAATATCAGACAGGTTCAG  60

Sbjct  662  ............................................................  603


Query  61   ACCGATCAGGGCCATTTCACCATGCTCGGAGTTCGCGATCGTTTGGCGGTGCTCCCGCGT  120

Sbjct  602  ............................................................  543
```

```
Query  121  CACTCTCAGCCAGGTAAAACGATTTGGGTTGAACATAAATTAATAAACATATTGGACGCC  180

Sbjct  542  ............................................................  483


Query  181  GTTGAGCTGGTTGATGAACAGGGTGTAAACTTAGAACTGACTTTAGTGACCCTGGATACC  240

Sbjct  482  ............................................................  423


Query  241  AATGAAAAGTTTCGCGACATTACCAAATTTATCCCCGAAAATATCTCAGCCGCCTCAGAT  300

Sbjct  422  ............................................................  363


Query  301  GCCACACTGGTTATCAACACTGAGCACATGCCCAGCATGTTTGTCCCGGTTGGAGATGTA  360

Sbjct  362  ............................................................  303


Query  361  GTTCAGTACGGTTTTCTTAACCTGAGCGGTAAGCCTACACATCGTACCATGATGTATAAC  420

Sbjct  302  ............................................................  243


Query  421  TTTCCGACGAAGGCTGGTCAGGCTGGGGGTGTGGTTACGAGTGTTGGTAAAGTGATCGGG  480

Sbjct  242  ............................................................  183


Query  481  ATACATATAGGAGGGAATGGTCGTCAGGGCTTTTGCGCTGGTCTGAAACGTTCGTATTTT  540

Sbjct  182  ............................................................  123


Query  541  GCATCAGAACAGCTTGAGCACCATCATCACCACCATTAATGA-CTCGAGCACCCGTCGAC  599

Sbjct  122  ................................................N................  63


Query  600  AAGC-TTGCGGCCGC   613

Sbjct  62   ....N..........  48
```

Score	Expect	Method	Identities	Positives	Gaps
398 bits(1022)	8e-149	Compositional matrix adjust.	192/192(100%)	192/192(100%)	0/192(0%)

```
Query  1    MGPSLDFALSLLRRNIRQVQTDQGHFTMLGVRDRLAVLPRHSQPGKTIWVEHKLINILDA  60

Sbjct  1    ............................................................  60


Query  61   VELVDEQGVNLELTLVTLDTNEKFRDITKFIPENISAASDATLVINTEHMPSMFVPVGDV  120

Sbjct  61   ............................................................  120
```

```
Query  121  VQYGFLNLSGKPTHRTMMYNFPTKAGQAGGVVTSVGKVIGIHIGGNGRQGFCAGLKRSYF  180

Sbjct  121  ............................................................  180


Query  181  ASEQLEHHHHHH  192

Sbjct  181  ............  192
```

Q5 inverse PCR

98 °C 30 seconds

25x

98 °C 10 seconds

Ta °C 20 seconds

72 °C 180 seconds

72 °C 2 min

12 °C hold

Clean up PCR reaction

Measure concentration of purified PCR product

Perform KLD reaction

100 ng of purified PCR product

xul of water, final volume 20 uL

2 uL of 10 x T4 DNA ligase Buffer

1uL of DPNI

1 uL of T4 Polynucleotide kinase

1 uL of T4 DNA polymerase

Run the following protocol on a thermocylcer with a heated lid:

30 minutes 18 °C

30 minutes 37 °C

20 minutes 80 °C

12 °C hold

Transform MACHI or similar with 3-5 uL of reaction mix

Transformation protocol

100 uL of MACH I cells

30-45 minutes on ice

42C 40 seconds

5 minutes on ice

Add 900 uL of RT SOC

Incubate 1h 37C (shaking optional)

Plate 500 uL on 90 mm LB-agar plate with relevant antibioitic

Incubate o/n 37C

Pick two colonies for minipreps and sequencing

Sequencing

Title missing - double click to edit

```
D68EV3CPROA-c001
```

```
ATGggaccgggattcgacttcgcacaagctattatgaaaaaaaatacggtgatcgcgcggaccgagaaaggcgagtttacca
tgctggggggtatatgaccgcgtggccgttatccctacacacgcttctgttggcgaaattatttacataaatgatgtagaaac
gcgcgttttggatgcgtgtgccttaagagacctgaccgacacaaatctcgaaattacaatagtgaagctggatcgaaaccag
aagtttagagacattcgtcatttcctgcccagatgtgaagacgactataacgatgcggttttgtccgtgcatactagtaagt
ttcccaacatgtatattccagtgggccaggtgaccaattatggttttcttaaccttggggggaactccgacgcatcgtatctt
gatgtataattttccaactagagcgggccagtgcggtggtgtggtcacaactacaggtaaagtaatcggcattcacgttggt
gggaatggggctcaggttttgcagcgatgctgctgcactcctatttcaccgatacccaaaagcaccatcatcaccaccatT
AATGACTCGAGCACCcgtcgacaagcttgcggccgcact
```

```
MGPGFDFAQAIMKKNTVIARTEKGEFTMLGVYDRVAVIPTHASVGEIIYINDVETRVLDACALRDLTDTNLEITIVKLDRNQ
KFRDIRHFLPRCEDDYNDAVLSVHTSKFPNMYIPVGQVTNYGFLNLGGTPTHRILMYNFPTRAGQCGGVVTTTGKVIGIHVG
GNGAQGFAAMLLHSYFTDTQKHHHHHH
```

Result

```
 H H H H H H G L N D I F E A Q K I E W H E * * L E H P

CACCATCATCACCACCATGgcctgaacgatattttgaagcgcagaaaattgaatggcatgaaTAATGACTCGAGCACCCG
GTGGTAGTAGTGGTGGTAccggacttgctataaaaacttcgcgtcttttaacttaccgtacttATTACTGAGCTCGTGGGC
```

Top of Form

Bottom of Form

Required Primers

Name (F/R)	Oligo (Uppercase = target-specific primer)	Len	% GC	Tm	Ta *
D68EV3CPROA_AVI_F	gcagaaaattgaatggcatgaaTAATGACTCGAGCACCCG	40	45	64°C	62°C
D68EV3CPROA_AVI_R	gcttcaaaaatatcgttcaggccATGGTGGTGATGATGGTG	41	46	61°C	

* Ta (recommended annealing temperature)

D68EV3CPROA-c002

```
atgggaccgggattcgacttcgcacaagctattatgaaaaaaaatacggtgatcgcgcggaccgagaaaggcgagtttacca
tgctgggggtatatgaccgcgtggccgttatccctacacacgcttctgttggcgaaattatttacataaatgatgtagaaac
gcgcgttttggatgcgtgtgccttaagagacctgaccgacacaaatctcgaattacaatagtgaagctggatcgaaaccag
aagtttagagacattcgtcatttcctgcccagatgtgaagacgactataacgatgcggttttgtccgtgcatactagtaagt
ttcccaacatgtatattccagtgggccaggtgaccaattatggttttcttaaccttgggggaactccgacgcatcgtatctt
gatgtataattttccaactagagcgggccagtgcggtggtgtggtcacaactacaggtaaagtaatcggcattcacgttggt
gggaatggggctcagggtttttgcagcgatgctgctgcactcctatttcaccgatacccaaaagCACCATCATCACCACCATG
GCCTGAACGATATTTTTGAAGCGCAGAAAATTGAATGGCATGAATAATGACTCGAGCACCCGtcgacaagcttgcggccgca
ct
```

```
MGPGFDFAQAIMKKNTVIARTEKGEFTMLGVYDRVAVIPTHASVGEIIYINDVETRVLDACALRDLTDTNLEITIVKLDRNQ
KFRDIRHFLPRCEDDYNDAVLSVHTSKFPNMYIPVGQVTNYGFLNLGGTPTHRILMYNFPTRAGQCGGVVTTTGKVIGIHVG
GNGAQGFAAMLLHSYFTDTQKHHHHHHGLNDIFEAQKIEWHE
```


Sequencing

600154701_3A_pLICR_E11.seq

```
NNNNNNNNNNNNNNNTCNNNNNNGGTGGNGGTGGTGCTCGAGTGCGGNCGNNAGCTTGTCGACGGGTGCTCGCNGTCATTA
TTCATGCCATTCAATTTTCTGCGCTTCAAAAATATCGTTCAGGCCATGGTGGTGATGATGGTGCTTTTGGGTATCGGTGAAA
TAGGAGTGCAGCAGCATCGCTGCAAAACCCTGAGCCCCATTCCCACCAACGTGAATGCCGATTACTTTACCTGTAGTTGTGA
CCACACCACCGCACTGGCCCGCTCTAGTTGGAAAATTATACATCAAGATACGATGCGTCGGAGTTCCCCCAAGGTTAAGAAA
ACCATAATTGGTCACCTGGCCCACTGGAATATACATGTTGGGAAACTTACTAGTATGCACGGACAAAACCGCATCGTTATAG
TCGTCTTCACATCTGGGCAGGAAATGACGAATGTCTCTAAACTTCTGGTTTCGATCCAGCTTCACTATTGTAATTTCGAGAT
TTGTGTCGGTCAGGTCTCTTAAGGCACACGCATCCAAAACGCGCGTTTCTACATCATTTATGTAAATAATTTCGCCAACAGA
AGCGTGTGTAGGGATAACGGCCACGCGGTCATATACCCCCAGCATGGTAAACTCGCCTTTCTCGGTCCGCGCGATCACCGTA
TTTTTTTTCATAATAGCTTGTGCGAAGTCGAATCCCGGTCCCATGTGTATATCTCCTTCTTAAGGTTAAACAAAATTATTTC
TAGAGGGGAATTGTTATCCGCTCACAATTCCCCTATAGTGAGTCGTATTAATTTCGCGGGATCGAGATCTCGATCCTCTACG
CCGGACGCATCGTGGCCGGCATCACCGGCGCCACAGGTGCGGTTGCTGGCGCCTATATCGCCGACATCACCGATGGGGAAGA
TCGGGCTCGCCACTTCGGGCTCATGAGCGCTTGTTTCGGCGTGGGTATGGTGGCAGGCCCCGTGGCCGGGGGACTGTTGGGC
GCCATCTCCTTGCATGCACCATTCCTTGCGGCGGCGGTGCTCAACGGCCTCAACCTACTACTGGGCTGCTTCCTAATGCAGG
AGTCGCATAAGGGAGAGCGTCGAGATCCCGGACACCATCGAATGGCGCAAAACCTTTCGCGGTATGGCANGATAGCGCCCGG
AAGAGAGTCAATTCAGGGTGGTGAATGTGAAACCAGTAACGTTNTNNNATGTCGCNAANTATGCCGGTGTTTCTTATCAGAA
CGTTTCCNNNNGGTGAACCAGGCCAGCCACGTTTCTGCGAAAACCNGGGAAAANNGNGGAANNGNNNAATGGCGGAACTGAA
NTTAATTTCCCAACCNNNNNNGCNNAACAACTGGGGGGGNNAANNAGTNGTTGCTTAATTGGNNTTGCCACCTCCANTTNGGG
CCNTGACCNGGCCNTCCNAAAATTGTNNNGGCGCANNAAAAATNC
```

```
MGPGFDFAQAIMKKNTVIARTEKGEFTMLGVYDRVAVIPTHASVGEIIYINDVETRVLDACALRDLTDTNLEITIVKLDRNQ
KFRDIRHFLPRCEDDYNDAVLSVHTSKFPNMYIPVGQVTNYGFLNLGGTPTHRILMYNFPTRAGQCGGVVTTTGKVIGIHVG
GNGAQGFAAMLLHSYFTDTQKHHHHHH==GLNDIFEAQKIEWHE==
```

Score	Expect	Identities	Gaps	Strand
1195 bits(647)	0.0	654/659(99%)	1/659(0%)	Plus/Minus

```
Query  1    ATGGGACCGGGATTCGACTTCGCACAAGCTATTATGaaaaaaaaTACGGTGATCGCGCGG  60

Sbjct  700  ...........................................................  641
```

```
Query  61   ACCGAGAAAGGCGAGTTTACCATGCTGGGGGTATATGACCGCGTGGCCGTTATCCCTACA  120
Sbjct  640  ............................................................  581


Query  121  CACGCTTCTGTTGGCGAAATTATTTACATAAATGATGTAGAAACGCGCGTTTTGGATGCG  180
Sbjct  580  ............................................................  521


Query  181  TGTGCCTTAAGAGACCTGACCGACACAAATCTCGAAATTACAATAGTGAAGCTGGATCGA  240
Sbjct  520  ............................................................  461


Query  241  AACCAGAAGTTTAGAGACATTCGTCATTTCCTGCCCAGATGTGAAGACGACTATAACGAT  300
Sbjct  460  ............................................................  401


Query  301  GCGGTTTTGTCCGTGCATACTAGTAAGTTTCCCAACATGTATATTCCAGTGGGCCAGGTG  360
Sbjct  400  ............................................................  341


Query  361  ACCAATTATGGTTTTCTTAACCTTGGGGGAACTCCGACGCATCGTATCTTGATGTATAAT  420
Sbjct  340  ............................................................  281


Query  421  TTTCCAACTAGAGCGGGCCAGTGCGGTGGTGTGGTCACAACTACAGGTAAAGTAATCGGC  480
Sbjct  280  ............................................................  221


Query  481  ATTCACGTTGGTGGGAATGGGGCTCAGGGTTTTGCAGCGATGCTGCTGCACTCCTATTTC  540
Sbjct  220  ............................................................  161


Query  541  ACCGATACCCAAAAGCACCATCATCACCACCATGGCCTGAACGATATTTTTGAAGCGCAG  600
Sbjct  160  ............................................................  101


Query  601  AAAATTGAATGGCATGAATAATGACT-CGAGCACCCGTCGACAAGCTTGCGGCCGCACT  658
Sbjct  100  ........................NG....................NN..N.......  42
```

	Score	Expect	Method	Identities	Positives	Gaps
	430 bits(1106)	3e-161	Compositional matrix adjust.	206/206(100%)	206/206(100%)	0/206(0%)

```
Query  1    MGPGFDFAQAIMKKNTVIARTEKGEFTMLGVYDRVAVIPTHASVGEIIYINDVETRVLDA  60
Sbjct  1    ............................................................  60


Query  61   CALRDLTDTNLEITIVKLDRNQKFRDIRHFLPRCEDDYNDAVLSVHTSKFPNMYIPVGQV  120
```

```
Sbjct   61   ...................................................   120


Query  121   TNYGFLNLGGTPTHRILMYNFPTRAGQCGGVVTTTGKVIGIHVGGNGAQGFAAMLLHSYF   180

Sbjct  121   ...................................................   180


Query  181   TDTQKHHHHHHGLNDIFEAQKIEWHE   206

Sbjct  181   .........................   206
```

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D68EV3CPROA-c001

```
ATGggaccgggattcgacttcgcacaagctattatgaaaaaaaatacggtgatcgcgcggaccgagaaaggcgagtttacca
tgctggggggtatatgaccgcgtggccgttatccctacacacgcttctgttggcgaaattatttacataaatgatgtagaaac
gcgcgttttggatgcgtgtgccttaagagacctgaccgacacaaatctcgaaattacaatagtgaagctggatcgaaaccag
aagtttagagacattcgtcatttcctgcccagatgtgaagacgactataacgatgcggttttgtccgtgcatactagtaagt
ttcccaacatgtatattccagtgggccaggtgaccaattatggttttcttaaccttggggggaactccgacgcatcgtatctt
gatgtataattttccaactagagcgggccagtgcggtggtgtggtcacaactacaggtaaagtaatcggcattcacgttggt
gggaatggggctcagggttttgcagcgatgctgctgcactcctatttcaccgatacccaaaagcaccatcatcaccaccatT
AATGACTCGAGCACCcgtcgacaagcttgcggccgcact
```

                              Top of Form

                              Bottom of Form

Result

   I L M Y N F P T R A G Q A G G V

```
GTATCTTGATGTATAATTTTCCAACTAGAGCGGGCCAGgcgGGTGGTGTGG
CATAGAACTACATATTAAAAGGTTGATCTCGCCCGGTCCGCCCACCACACC
```

                              Top of Form

                              Bottom of Form

Required Primers

Name (F/R)	Oligo (Uppercase = target-specific primer)	Len	% GC	Tm	Ta *
D68EV3CPROA_C2A_F	AGCGGGCCAGgcgGGTGGTGTGG	23	78	65°C	61°C
D68EV3CPROA_C2A_R	CTAGTTGGAAAATTATACATCAAGATAC	28	29	60°C	

* Ta (recommended annealing temperature)

D68EV3CPROA-c003

```
atgggaccgggattcgacttcgcacaagctattatgaaaaaaaatacggtgatcgcgcggaccgagaaaggcgagtttacca
tgctggggggtatatgaccgcgtggccgttatccctacacacgcttctgttggcgaaattatttacataaatgatgtagaaac
gcgcgttttggatgcgtgtgccttaagagacctgaccgacacaaatctcgaaattacaatagtgaagctggatcgaaaccag
aagtttagagacattcgtcatttcctgcccagatgtgaagacgactataacgatgcggttttgtccgtgcatactagtaagt
ttcccaacatgtatattccagtgggccaggtgaccaattatggttttcttaaccttggggggaactccgacgcatcGTATCTT
GATGTATAATTTTCCAACTAGAGCGGGCCAGGCGGGTGGTGTGGtcacaactacaggtaaagtaatcggcattcacgttggt
gggaatggggctcagggttttgcagcgatgctgctgcactcctatttcaccgatacccaaaagcaccatcatcaccaccatt
aatgactcgagcacccgtcgacaagcttgcggccgcact
```

```
MGPGFDFAQAIMKKNTVIARTEKGEFTMLGVYDRVAVIPTHASVGEIIYINDVETRVLDACALRDLTDTNLEITIVKLDRNQ
KFRDIRHFLPRCEDDYNDAVLSVHTSKFPNMYIPVGQVTNYGFLNLGGTPTHRILMYNFPTRAGQAGGVVTTTGKVIGIHVG
GNGAQGFAAMLLHSYFTDTQKHHHHHH
```

Sequencing

600154701_4A_pLICR_G11.seq

NNNNNNNNNNNNNNNGNNNCTCAGTGGTGGTGGTGGTGGTGCTCGAGTGCGGCCNNNANGCTTGTCGACGGGTGCTCGAGTCATTAATGGTGGTGATGATGGTGCTTTTGGGTA
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TCTAGTTGGAAAATTATACATCAAGATACGATGCGTCGGAGTTCCCCCAAGGTTAAGAAAACCATAATTGGTCACCTGGCCCACTGGAATATACATGTTGGGAAACTTACTAG
TATGCACGGACAAAACCGCATCGTTATAGTCGTCTTCACATCGGGCAGGAAATGACGAATGTCTCTAAACTTCTGGTTTCGATCCAGCTTCACTATTGTAATTTCGAGATTT
GTGTCGGTCAGGTCTCTTAAGGCACACGCATCCAAAACGCGCGTTTCTACATCATTTATGTAAATAATTTCGCCAACAGAAGCGTGTGTAGGGATAACGGCCACGCGGTCATA
TACCCCCAGCATGGTAAACTCGCCTTTCTCGGTCCGCGCGATCACCGTATTTTTTTTCATAATAGCTTGTGCGAAGTCGAATCCCGGTCCCATGTGTATATCTCCTTCTTAAG
GTTAAACAAAATTATTTCTAGAGGGGAATTGTTATCCGCTCACAATTCCCCTATAGTGAGTCGTATTAATTTCGCGGGATCGAGATCTCGATCCTCTACGCCGGACGCATCGT
GGCCGGCATCACCGGCGCCACAGGTGCGGTTGCTGGCGCCTATATCGCCGACATCACCGATGGGGAAGATCGGGCTCGCCACTTCGGGCTCATGAGCGCTTGTTTCGGCGTGG
GTATGGTGGCAGGCCCCGTGGCCGGGGGACTGTTGGGCGCCATCTCCTTGCATGCACCATTCCTTGCGGCGGCGGTGCTCAACGGCCTCAACCTACTACTGGGCTGCTTCCTA
ATGCAGGAGTCGCATAAGGGAGAGCGTCGAGATCCCGGANNCCATCGAATGGCGCAAAACCTTTCGCGGTATGGCATGAAAGCGCCCGGAAGAGAGTCAATTCAGGGTGGTGG
AATGTNAAACCCAGTAACGTTATACGATGTCNCANANNTATGCCGGNGTNCTTTTATCNAACCGTTTCCNGGGTGGGNAACCAGGCCAGCCANGTTTNCTGNNAAAACGCGGN
AAAAAGTGGAAGNGGGNAATGGCGGAGCTNNAATTACATTCCCAACCNGNNGGNNCNAAANAACTNGGNGGNCAAAACANNCGTTGCTGAATTGGNGGTTGCCACCNTCCNAG
TCTGGGCCCTGCCNGCGCCCGNNCCCAAAATTTNNNCG

MGPGFDFAQAIMKKNTVIARTEKGEFTMLGVYDRVAVIPTHASVGEIIYINDVETRVLDACALRDLTDTNLEITIVKLDRNQKFRDIRHFLPRCEDDYNDAVLSVHTSKFPNM
YIPVGQVTNYGFLNLGGTPTHRILMYNFPTRAGQ<mark>A</mark>GGVVTTTGKVIGIHVGGNGAQGFAAMLLHSYFTDTQKHHHHHH

Score	Expect	Identities	Gaps	Strand
1116 bits(604)	0.0	610/614(99%)	1/614(0%)	Plus/Minus

```
Query  1    ATGGGACCGGGATTCGACTTCGCACAAGCTATTATGaaaaaaaaaTACGGTGATCGCGCGG  60

Sbjct  658  .......................................................  599



Query  61   ACCGAGAAAGGCGAGTTTACCATGCTGGGGGTATATGACCGCGTGGCCGTTATCCCTACA  120

Sbjct  598  .......................................................  539



Query  121  CACGCTTCTGTTGGCGAAATTATTTACATAAATGATGTAGAAACGCGCGTTTTGGATGCG  180

Sbjct  538  .......................................................  479



Query  181  TGTGCCTTAAGAGACCTGACCGACACAAATCTCGAAATTACAATAGTGAAGCTGGATCGA  240

Sbjct  478  .......................................................  419



Query  241  AACCAGAAGTTTAGAGACATTCGTCATTTCCTGCCCAGATGTGAAGACGACTATAACGAT  300

Sbjct  418  .......................................................  359



Query  301  GCGGTTTTGTCCGTGCATACTAGTAAGTTTCCCAACATGTATATTCCAGTGGGCCAGGTG  360

Sbjct  358  .......................................................  299



Query  361  ACCAATTATGGTTTTCTTAACCTTGGGGGAACTCCGACGCATCGTATCTTGATGTATAAT  420

Sbjct  298  .......................................................  239



Query  421  TTTCCAACTAGAGCGGGCCAGGCGGGTGGTGTGGTCACAACTACAGGTAAAGTAATCGGC  480

Sbjct  238  .......................................................  179
```

```
Query  481   ATTCACGTTGGTGGGAATGGGGCTCAGGGTTTTGCAGCGATGCTGCTGCACTCCTATTTC   540

Sbjct  178   ............................................................   119


Query  541   ACCGATACCCAAAAGCACCATCATCACCACCATTAATGACTCGAGCACCCGTCGACAAGC   600

Sbjct  118   ............................................................   59


Query  601   TTGC-GGCCGCACT   613

Sbjct  58    N.NNN........   45
```

Score	Expect	Method	Identities	Positives	Gaps
396 bits(1017)	4e-148	Compositional matrix adjust.	191/191(100%)	191/191(100%)	0/191(0%)

```
Query  1     MGPGFDFAQAIMKKNTVIARTEKGEFTMLGVYDRVAVIPTHASVGEIIYINDVETRVLDA   60

Sbjct  1     ............................................................   60


Query  61    CALRDLTDTNLEITIVKLDRNQKFRDIRHFLPRCEDDYNDAVLSVHTSKFPNMYIPVGQV   120

Sbjct  61    ............................................................   120


Query  121   TNYGFLNLGGTPTHRILMYNFPTRAGQAGGVVTTTGKVIGIHVGGNGAQGFAAMLLHSYF   180

Sbjct  121   ............................................................   180


Query  181   TDTQKHHHHHH   191

Sbjct  181   ...........   191
```

Title missing - double click to edit

A71EV2A-c012

**atgcaccaccatcatcaccatggctcaggagatcaggaagcgaaaccttcgaccgaggacctgggcgacaagaaagaagggg
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cacacacctaaggaacttggtatggaagaggaagacgtcattgaggtgtacCAGGAGCAGACAGGCGGCTCGGGTGCCATAT
ATGTGGGtaactatcgtgtggttaatcgccacctcgctactcacaatgattgggccaacttagtgtgggaggacagctctcg
cgatctccttgtatcatcgaccactgcgcagggttgcgacaccattgcgcgctgtgattgccagacaggcgtgtattattgc
tcgtctcgtcgtaaacattatccggtttctttttctaagccgtcactgatctttgttgaagcgtcagagtactatccagccc
gttatcagtcccatctgatgctggctgtgggtcacagcgaacccggggattgtggcggtatactgcgctgtcagcacggtgt
agttggcattgtcagtacaggaggtaatgggttagtcggatttgccgatgtgcgggatttactgtggttggacgaggaggct**

atggaacagtaatga

```
 A  V  G  H  S  E  P  G  D  A  G  G  I  L
```

```
GGCTGTGGGTCACAGCGAACCCGGGGATgctGGCGGTATACTGC
CCGACACCCAGTGTCGCTTGGGCCCCTACGACCGCCATATGACG
```

Required Primers

Name (F/R)	Oligo (Uppercase = target-specific primer)	Len	% GC	Tm	Ta *
A71EV2A_C2A_F	ACCCGGGGATgctGGCGGTATACTGC	26	65	66°C	67°C
A71EV2A_C2A_R	TCGCTGTGACCCACAGCC	18	67	70°C	

* Ta (recommended annealing temperature)

A71EV2A-c016

```
atgcaccaccatcatcaccatggctcaggagatcaggaagcgaaaccttcgaccgaggacctgggcgacaagaaagaagggg
agtatattaagttaaaggtgatcggccaggactcaagcgagatccacttcaaagtcaaaatgaccacgcacctcaagaagtt
gaaggagagttactgtcaacggcaaggggttcctatgaactcgcttcgcttcctgttcgagggccagcgaattgcggacaac
cacacacctaaggaacttggtatggaagaggaagacgtcattgaggtgtaccaggagcagacaggcggctcgggtgccatat
atgtgggtaactatcgtgtggttaatcgccacctcgctactacaatgattgggccaacttagtgtgggaggacagctctcg
cgatctccttgtatcatcgaccactgcgcagggttgcgacaccattgcgcgctgtgattgccagacaggcgtgtattattgc
tcgtctcgtcgtaaacattatccggtttcttttttctaagccgtcactgatctttgttgaagcgtcagagtactatccagccc
gttatcagtcccatctgatgctGGCTGTGGGTCACAGCGAACCCGGGGATGCTGGCGGTATACTGCgctgtcagcacggtgt
agttggcattgtcagtacaggaggtaatgggttagtcggatttgccgatgtgcgggatttactgtggttggacgaggaggct
atggaacagtaatga
```

```
MHHHHHHGSGDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTTHLKKLKESYCQRQGVPMNSLRFLFEGQRIADN
HTPKELGMEEEDVIEVYQEQTGGSGAIYVGNYRVVNRHLATHNDWANLVWEDSSRDLLVSSTTAQGCDTIARCDCQTGVYYC
SSRRKHYPVSFSKPSLIFVEASEYYPARYQSHLMLAVGHSEPGDAGGILRCQHGVVGIVSTGGNGLVGFADVRDLLWLDEEA
MEQ
```

Sequencing

600154701_6B_pLICR_B12.seq

```
NNNNNNNNNNNNNNNNNTNNNNGGTGGTGGTGGTGGTGCTCGAGTGCGGNCGNNNNNTTGTCGACGGGTGCNCNNNTNANTACTGTTCCATAGCCTCCTCGTCCAACCACAGT
AAATCCCGCACATCGGCAAATCCGACTAACCCATTACCTCCTGTACTGACAATGCCAACTACACCGTGCTGACAGCGCAGTATACCGCCAGCATCCCCGGGTTCGCTGTGACC
CACAGCCAGCATCAGATGGGACTGATAACGGGCTGGATAGTACTCTGACGCTTCAACAAAGATCAGTGACGGCTTAGAAAAAGAAACCGGATAATGTTTACGACGAGACGAGC
AATAATACACGCCTGTCTGGCAATCACAGCGCGCAATGGTGTCGCAACCCTGCGCAGTGGTCGATGATACAAGGAGATCGCGAGAGCTGTCCTCCCACACTAAGTTGGCCCAA
TCATTGTGAGTAGCGAGGTGGCGATTAACCACACGATAGTTACCCACATATATGGCACCCGAGCCGCCTGTCTGCTCCTGGTACACCTCAATGACGTCTTCCTCTTCCATACC
AAGTTCCTTAGGTGTGTGGTTGTCCGCAATTCGCTGGCCCTCGAACAGGAAGCGAAGCGAGTTCATAGGAACCCCTTGCCGTTGACAGTAACTCTCCTTCAACTTCTTGAGGT
GCGTGGTCATTTTGACTTTGAAGTGGATCTCGCTTGAGTCCTGGCCGATCACCTTTAACTTAATATACTCCCCTTCTTTCTTGTCGCCCAGGTCCTCGGTCGAAGGTTTCGCT
TCCTGATCTCCTGAGCCATGGTGATGATGGTGGTGCATATGTATATCTCCTTCTTAAAGTTAAACAAAATTATTTCTAGAGGGGAATTGTTATCCGCTCACAATTCCCCTATA
GTGAGTCGTATTAATTTCGCGGGATCGAGATCTCGATCCTCTACGCCGGACGCATCGTGGCCGGCATCACCGGCGCCACAGGTGCGGTTGCTGGCGCCTATATCGCCGACATC
ACCGATGGGAAGATCGGGCTCGCCCACTTCGGGCTCATGAGCGCTTGTTTCGGCGTGGGTATGGTGGCAGGCCCCGTGGCCGGGGGACTGTTGGGCGCCATCTCCTTGCATGC
ACCATTCCTTGCGGCGGCGGTGCTCAACGGCCTCAACCTACTACTGGGCTGCTTCCTAATGCAGGAGTCGCATAAGGGAAACGTCCGAGATCCCGGGANNCCATCGAATGGC
NGAAAACC
```

```
MHHHHHHGSGDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTTHLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIEVYQEQTGGSGAIYVGN
YRVVNRHLATHNDWANLVWEDSSRDLLVSSTTAQGCDTIARCDCQTGVYYCSSRRKHYPVSFSKPSLIFVEASEYYPARYQSHLMLAVGHSEPGDAGGILRCQHGVVGIVSTG
GNGLVGFADVRDLLWLDEEAMEQ
```

Score	Expect	Identities	Gaps	Strand
1384 bits(749)	0.0	749/749(100%)	0/749(0%)	Plus/Minus

```
Query  1    ATGCACCACCATCATCACCATGGCTCAGGAGATCAGGAAGCGAAACCTTCGACCGAGGAC  60

Sbjct  829  ............................................................  770
```

```
Query   61   CTGGGCGACAAGAAAGAAGGGGAGTATATTAAGTTAAAGGTGATCGGCCAGGACTCAAGC   120

Sbjct   769   ............................................................   710


Query   121  GAGATCCACTTCAAAGTCAAAATGACCACGCACCTCAAGAAGTTGAAGGAGAGTTACTGT   180

Sbjct   709   ............................................................   650


Query   181  CAACGGCAAGGGGTTCCTATGAACTCGCTTCGCTTCCTGTTCGAGGGCCAGCGAATTGCG   240

Sbjct   649   ............................................................   590


Query   241  GACAACCACACACCTAAGGAACTTGGTATGGAAGAGGAAGACGTCATTGAGGTGTACCAG   300

Sbjct   589   ............................................................   530


Query   301  GAGCAGACAGGCGGCTCGGGTGCCATATATGTGGGTAACTATCGTGTGGTTAATCGCCAC   360

Sbjct   529   ............................................................   470


Query   361  CTCGCTACTCACAATGATTGGGCCAACTTAGTGTGGGAGGACAGCTCTCGCGATCTCCTT   420

Sbjct   469   ............................................................   410


Query   421  GTATCATCGACCACTGCGCAGGGTTGCGACACCATTGCGCGCTGTGATTGCCAGACAGGC   480

Sbjct   409   ............................................................   350


Query   481  GTGTATTATTGCTCGTCTCGTCGTAAACATTATCCGGTTTCTTTTTCTAAGCCGTCACTG   540

Sbjct   349   ............................................................   290


Query   541  ATCTTTGTTGAAGCGTCAGAGTACTATCCAGCCCGTTATCAGTCCCATCTGATGCTGGCT   600

Sbjct   289   ............................................................   230


Query   601  GTGGGTCACAGCGAACCCGGGGATGCTGGCGGTATACTGCGCTGTCAGCACGGTGTAGTT   660

Sbjct   229   ............................................................   170


Query   661  GGCATTGTCAGTACAGGAGGTAATGGGTTAGTCGGATTTGCCGATGTGCGGGATTTACTG   720

Sbjct   169   ............................................................   110


Query   721  TGGTTGGACGAGGAGGCTATGGAACAGTA   749

Sbjct   109   .............................   81
```

Score	Expect	Method	Identities	Positives	Gaps
522 bits(1344)	0.0	Compositional matrix adjust.	249/249(100%)	249/249(100%)	0/249(0%)

```
Query  1    MHHHHHHGSGDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTTHLKKLKESYC  60

Sbjct  1    ...........................................................  60


Query  61   QRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIEVYQEQTGGSGAIYVGNYRVVNRH  120

Sbjct  61   ...........................................................  120


Query  121  LATHNDWANLVWEDSSRDLLVSSTTAQGCDTIARCDCQTGVYYCSSRRKHYPVSFSKPSL  180

Sbjct  121  ...........................................................  180


Query  181  IFVEASEYYPARYQSHLMLAVGHSEPGDAGGILRCQHGVVGIVSTGGNGLVGFADVRDLL  240

Sbjct  181  ...........................................................  240


Query  241  WLDEEAMEQ  249

Sbjct  241  .........  249
```

Title missing - double click to edit

A71EV2A-c012

**atgcaccaccatcatcaccatggctcaggagatcaggaagcgaaaccttcgaccgaggacctgggcgacaagaaagaagggg
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cacacacctaaggaacttggtatggaagaggaagacgtcattgaggtgtacCAGGAGCAGACAGGCGGCTCGGGTGCCATAT
ATGTGGGtaactatcgtgtggttaatcgccacctcgctactcacaatgattgggccaacttagtgtgggaggacagctctcg
cgatctccttgtatcatcgaccactgcgcagggttgcgacaccattgcgcgctgtgattgccagacaggcgtgtattattgc
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gttatcagtcccatctgatgctggctgtgggtcacagcgaacccgggggattgtggcggtatactgcgctgtcagcacggtgt
agttggcattgtcagtacaggaggtaatgggttagtcggatttgccgatgtgcgggatttactgtggttggacgaggaggct
atggaacagtaatga**

```
E  E  A  M  E  Q  G  L  N  D  I  F  E  A  Q  K  I  E  W  H  E  *  *
GAGGAGGCTATGGAACAGGgcctgaacgatattttttgaagcgcagaaaattgaatggcatgaaTAATGAATGCACCACCATC
CTCCTCCGATACCTTGTCccggacttgctataaaaacttcgcgtcttttaacttaccgtacttATTACTTACGTGGTGGTAG
```

Top of Form

Bottom of Form

Required Primers

Name (F/R)	Oligo (Uppercase = target-specific primer)	Len	% GC	Tm	Ta *
A71EV2A-c012_AVITAG_F	gcagaaaattgaatggcatgaaTAATGAATGCACCACCATC	41	39	60°C	61°C
A71EV2A-c012_AVITAG _R	gcttcaaaaatatcgttcaggccCTGTTCCATAGCCTCCTC	41	49	62°C	

* Ta (recommended annealing temperature)

A71EV2A-c017

```
ATGCACCACCATCatcaccatggctcaggagatcaggaagcgaaaccttcgaccgaggacctgggcgacaagaaagaagggg
agtatattaagttaaaggtgatcggccaggactcaagcgagatccacttcaaagtcaaaatgaccacgcacctcaagaagtt
gaaggagagttactgtcaacggcaagggggttcctatgaactcgcttcgcttcctgttcgagggccagcgaattgcggacaac
cacacacctaaggaacttggtatggaagaggaagacgtcattgaggtgtaccaggagcagacaggcggctcgggtgccatat
atgtgggtaactatcgtgtggttaatcgccacctcgctactcacaatgattgggccaacttagtgtgggaggacagctctcg
cgatctccttgtatcatcgaccactgcgcaggggttgcgacaccattgcgcgctgtgattgccagacaggcgtgtattattgc
tcgtctcgtcgtaaacattatccggtttcttttttctaagccgtcactgatctttgttgaagcgtcagagtactatccagccc
gttatcagtcccatctgatgctggctgtgggtcacagcgaacccggggattgtggcggtatactgcgctgtcagcacggtgt
agttggcattgtcagtacaggaggtaatgggttagtcggatttgccgatgtgcgggatttactgtggttggacGAGGAGGCT
ATGGAACAGGGCCTGAACGATATTTTTGAAGCGCAGAAAATTGAATGGCATGAATAATGA
```