

Species Identification: Primates

6. Aotus: The Owl Monkey Mystery

Name: _____

**Objective**

Using both chromosomes and DNA sequence, determine the species of Aotus provided in the karyotype.

Background

Owl monkeys (genus *Aotus*) are a small primate that live in Central and South America, earning their name by their large, owl-like eyes. These monkeys live in family groups of two to five individuals and spend the day hidden in tree hollows and vine tangles. At sundown, they set out in search for food, as they are the only South American monkeys that hunt at night. They are completely omnivorous, as they eat fruits, leaves, insects, small birds, and eggs and even small mammals. As dawn breaks, they return to their hiding place. These monkeys make a host of different sounds to communicate, although, when in danger, they emit a high-pitched shriek. Unlike many monkeys, owl monkeys do not groom each other except immediately before mating.

Although the different species live in close proximity to one another, they can have significant genetic differences. Scientists studying these monkeys have found them to have different diploid numbers and different gene sequences. Below is a key that will help you when you receive your monkey's sample:

Species	Type	Diploid Number	Species	Type	Diploid Number
<i>Aotus azarae</i>	KVI	2n = 49/50	<i>Aotus miconax</i>	KXII, XIII	2n = 57, 58 ?
<i>Aotus brumbacki</i>	?	2n = 50	<i>Aotus nancymai</i>	KI	2n = 54
<i>Aotus herskovitzi</i>	?	?	<i>Aotus nigriceps</i>	KVII	2n = 51/52
<i>Aotus infulatus</i>	KVI	2n = 49/50	<i>Aotus trivirgatus</i>	?	?
<i>Aotus lemurinus griseimembra</i>	KII, III, IV	2n = 52, 53, 54	<i>Aotus vociferans</i>	KV, X, XI	2n = 46, 47, 48
<i>Aotus lemurinus lemurinus</i>	KVIII, IX	2n = 55, 56			

You may notice that some individuals have an odd number of chromosomes. It has been found that when an odd-numbered karyotype was discovered, it was from a male. Upon further investigation, researchers discovered that the Y chromosome was somehow attached to one of the autosomes! Others have an unpaired large autosomal chromosome due to the homologue splitting into two acrocentric chromosomes. This illustrates the variability that can be found among species throughout the geographic distribution of the genus. Keep this in mind when you receive your sample.

Procedure

You will be doing two analyses in this lab: a karyotype and a DNA sequence analysis.

- 1) Your instructor will provide four chromosome spreads, one for each member of your group. You will cut the chromosomes out and arrange them according to their size and centromere placement. Metacentric chromosomes have a centromere at or very close to the center of the chromosome. Submetacentric chromosomes have a centromere above the center of the chromosome. Acrocentric chromosomes have a centromere at the top of the chromosome.
- 2) When your karyotype is complete, write your "Species ID" hypothesis on your form in the space provided.
- 3) Once your karyotype is complete, have your teacher initial your form so you can move to the next step in the identification process.

4) You will now receive your individual’s COII sequence. COII is a gene in the mitochondria and, since all animals get their mitochondria from their mother’s egg, this gene sequence is usually identical to its mother’s. Mitochondrial DNA (or mtDNA) is circular and has more than 12 genes made of G, C, A, and T. Since the nuclear DNA is a mixture of both parents, using mtDNA is a helpful check when trying to identify a species. Here is a gene map of the mitochondrial DNA of most mammals, though the GCATs are not shown:

5) Using an enzyme called DNA polymerase, COII has been copied and sequenced so you can look at the order of nucleotides. Look at the COII gene that has been sequenced for you. In order to differentiate between species, we use restriction enzymes that cut each sequence in a different place. You will simulate this activity by using a different color ink or pencil for each enzyme. Whenever you come to one of the sequences shown below, make the cut by drawing a slash mark (/) in the appropriate site.



Use the following table as your guide when cutting your sequence:

Restriction Enzyme	Recognition Sequence
Alu I	AG/CT
Bgl II	A/GATCT
Bst NI	CC/WGG
Eco RII	/CCWGG
Hae III	GG/CC

Restriction Enzyme	Recognition Sequence
Msp I	C/CGG
Sau 3AI	/GATC
Sst III	/ACGT
Stu I	AGG/CCT
Taq I	T/CGA

Note: W is a variable base and can be G, C, A, or T as long as the rest of the sequence is recognized as a site for the restriction enzyme.

- 6) After “cutting” each sequence into fragments, record the base lengths into your data table.

Data Table:

Restriction Enzyme	Number of Fragments	Fragment Lengths
Alu I		
Bgl II		
Bst NI		
Eco RII		
Hae III		
Msp I		
Sau 3AI		
Sst III		
Stu I		
Taq I		

- 7) Gel electrophoresis is used to separate the different fragment lengths by using an electric charge and running it through a gel. Longer fragments move slower through the gel, whereas shorter fragments move quickly. On your gel simulation sheet, fill in each band completely for each of the base lengths you recorded. You will record these by filling in the set of 10 to which the length belongs. For example, if a base length is 245, fill in the band at 240. This will give a series of bands to compare.
- 8) After you have filled in all bands for each enzyme, match your “gel” with the known gels. Your instructor should have these gels for comparison.
- 9) Complete the analysis form.
- 10) After you have determined the species type, consult the map and mark an X on your species’ origin.

Analysis Form: Data Record

Species Common Name	
Species Scientific Name	
Diploid Number	
Type ID (KI-KVIII)	
Geographic Location	
Hybrid?	Yes No Need More Data
Most Useful Restriction Enzymes for Your Individual	(See question 3 on the next page for more info.)

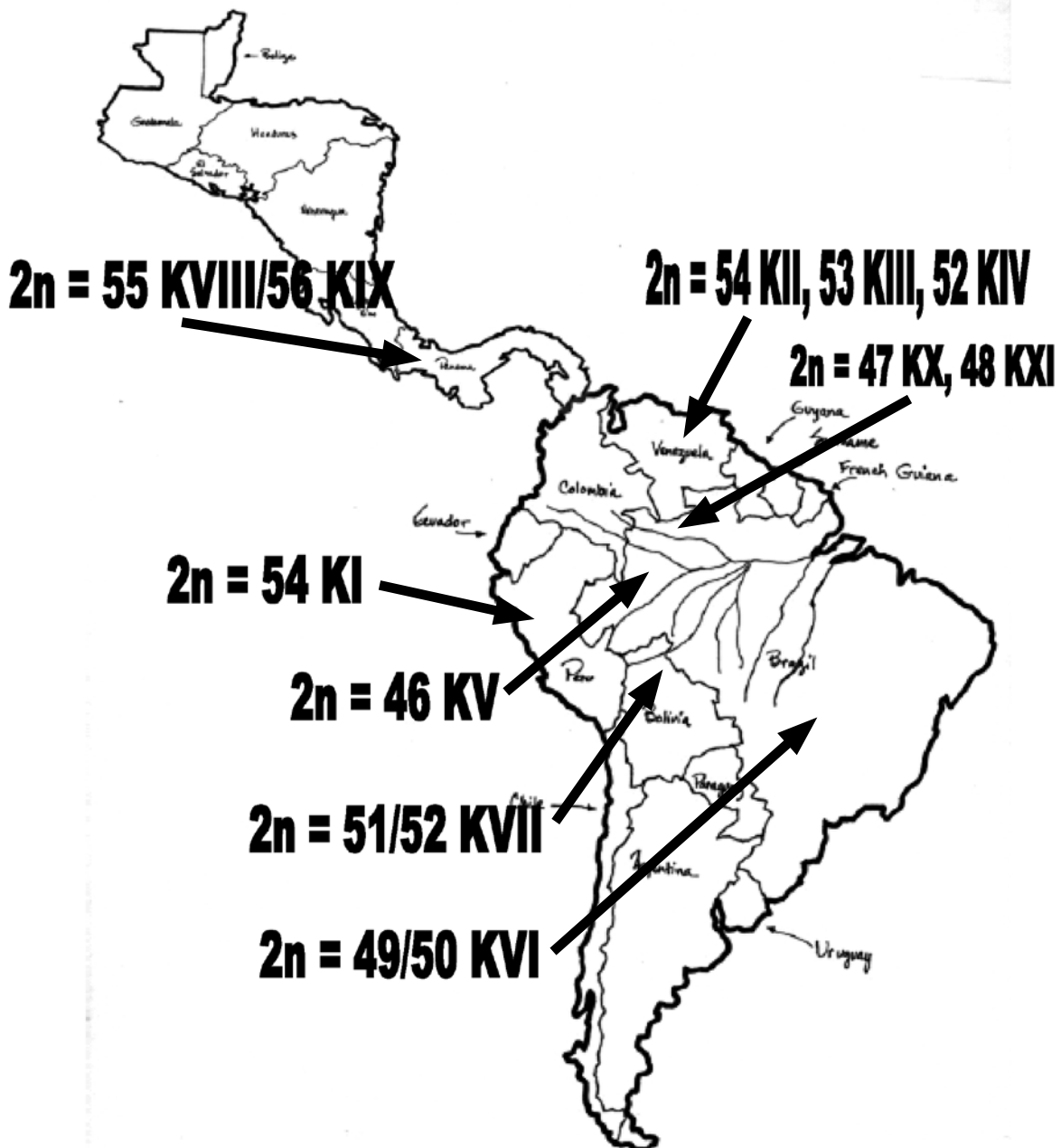
Forming a Conclusion

Using the colored pins and the map that has been posted, put your pin into the region where you believe your individual originated.

Further Analysis

- 1) What was the first point in this lab activity when you formed a hypothesis as to what species' sample you had? What made you form this hypothesis at this moment?
- 2) Was each chromosome in the karyotype identical to its homologue? _____
Why or why not?
- 3) Each of the known DNA fragment patterns used many different restriction enzymes. After looking at your sample, why was it necessary to use many of these enzymes? Which ones worked best for you?

Distribution of Aotus Types Based on Chromosome Counts



Use the following table as your guide when cutting your sequence:

	Hae III	Rsa I	Sau 3AI	Sst III	Stu I	Taq I
540						
530						
520						
510						
500						
490						
480						
470						
460						
450						
440						
430						
420						
410						
400						
390						
380						
370						
360						
350						
340						
330						
320						
310						
300						
290						
280						
270						
260						
250						
240						
230						
220						
210						
200						
190						
180						
170						
160						
150						
140						
130						
120						
110						
100						
90						
80						
70						
60						
50						
40						
30						
20						
10						
0						

SOLUTION

Aotus KII 2n=54						
Fragment Size	Hae III	Rsa II	Sau 3AI	Sst III	Stu II	Taq II
540						
530						
520						
510						
500						
490			■			
480		■				
470						
460					■	
450						
440						
430						
420						
410						
400						
390						
380						
370						
360						
350						
340						
330						
320						
310						
300						
290						
280						
270						
260						
250	■					
240						
230				■		■
220						
210						■
200	■					
190						
180						
170				■		
160						
150						
140				■		
130						
120						
110						
100						
90						
80						■
70	■				■	
60		■				
50						
40			■			
30						
20						
10	■					
0			■			■

Aotus KIII 2n=54, 53, 52						
Fragment Size	Hae III	Rsa II	Sau 3AI	Sst III	Stu II	Taq II
540		■				
530						
520						
510						
500						
490						
480						
470						
460					■	
450						
440						
430						
420						
410						
400						
390						
380						
370						
360						
350						
340						
330						
320						
310						
300						
290						
280						
270						
260						
250	■		■			
240			■			
230				■		■
220						
210						■
200						
190						
180						
170				■		
160						
150						
140				■		
130						
120						
110						
100						
90						
80						■
70	■				■	
60						
50						
40			■			
30						
20						
10	■					
0						■

SOLUTION

Aotus KVII 2n=49, 50						
Fragment Size	Hae III	Rsa II	Sau 3AI	Sst III	Stu II	Taq II
540		■				
530						
520						
510						
500						
490						
480						
470						
460					■	
450						
440						
430						
420						
410						
400						
390						
380						
370						
360						
350						
340						
330						
320						
310				■		
300						
290						■
280						
270						
260	■					
250			■			
240			■			
230						
220						■
210						
200	■					
190						
180						
170						
160						
150						
140				■		
130						
120						
110						
100						
90						
80				■		
70	■				■	
60						
50						
40			■			
30						
20						
10						■
0						■

Aotus KVIII 2n=51, 52						
Fragment Size	Hae III	Rsa II	Sau 3AI	Sst III	Stu II	Taq II
540						
530						
520						
510						
500						
490						
480						
470						
460						
450						
440						
430						
420						
410						
400						
390						
380						
370						
360						
350						
340						
330						
320						
310						
300						
290						
280						
270						
260						
250						
240						
230						
220						
210						
200						
190						
180						
170						
160						
150						
140						
130						
120						
110						
100						
90						
80						
70						
60						
50						
40						
30						
20						
10						
0						

SOLUTION

Aotus KVIII 2n=55, 56						
Fragment Size	Hae III	Rsa II	Sau 3AI	Sst III	Stu II	Taq II
540					■	
530						
520						
510						
500						
490						
480		■				
470						
460						
450						
440						
430						
420						
410						
400						
390						
380						
370						
360						
350						
340						
330						
320						
310						
300						
290						■
280						
270						
260	■					
250			■			
240						
230						
220						■
210						
200	■					
190						
180						
170						
160						
150						
140				■		
130						
120						
110	■					
100	■					
90	■					
80	■			■		■
70	■					
60		■				
50						
40			■			
30						
20						
10	■					
0						■

Aotus KI 2n=54			
1 - 540 Base Pairs Total			
Enzyme Name	Sequence	# Cutting Sites	Cutting Positions
Hae III	GG ! CC	3	200 451 467
Rsa I	GT ! AC	1	60
Sau 3AI	! GATC	2	491 499
Sst III	! ACGT	2	140 370
Stu I	AGG ! CCT	1	467
Taq I	T ! CGA	3	80 297 305

Aotus KII 2n=54,53,52			
1 - 540 Base Pairs Total			
Enzyme Name	Sequence	# Cutting Sites	Cutting Positions
Hae III	GG ! CC	3	200 451 467
Rsa I	GT ! AC	0	
Sau 3AI	! GATC	2	240 499
Sst III	! ACGT	2	140 370
Stu I	AGG ! CCT	1	467
Taq I	T ! CGA	3	80 297 305

Aotus KVI 2n=49,50			
1 - 540 Base Pairs Total			
Enzyme Name	Sequence	# Cutting Sites	Cutting Positions
Hae III	GG ! CC	2	200 467
Rsa I	GT ! AC	0	
Sau 3AI	! GATC	2	240 499
Sst III	! ACGT	2	140 458
Stu I	AGG ! CCT	1	467
Taq I	T ! CGA	3	297 305 318

Aotus KVII 2n=51,52			
1 - 540 Base Pairs Total			
Enzyme Name	Sequence	# Cutting Sites	Cutting Positions
Hae III	GG ! CC	2	200 467
Rsa I	GT ! AC	0	
Sau 3AI	! GATC	2	240 499
Sst III	! ACGT	3	140 370 458
Stu I	AGG ! CCT	1	467
Taq I	T ! CGA	4	80 297 305 318

Aotus KVIII 2n=55,56			
1 - 540 Base Pairs Total			
Enzyme Name	Sequence	# Cutting Sites	Cutting Positions
Hae III	GG ! CC	3	200 451 467
Rsa I	GT ! AC	1	60
Sau 3AI	! GATC	2	240 499
Sst III	! ACGT	3	140 370 458
Stu I	AGG ! CCT	0	
Taq I	T ! CGA	3	80 297 305

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File: AOTUS KVIII/KIX55/56 J
Mode: Normal 1 - 540
Table: DNASIS1 Style: Linear Indication Mode: Actual Cutting Site

      10      20      30      40      50      60
5' GCTCACTAGTCCTATATATTATTTCCCTAATACTTACTACAAAATTAACCCACACCAGTA
                                         ^
                                         RsaI

      70      80      90      100     110     120
CCATAAACGCCCGAGGAAATCGAAATAATCTGAACTATTCTGCCCGCTATCATTCTTATTA
                                         ^
                                         TaqI

      130     140     150     160     170     180
TAATTGCTCTCCCATCCCTACGTATCCTCTATATAACAGACGAATTCAATAAACCCCTACC
                                         ^
                                         SstIII

      190     200     210     220     230     240
TAACCCTTAAAGCAATTGGCCACCAATGATACTGGAGCTATGAATACTCAGACTATGAAG
                                         ^
                                         HaeIII
                                         ^
                                         Sau3AI

      250     260     270     280     290     300
ATCTAGCATTGATTCCCTATATTACGCCAACCTACTTCCTTGAACCTGGCGAGTTTCGCAC
                                         ^
                                         TaqI

      310     320     330     340     350     360
TCCTCGAAGTAGATAACCGGACAACCCCTCCCAATAGAAGCAGATATTCGTATACTAATTA
^
TaqI

      370     380     390     400     410     420
CATCACAAGACGTCTTACACTCATGAGCTGTTCCATCGTTAGGTGTCAAAACAGACGCAA
^
SstIII

      430     440     450     460     470     480
TCCCCGGACGCTTAAATCAAGCTATACTGGCCTCCATACGTCCTGGCCTATTCTATGGAC
                                         ^           ^           ^
                                         HaeIII SstIII HaeIII

      490     500     510     520     530     540
AATGCTCAGAAATCTGCGGATCAAACCATAGCTTCATACCTATCGTTCTAGAATTTATCT
^
Sau3AI

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File: AOTUS KVII51/52J
 Mode: Normal 1 - 540
 Table: DNASIS1 Style: Linear Indication Mode: Actual Cutting Site

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        10      20      30      40      50      60
5' GCTCACTAGTCCTATATATTATTTCCGTAATACTTCCTACAAAATTAACCCACACCAGCA

        70      80      90      100     110     120
CCATAAACGCCCAAGAAATCGAAATAATCTGAACTATTCTGCCCGCTATCATTCTTATTA
      ^
      TaqI

        130     140     150     160     170     180
TAATTGCTCTCCCATCCCTACGTATCCTCTATATAACAGACGAATTTAATAAACCTACC
      ^
      SstIII

        190     200     210     220     230     240
TAACCCTTAAAGCAATCGGCCATCAATGATACTGAAGCTATGAATACTCAGACTATGAAG
      ^                               ^
      HaeIII                           Sau3AI

        250     260     270     280     290     300
ATCTAGCATTGACTCCTATATTACACCAACCTACTTCCTTGAACCTGGCGAATTTGAC
                                             ^
                                             TaqI

        310     320     330     340     350     360
TTCTCGAAGTAGATAATCGAACAACCCTCCCAATAGAAGCAGATATTCGTATACTAATTA
      ^           ^
      TaqI       TaqI

        370     380     390     400     410     420
CATCACAAGACGTCTTACACTCATGAGCTGTTCCATCGTTGGGTGTTAAAACAGACGCAA
      ^
      SstIII

        430     440     450     460     470     480
TCCCCGGACGCTTAAACCAAGCTATACTAGCCTCCATACGTCCAGGCCTATTCTACGGAC
                                   ^           ^
                                   SstIII   StuI
                                   HaeIII

        490     500     510     520     530     540
AATGCTCAGAAATCTGCGGATCAAATCATAGCTTCATGCCAATCGTTCTAGAATTTATCT
      ^
      Sau3AI
    
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File: AOTUS KVI49/50J
Mode: Normal          1 -      540
Table: DNASIS1  Style: Linear  Indication Mode: Actual Cutting Site

      10      20      30      40      50      60
5'  GCTCACTAGTCCTATATATTATCTCCCTAATACTTACTACAAAATTAACCCACACCAGCA

      70      80      90      100     110     120
    CCATAAACGCCCAAGAAATTGAAATAATCTGAACTATTCTGCCCGCTATCATTCTTATTA

      130     140     150     160     170     180
    TAATTGCTCTCCCATCCCTACGTATCCTCTATATAACAGACGAATTTAATAAACCCCTACC
          ^
          SstIII

      190     200     210     220     230     240
    TAACCCTTAAAGCAATCGGCCATCAATGATACTGAAGCTATGAATACTCAGACTATGAAG
          ^                               ^
          HaeIII                         Sau3AI

      250     260     270     280     290     300
    ATCTAGCATTGACTCCTATATTACACCAACCTACTTCCTTGAACCTGGTGAGTTTCGAC
                                               ^
                                               TaqI

      310     320     330     340     350     360
    TTCTCGAAGTAGATAATCGAACCAACCCTCCCGATAGAAGCAGATATTCGTATACTAATTA
    ^           ^
    TaqI       TaqI

      370     380     390     400     410     420
    CATCACAAGATGTCTTACACTCATGAGCTGTTCCATCATTGGGTGTTAAAACAGACGCAA

      430     440     450     460     470     480
    TCCCCGACGCTTAAACCAAGCCATACTAGCCTCTATACGTCCAGGCCTATTCTATGGAC
          ^           ^
          SstIII     StuI
                  HaeIII

      490     500     510     520     530     540
    AGTGCTCAGAAATCTGCGGATCAAATCATAGCTTCATGCCTATCGTTCTAGAATTTATCT
          ^
          Sau3AI

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File: AOTUS KII54J
Mode: Normal
Table: DNASIS1 Style: Linear Indication Mode: Actual Cutting Site
1 - 540

      10      20      30      40      50      60
5' GCTCACTAGTCCTATATATTATCTCCCTAATACTTACTACAAAATTAACCCACACCAGCA

      70      80      90      100     110     120
CCATAAACGCCAGGAAATCGAAATAATCTGAACTATTCTGCCCGCTATCATTCTTATTA
      ^
      TaqI

      130     140     150     160     170     180
TAATTGCTCTCCCATCCCTACGTATCCTATATATAACAGACGAATTTAATAAACCTACC
      ^
      SstIII

      190     200     210     220     230     240
TTACCCTTAAAGCAATTGGCCATCAATGATACTGAAGCTATGAATACTCAGACTATGAAG
      ^                               ^
      HaeIII                           Sau3AI

      250     260     270     280     290     300
ATCTAGCATTGATTCCTATATTACGCCAACCTACTTCCTTGAACCTGGCGAATTTGAC
      ^
      TaqI

      310     320     330     340     350     360
TTCTCGAAGTAGATAACCGAACAACCCTCCCAATAGAAGCAGATATTCGTATACTAATTA
      ^
      TaqI

      370     380     390     400     410     420
CATCACAAGACGTCTTACACTCATGAGCTGTTCCATCGTTAGGTGTTAAACAGACGCAA
      ^
      SstIII

      430     440     450     460     470     480
TCCCCGGACGCTTAAATCAAGCTATACTGGCCTCTATACGCCAGGCCTATTCTATGGAC
      ^                               ^
      HaeIII                           StuI
      HaeIII

      490     500     510     520     530     540
AGTGCTCAGAAATCTGCGGATCAAACCATAGCTTCATACCTATCGTTCTAGAATTTATCT
      ^
      Sau3AI
    
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File: AOTUS KI54J2
Mode: Normal          1 -      540
Table: DNASIS1 Style: Linear Indication Mode: Actual Cutting Site

      10      20      30      40      50      60
5' GCTCACTAGTCCTATATATTATTTCCCTAATACTTACTACAAAATTAACACACACTAGTA
                                     ^
                                     RsaI

      70      80      90      100     110     120
CCATAAACGCCCAAGAAATCGAAATAATCTGAACTATCCTGCCCGCAATCATTCTTATTA
                                     ^
                                     TaqI

     130     140     150     160     170     180
TAATTGCTCTCCCATCCCTACGTATCCTCTACATAACAGACGAATTTAATAAACCTACC
                                     ^
                                     SstIII

     190     200     210     220     230     240
TAACCCTCAAAGCAATTGGCCATCAATGATATTGAAGCTATGAATACTCAGACTATGAAG
                                     ^
                                     HaeIII

     250     260     270     280     290     300
ACCTAGCATTGATTCTATATTACACCAACCTACTTCCTTGAACCTGGCGAATTTGAC
                                                     ^
                                                     TaqI

     310     320     330     340     350     360
TTCTCGAAGTAGATAACCGGACAACCCTCCCAATAGAAGCAGATATTCGTATACTAATTA
^
TaqI

     370     380     390     400     410     420
CATCACAAGACGTCTTACACTCATGAGCTGTTCCATCATTAGGTGTTAAAACCGACGCAA
^
SstIII

     430     440     450     460     470     480
TCCCCGGACGCTTAAATCAACAGATACTGGCCTCTATGCGCCCAGGCCTATTCTACGGAC
                                     ^
                                     HaeIII
                                     ^
                                     StuI
                                     HaeIII

     490     500     510     520     530     540
AATGCTCAGAGATCTGCGGATCAAATCATAGCTTCATGCCTATCGTTCTAGAATTTATCT
^      ^
Sau3AI Sau3AI

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