1. My autoencoder is learning, but is not able to predict the correct outputs 100%. I do not know why (Fig 1). I decided to move forward with this neural network for the rest of the project, and it seems to be working just fine.

```
[[0 0 0 0 0 0 0 0]
 [0 1 0 0 0 0 0 0]
 [0 0 1 0 0 0 0 0]
 [0 0 0 0 0 0 0 0]
 [0 0 0 0 0 0 0 0]
 [0 0 0 0 0 0 0 0]
 [0 0 0 0 0 0 1 0]
 [0 0 0 0 0 0 0 1]]
[[1 0 0 0 0 0 0 0]
 [0 1 0 0 0 0 0 0]
 [0 0 1 0 0 0 0 0]
 [0 0 0 1 0 0 0 0]
 [0 0 0 0 1 0 0 0]
 [0 0 0 0 0 1 0 0]
 [0 0 0 0 0 0 1 0]
 [0 0 0 0 0 0 0 1]]

Correctly predicted:
[[False  True  True  True  True  True  True  True]
 [ True  True  True  True  True  True  True  True]
 [ True  True  True  True  True  True  True  True]
 [ True  True  True False  True  True  True  True]
 [ True  True  True  True False  True  True  True]
 [ True  True  True  True  True False  True  True]
 [ True  True  True  True  True  True  True  True]
 [ True  True  True  True  True  True  True  True]]
```

Figure 1: Top matrix is predicted. Second matrix is actual (also training data)

2. I am using this ([http://file.scirp.org/Html/3-9102277_65923.htm)](http://file.scirp.org/Html/3-9102277_65923.htm) method for turning DNA sequences into number arrays. It is based on creating a sliding window (with size 3) that turns each set of 3 DNA bases into a unique binary vector. The Region Size defines how many of these windows you fuse together into a single vector. I started with variable region sizes, but it was far simpler to turn the whole 17 bases into a single region.

I am feeding these vectors into the NN that I developed for the autoencoder. The NN has 64*region_size input neurons. 64 is 4^3 because of the 4 unique DNA bases, and 3 bases to a codon. So, 64 unique codons, and each one needs to be different when encoded into a binary vector, with a single 1 apiece, so each codon vector needs to be 64 long. The region size is how many of these codons get strung together.

The middle layer of the neural network is variable, but I have it initially set to log2(input_layer_size), which should be the minimal size needed to respond to each input.

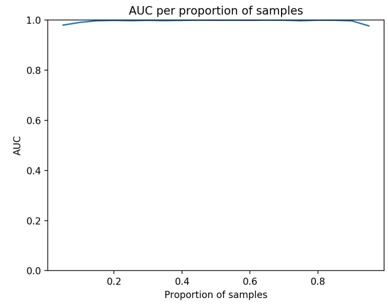
The output layer is size 1. A single output prediction of binding site.

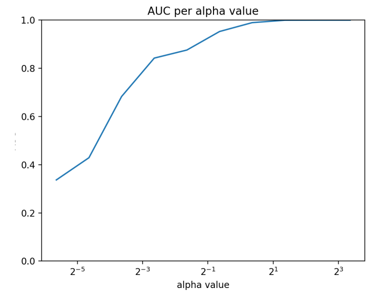
The Neural network is using sigmoid activation.

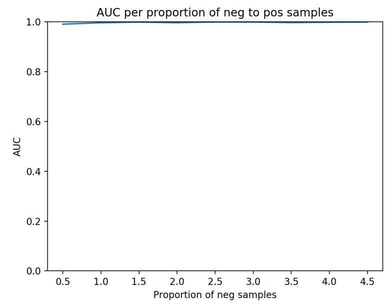
3. My training regime revolves around taking a subset of random samples from the positive sequences, the size of this subset is variable, the samples that are not included in the subset are set aside for testing and validation. I do the same thing for the negative samples, but the amount of samples taken is set to a proportion of the positive samples taken, with the default being 1:1. Since the negative samples are much longer (1000bp) than the positive samples, I also randomly take a 17bp region from the selected negative samples. The rest of the negative samples are held back for testing and validation (also with the random 17bp region selection). The positive and negative training data is concatenated into a single array, with the positive and negative true values (1's and 0's) being concatenated into a corresponding true array. The withheld positive and negative samples are concatenated into a test array, with the true values also being put into a label array.

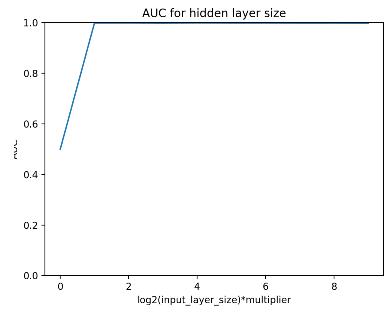
The training array is used to train the neural network, then the test array is used to validate the network performance. Every time the nn_test function is called, a different random subset of training and test data is used. With the method I have used to bring in the negative data, it is not overwhelming the pos data at all. I have been able to go up to 5x neg data to pos data without overwhelming the network.

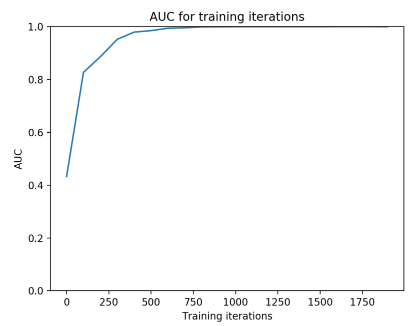
4. To test performance of the network, I calculated the area under the ROC curve for the withheld samples, this was my metric. The different parameters I varied were; hidden layer size, training iterations, subset proportion (to positive samples), negative to positive sample proportion, lambda and alpha. To test these different parameters, I varied each one over a range that I thought would be significant, graphing the area under the curve (AUC) for each iteration.

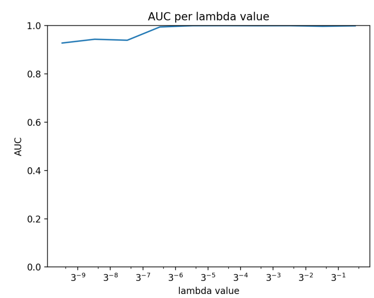






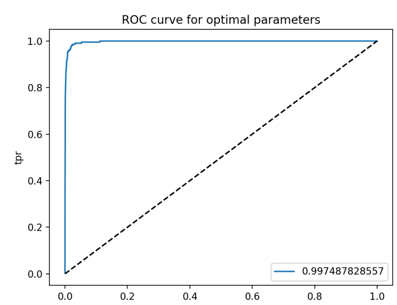






The point that I chose was any point close to an AUC of 1, with preference given to points that were on the edge of the curve (so closer to a lower AUC), because I thought those points would still have good performance, but have a lower chance of overfitting.

The set of learning parameters that seemed to work best are as follows; hidden layer size (2), training iterations (1000), subset proportion (0.75), neg to pos sample ratio (5), lambda (0.001), alpha (1). For training subset proportion I wanted to grab a large amount of the positive samples for training since there are not many, and for the neg to pos sample ratio, I also wanted to grab a large amount of negative samples since there is so much variation in the sequences after taking random subsets.



I have included the final ROC curve for these parameters based on the withheld test data.

For a lot of the parameters, changing them below a certain threshold has a large effect on the corresponding ROC (like for training iterations, hidden layer size, and alpha value). All of these values have a large effect on the training and information holding capabilities of the network, so it makes sense that if they are too small, the network performance seriously suffers.

5. Here are my predictions, they are also in predictions.txt

CCACCCACGCATCCAAA	0.797964209201
AAAGCCAGACACATCTT	0.173261432538
AAATCCGTGCACCCCGT	0.92664182296
GCATGCACTCAACTGAA	0.0562575776959
ACTTCCATATACCTAAA	0.361885421529
ACACCCTTACCAATCAG	0.854245226194
GCACCCGTCAACATCGC	0.721376209344
AGGCCCAATCACCTAGT	0.563148290247
TTGTCCATGAACTTTGG	0.155322138048
GCCTACGTACACTTCCA	0.799126154741
GAATCAAGACACCTGGT	0.318325904934
TCCGCCATATACTCGAA	0.203068556849
TCTTCCAGGCCCTCCAC	0.573183288561
AAACCCATAGAGCACGC	0.658937357764
TGGCTCATACCTGTCAT	0.19236809169
AGGCACATGCACTAGAG	0.351185453365
GGACCCATGCACCACAT	0.948409081526
ACATCCCAGCCTCTCAT	0.428136287174
ACACCAAAACACTTGAT	0.369532503406
ACGCTCATGCCTCAAAA	0.151332712969
ACACCCACATCCCACAG	0.949857702252
AAACGCATGCGCACGGC	0.159111797479
ATATCCATACAATAGAA	0.711952820228
GAACCAGTACATAAAAG	0.724024618227
GAAACCTTACCTGGAAC	0.127654831195
GCGCCCCAAGACGTTAT	0.227542079863
AAGTTCAGACACCAACC	0.430508221574
AGCCACAAACACTTTTA	0.109326418392
ACACCCAGTAACCCTAT	0.746665382898
AAACGCATGCAATTTAA	0.0565113769005
GACCACATACACCTTTT	0.432473934062
TGGTCCATATACTCAGT	0.129370582686
GCATCAGCTCATCTTGG	0.416273402303
TGAGACATACTCCACAA	0.198181569106
ATAACCATGCATATCAC	0.373668664149
TTGTACATATACCTCAC	0.327239478205
CCCCCCTGTCATCTAAG	0.326173686745
GCACCAGAACATCCCGA	0.823727534158
TGACCCATACATTTTGG	0.886835190694
AAATCCATCCATTACCT	0.414672010754
ACAGCCATTCAACTCAG	0.253367068449
GTATCAATAAACTTCCC	0.0643796297572
GAACCCAGCCCCTATGC	0.794756132585
TGACCCAGTCATTTCTT	0.590110184613

GAATCCATGAAACTCAT	0.659027316633
AAACCCAAACAATTGTT	0.720958704798
TGACCCATACAAGTACA	0.832700380252
GACTCCATGTATTCTGT	0.154037060632
ACAACCACGCATAGATT	0.0911037849257
ACATCCGAACATTTCCA	0.804427349443
AGGCACATACCCATTTC	0.391094421171
AGGCTCATACCCACATT	0.0219819859939
AAACCCAAACAGGTCGT	0.912473066524
ACAACCGTTCATCAAGC	0.383596400374
ATTTCCGTGCACTTCGT	0.712325927472
GAAGTCAGAAACCCCGC	0.0590601792773
AGACACATCAACTCCTC	0.0437969237925
ACCTACAGGCAGTCCGA	0.148722324245
TCGCCGAGACCCTTTTA	0.0444887698238
TCGTGCCTACACGCGCA	0.36444762536
TAATCCTGACGTTATGT	0.2870535915
TCATCCATTCATTGGAG	0.599020316598
GTGCTCATCCTCCCCAA	0.0620136832013
TAATACATACCCCACTC	0.736784729291
AGCTGCAGACATCTTGT	0.295003965498
AGATCCACAGACTTTCT	0.407092172465
CAACCCAAGCCCTAGAG	0.446816081726
ACATCCGGTCACACAAC	0.206900501049
AGACCCATACATCACAA	0.910306771952
AGTTCCGCACCACTGAC	0.151795439077
AAGTACATACCTTTTTC	0.158096096443
TCGGTCATACATACGTA	0.168915705596
GCGACCATATACCCCTC	0.260402726311
TCATGCAGACCCTCAAG	0.0669561342137
AGCTTCATTCTCTTCGC	0.0650766591692
AAATCCGGAAATAATAT	0.491155945791
GCATCCATGGAATTTGA	0.615141094354
CAGCCCTTACATTTCAG	0.935710309843
TCATGCAAGCAATCTTC	0.00907048915738
ACTCCCTTACCCGACGG	0.484967783059
ACGCCCGTGCCCCGCGA	0.813946543458
TCGCTCATAAACCCCAG	0.106073404386
AAACCCAGGAACTACGC	0.469523607478
AAATCCGAACATAAAGC	0.682961457864
ACACACACACACATAAA	0.481823193661
TTCCGCATGCATCAGAC	0.140317988626
ACACCCTCACAGAACAT	0.831540463996
AGACCCAAAACTTCAGC	0.783515519808

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ATCTCCAAAGACCTTTA	0.196613888885
GTGTCCATGCACTTTAA	0.623694703004
GAATGCATCCATTTCGC	0.12947021904
CCATCCAATACCTTGGA	0.351178651112
ACACCCAGACCGCTGAG	0.791579286564
GCATACAAACAACTCAT	0.472413577746
AAGCCCATCCACGTAAC	0.338824546066
ACCTCCAGACTCTTCCA	0.427871745211
CCACCAGGACATTTCCA	0.858226500906
GGTCCCAAACATCAGGG	0.880158781402
ACACGCACACATTCACA	0.484772741676
ATAAAGGCGCAACTTCG	0.0339304307954
CACCCCAAACACCCTGA	0.868994741448
AGGCCCTTACAGAGCAG	0.756804241961
AAACTCACGGACTCGAG	0.0839943439944
GAAACCATACCCTTCGG	0.70915847314
GACACCATACATCACCT	0.28073401045
GCGCCCTAACATCCGCA	0.299874989494
TCATCCTTGCATTACCC	0.538437957227
GGACTCAAAAATCCCAA	0.23731862287
ACTTCCGGACATTTCCT	0.498540740725
ACCCCCACACATAGGGT	0.573445850259
GCAGCCGCACATCCAGC	0.32322735674
AACTGCATACTCTTCTC	0.289153583229
GTGTTCATAAACGTGGT	0.136370798259
GCACCCATACATTATAA	0.969163483279
TGATTCACACACGGTGC	0.634883558893
GCACCCATACCTTCCTT	0.885160538298
ACACCCTTACCCATCCA	0.846015249931
GCCACCATACGTTACCA	0.542363306148
TTACCCATACTCTTCAT0.826157718543	
TGGGCCATATACACTAA	0.0491966248348
TCATCCACACCTGAAAT	0.551377562561
ACACTCAATCACGTGGG	0.0773974832019
GAATTCATACGCCTCTA	0.105956560804
ACGATCATACATTCTGT	0.763596049967
TCACCCAAACATAGCAC	0.943657075351
ACACTCATACATCCGCT	0.500229111883
TTACGCATGAACTCGTA	0.0886523520624
AAATCCGTACATTTTGA	0.913614217599
GTCTCCAGACACTGCTC	0.507234501779
AATTACACGCACCCGAT	0.253843349676
ACATCCGGGCATGCTCT	0.132342746156

GAAACCAAGCATCAAAC	0.197068284635
AGCTCCATATCCCTTAA	0.425443090368
AACGCCGTGCACGTGAC	0.346854580457
ATACGCATACAAATCAA	0.359285106197
TTCTGTATACACCCGCA	0.282634350859
CCATCCGCTCACCTGAA	0.37715380143
ACTCCCATATACACCAT	0.570219931809
GAGATGATGCCCTCCTA	0.208915551002
TACTACTTACACCTGAC	0.132494540437
AGCTCCAAACCCTTTAA	0.199779681021
TGCTGCACACCCTAGGT	0.15779643169
GAAGCCATAAACGCTCC	0.105099415151
ACATCCTGACAAGTCTC	0.298976260016
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GTGTCCATGACCTTAGG	0.393672318075
TCTCCCATACCATCGAA	0.676628897364
TGATCCATTCAGTCCAT	0.771940076204
TATCCCTTGCCTCGTCT0.449259782447	
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TCACTCAAGTACTACAA	0.0176182582579
AAATACATACAATTTAG	0.418126169451
AAAACCATACACATTCG	0.627238151159
TAAACCAGAGACTCCGA	0.214930180559
TGGTCCCTCCATCCCAG	0.0948819583479
ACACTCAGGCATTCTAC	0.0965473162241
ATATACAAACACCCGGT	0.500387300492
TAATACATACAACTTAG	0.573572907616
TGGCCCATACTTCTTAA	0.817430749541
AATTCCATAGACTTGAT	0.61971301845
GAAACGATACACCTGCA	0.263707386071
AGGCGCACGCACCTTAA	0.255615231612
GGGTTCACAGACACAAA	0.0312442208855
GCAGCCAAGCCCATTAA	0.100915971878
GACCACGTACACACCAG	0.787584831004
ATATCCGCGCAATTTAA	0.0706226552644
GGAGCCATACACGCGTC	0.503906098701
ACCTCCGTACACCTTCT	0.832544669867
ACGCCCACAAACCGGTA	0.468055440251
ACACCGTGACATACTAC	0.554812273513
AGATTCACACCCTCTTG	0.0888251867636
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TACTCCATGCAAGTCAG	0.429722638123
AAATCCATAAACGTATA	0.62721276762
CAATCCTGACATATATT	0.224974184637

AGATCCAAAGATCCCAT	0.470661344938
AAACCCAGCCATCAATT	0.409000589765
GAGTTCATAGATGTGAC	0.0207594704506
GCGCCTGTGCACTACAT	0.196587775736
TATTCCATACCTCAAAA	0.840582504186
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GCATTCATTAACTTCAA	0.130988545426
GAATCCATCCACAGCGT	0.778790844331
GAGCCAATGCACTAAAC	0.0472769007351
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TGAACCATTCATCAGAT	0.236218934346
ACCTCCGGCCACAACAT	0.0229808484601
AAATCCATAAATTCCTC	0.734409714691
GGATACACACCTCCCTC	0.501429386091
ACACCCACACCCACCCA	0.850376083723
ATCTACAGATACTCCGA	0.0466796637945
TCAGCCAAGCATCTGTA	0.102150841844
TGAACCAAACACCAAGG	0.648345664134
TGCTCTATGAATCCCAA	0.146434718262
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GGGTCCACTTACCCCTC	0.230745100535
ACATCGTTACATTGTAT	0.564939607423
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TCCACCATAGACTTGGC	0.572543971533
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CCATCCGTACGCACAAA	0.342976112483
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AGCTCCATTCAATCAGA	0.156649789206
GACCCCAAACCTTTTCC	0.321943925985
TCTTCCAAGCATTCAGA	0.223108819348
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AACAGCAAACACTCCGA	0.128800418689
GCTTACAGTCACTCCGC	0.147925415399
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AACCCCATACAGTGTTT	0.899273377506
AACTCCATGCATTATTA	0.720128689868

GTGACCATGGACCTGTC	0.27637691802
GTCGTCATACACTCCTC	0.0807147560492
ACATCCGTACATTACTA	0.893108630096
ACACCGTTGCCTACAGT	0.403971660806
GCTCCCAGGCACCGCAT	0.783152866206
TGGTGCATATACATGGA	0.149853363955
TCGCACACACAACCAAT	0.0574185159086
CAATCCAAACCTTTAAT	0.740350669727
GCATCCCTTCCCCACGT	0.647190382828
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CCAACCATTCCTTCCTA	0.0732978643116
ACACACACACAGACGGA	0.406803002606
TCGCCCAAACCTTCTAG	0.411216403443
GTAGCCACACCCTTCGC	0.170980767373
AAGGCCATGCACATAAT	0.123254997543
GCATCCAAGACCCTCCC	0.645525680611
CCTCTCACGCACCTGAA	0.129195157695
GGCTCCATTCCTTCTTT	0.253639984054
GAGTACAAACAAGTCGC	0.160130750989
TCTTCCACACACACAAA	0.374278509936
GCCTTCATGCCTGCCTC	0.385750568298
TAATCCACACGTACCCA	0.485439087359
AGCAGCAGACACTCCTT	0.0290604216056
TCGGCCAGGCATCATAT	0.183461812173
GGCTCCCAGCCCTTGGA	0.154887798917
ACACACACACACATATA	0.314845785988
AAACCCAATCCCTTCAG	0.772148437624
ACCTCCAACCCTCCTGA	0.420244325925
ACCAGCATACTCTCCAT	0.21477898182
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CCATCCAAACATAGATA	0.553207160788
GAATGCAAACAACTGCA	0.218505663338
ATACACGTACATATTTT	0.684744924634
ACACCCAAACACCTCTT	0.897566989971
GAGAACGCACATCCCAA	0.556836435098
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GAGCCGGTACAAGTCTT	0.086082031503
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GGATTCAAAACCTTGAA	0.0892440487468
AGACCCATATCTTCGTC	0.673316157045
AAATCCGCAAACATCAA	0.441977507906
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GCAACCATACAAGCGGT	0.627608604862
GCATCCGTGCCTCCTGG	0.879015195371
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ACACGCGTACCTGAAAA	0.830569662822
GCATCCACACGTCACAA	0.685674768907
ACACCACGACATCACCT	0.480833661245
TGGTACATAGACTTGAC	0.144424835768
CAGCCCACACATGAGGC	0.784907546302
GCACCCACACCTCGCAT	0.850819367593
GCCTCCGTACCCTCACA	0.624193979634
TATTGCATGCCCTCTAC	0.160277810254
CAATCCATGCACAAAAA	0.876945070604
AAGTCGATACACTATAT	0.116814308577
ACACCCACACACACCAC	0.973521700895
GGCTTCATACATAATAA	0.443567924461
CAACCAGTACAGAGAGT	0.691022205303
GAGCCCAGGCCTCCGTG	0.592519329338
AAATCGGCAGATCATCC	0.0859562456064
GCGTCCAAGCACGCCCG	0.394545759328
ACTTGCATACACGTAAC	0.384366583184
GTAACAATACCCTCGAA	0.0348579312473
ACAACCAAAGCCTACGA	0.232274874586
TCGGCCACACCGCTCAT	0.109276529989
GTCACCAGGCACGTCGA	0.315812464658
CAACCCATTCATCAAAT	0.454103177085
ACGACCATACCAGTGGA	0.299056458588
TGGTGCATATATTTAAA	0.127892040568
GCTTCCACACATAATAA	0.468883713761
CCCCCCGTTCGTTGCAA	0.84004626323
GAATTCTTACCCCACCA	0.585481248802
GCATCCTTACCCAACAG	0.704047360197
ACATCCATACATACGCC	0.850486248328
TACCCCTTACCTCTCGC	0.422260655726
GCACCAGTACACTTATC	0.915469375152
GCGTACATACACCAGCA	0.759401667214
TGTTCCATACATTGCAG	0.893278460812
AGACCCGTCCCTAGAGT	0.659913376538
AAACCCATACAATAGCC	0.867518235356
TCCCCCCTGCATTCCCA	0.469325888247
ACGCCCACGCACCCCAC	0.877683947796
AAGGTCACACATCACAA	0.108235292332
GCCACCATGCATCGTTC	0.362929863598
GCGCTCATGCCACTGAT	0.0544540708445

TCATCCTCTCGTCGTCT	0.122617764831
ACACCCAAACATATTAT	0.929582339898
AACTCCGTACACCCGGC	0.874349935345
AAACACACACACTCACC	0.162846817485
ACCTTCATACAGTTGTT	0.309303679405
AAACCCAAGCCCAAGCT	0.449453558156
GAACCCATACATTACTT	0.902291966687
AGTTCCATAAACCTTTC	0.281279547649
CCATGCAGACACTTTGT	0.241045647169
GCAGCCAGAACTCTTGC	0.453084408409
AAAAACACACATCTGGC	0.153823111952
ACATGCAGGCATTCTCA	0.263800812911
ACACCAGCACCCAAAGC	0.525060502306
TACCATATACCCCTCAA	0.202087912266
ATACGCATACACGGGAT	0.533953694368
ACATCCTTCCATGCCCT	0.0783276564495
GCATCCGTACTCTCATA	0.677312922497
TCTTCCAAGCATCTCAT	0.343340107287
AAATCCAAGCATTATCC	0.358584254444
CAATCCATACAATTCTT	0.501671273469
AGGCCCAACCATCTCTG	0.296768384201
CCACCGTGACATCCTCT	0.604478816949
TAACCCAAGCATCAATT	0.28466671743
TGCTCCATTCAACCGAG	0.282465197
TGACCCAAACTTTGCGA	0.897676613361
ACAACCGAACACCACCT	0.459066817064
TAGCCCAGAGCCCTCTT	0.466809861271
AAATCCATACATTTTAA	0.843919940843
AAGCCCATGCTACTCAA	0.444481338748
ACACCCATACATCTAGA	0.948308759755
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ACGAGCACACACGTGAT	0.162155517424
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