Finding a Tandem Repeats Motifs in the Completed Genomes of Human Coronavirus (hku1) Which is Identified as a Hotspot Region for the Viruses Recombination by Using Regular Expression Language

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Abstract

A lot of research studies have been surveyed the completed genomes of prokaryotic and eukaryotic and focused on the correlation between the percentage of microsatellite sequences in completed genomes and the whole size of the organism genomes. There are fewer studies made in repetitive sequences otherwise simple sequence repeats or long tandem repeats of virus genomes. simple sequence repeats (SSRs) are the most important regions for recombination and moving repeats blocks from site to another site in the genomes. A tool was programmed and designed by visual basic 6.0 to find the long tandem repeats in DNA sequences of the small genomes. The tool named "Repeater Finder Regular Expression", (RFRE) Version 1.0, 2016. The tool was utilized to discover different pattern of long tandem repeats (LTR) motifs on the completed genomes of human corona virus strains by using a joined regular expression language. In this study, a twenty-nine accession numbers of human coronavirus completed genomes, (hku1) strains were retrieved from the Genbank. The researcher can write a different regular expression patterns and joined regular expression patterns through the designed tool to search and find a specific motif of nucleotide sequences inside the complete genomes. The RFRE tool searched and found three different total lengths of a perfect long tandem repeats (240bp, 300bp and 480bp). A Dot plot gave a picture view for the long tandem repeat sequences in the completed genome sequence (KF430201.1) of human coronavirus. The genomic dot plot tool YASS was used as a genomic similarity searching tool to check for the uninterrupted repeats and confirm the sensitivity of the (RFRE) tool. To identify the recombination site in the genomes of human coronavirus the RAT tool was applied to find the recombination sites between the completed genomes of human corona viruses .The RAT tool recognized the recombination site in the nucleotide position (3012) and at the same time this

recombination site position (3012) was also recognized as a beginning position of a long tandem repeat. A precise motif was predicted from the translated repeats of Human Corona Virus which found by PRATT tool. There was a relationship between the total length of long tandem repeats and genome size of Human Corona Virus and the correlation value R^2 was equal to (0,451). In conclusion, this study presented the importance of finding the long tandem repeats of human coronavirus and gives a relationship between the completed genome size of human coronavirus types and long tandem repeats. The nucleotide position (3012) was a hot spot site for a recombination among the complete genomes of human coronavirus and also identified as a repetitive site in the genomes of human corona virus (*hku1*). The repeats in human coronavirus (*hku1*) were predicated to be a main major role of virus evolution.

Key words: Repeats Blocks; Repeater Finder Regular Expression; Tandem Repeats of Human Coronavirus

1. Introduction

A lot of studies had been made in the finished genomes of prokaryotic and eukaryotic to illustrate a correlation between the total length of different repeats patterns in the completed genomes and genome size but there are a small numbers of research studies are made in the completed genomes of virus to illustrate the correlation between the total length of repetitive sequences in the virus genome and genome size of viruses [1]. Twenty-two strains of human coronavirus, (HKU1) completed genomes were matched after sequencing and categorized in different genotypes (A, B and C) [2]. The human coronavirus, (HKU1) strains contain an acidic tandem repeats, which encodes the following amino acids residues - NDDEDVVTGD [2]. The recombination mechanism in human coronavirus lead to form a new four genotypes of human coronavirus (A,

B, C and D) [3]. The genotypes of human coronavirus, (HKU1) linked with human infection and this linkage was responsible for new genotype of human coronavirus, (HKU1) [3]. Recombination between the repeat regions in the finished genomes of different human coronavirus, (HKU1) strains was observed and considered to be a main major role of human coronavirus evolution [2]. Imperfect microsatellites repeats are detected in the different strains of coronavirus (HKU1) that could be found due to the incorrect replication by the viral polymerase [2]. Recombination considered as one of the procedure of virus evolution [4]. Simple sequence repeats are the essential sites for virus recombination and exchange repeats blocks from region to another region in the human genome [5]. There is a correlation between the size of viruses' genome and SSR density [1]. The great variances in genome size of viruses give a wide possible to study the perfect and imperfect repeats length of virus's genome [1]. The number of repeats was caused by an error rates of viral polymerase replication or by recombination evidence between the repeat blocks of different virus's strains [6]. A number of herpes viruses' telomeric repeats (TMRs) are identical to the telomeric repeat sequences of the host in their genomes, TMRs although they are genetically classifying herpes virus to classes A, C, D, or E genome [7]. Some SSR sequences can stimulate the recombination and special effects on DNA structure where the repeats (GT, CA, CT, GA, GC, or AT) were considered as repeat-binding proteins [8].

2. Material and Methods

2.1 Retrieving (hku1) strains of coronavirus

A twenty-nine completed genome records were retrieved from the primary database of Genbank, NCBI core nucleotide database and downloaded in fasta format file then organized in (Table 1).

Table 1. The Retrieved Accession Numbers (twenty-nine records) of Human coronavirus Completed Genome ((HKU1)) from Archived Genbank of NCBI.

Accession number	Genome Size	Accession number	Genome Size
DQ415904.1	29295	HM034837	29862
KF686344.1	29695	DQ415914.1	29887
KF686342.1	29742	DQ415911.1	29902
DQ415909.1	29767	DQ415903.1	29920
DQ415913.1	29785	KF686340.1	29923
DQ415910.1	29797	AY597011.2	29926
DQ415900.1	29812	NC_006577.2	29926
DQ415902.1	29845	KF430201.1	29934
DQ415912.1	29845	DQ415908.1	29947
DQ415905.1	29857	KF686346.1	29982
DQ415906.1	29857	KF686343.1	29983
DQ415901.1	30097	DQ415897.1	29845
DQ339101.1	29755	DQ415898.1	29815
KF686341.1	29832	DQ415899.1	29905
DQ415896.1	29860		

2.2 Analyzing the completed genomes

The repeated sequences of the (hku1) strain of coronavirus completed genomes were found and analyzed by a new-programmed tool, RFRE Version 1.0 (Repeater Finder Regular Expression). The tool can be downloaded via the following link (<https://sourceforge.net/projects/rfret/>). The RFRE tool found the lengths of repeat, positions of repeat, frequencies of repeat and the densities of repeat. The tool used the regular expression language to find different types of patterns and the user could easily change the syntax of regular expression command to retrieve different forms of specific repeats. The tool had an easily GUI (Graphical User Interface) which could be used by a nonprofessional user. For example the regular expression pattern **([agct]{30})\1** was accepted by the RFRE tool to find a different repeated patterns with sixty characters in length. The input pattern

([agct]{30})\1 was used to match any pattern of characters a or g or c or t [agct] and repeated two times by a length thirty characters {30}. The Metacharacter back reference \1 was used to match the same text again. Back references match the same text as previously matched by a catching group, for example the same back reference can be used again more than once **([y|c])x\1** matches yxyxa, cxcxb and cxcxc. The outputs results of using RFRE tool could be saved in word file or excel sheet.

2.3 Organizing the output of the results in excel sheets

The results were archived and recorded in the excel sheet to finalize the results in charts to show the relationship between the genome size and the repeat length.

2.4 Predict a specific motif for the extracted repeats

All the extracted perfect repeats were aligned by using MAFFT tool, which was used as a plugin of Unipro UGENE version (1.16.1, Mar 17-2015) to find a highly conserved sequence. The conserved sequence was saved in a fasta format file and converted to a protein sequence by using ORF finder tool (<http://www.ncbi.nlm.nih.gov/projects/gorf/>). The translated frame was used to hit the protein sequence database by using PSI-BLAST of Primary database of Genbank, European bioinformatics institute (EBI) which had the following web address (<http://www.ebi.ac.uk/Tools/sss/psiblast>). EBI had The PRATT tool, (<http://www.ebi.ac.uk/Tools/pfa/pratt/>) which was used to search for a conserved patterns in sets of aligned protein sequences, which aligned to the translated pattern of the conserved perfect repeat. The scanprosite of Expasy, bioinformatics resource portal server (<http://prosite.expasy.org/scanprosite/>)

was fed by the expected pattern which produced by PRATT tool to find a specific motif in the Human coronavirus (*hku1*).

2.5 Using the Dot plot to present the repeats in a picture view

To find a picture view for a repeated sequences in the completed genome sequence (KF430201.1) of human coronavirus the YASS online tool (<http://bioinfo.lifl.fr/yass>) was used to produce this picture view in dot plot view. YASS is a genomic similarity search tool to produce a local alignment results. YASS, Like most of the heuristic pairwise local alignment tools for DNA sequences (FASTA, BLAST, PATTERNHUNTER, BLASTZ/LASTZ, LAST).

2.6 Detecting recombination among genomes

The RAT (**Recombination analysis tool**) program was used to detect the recombination between multiple sequences, in a straightforward graphical user interface. The fasta format of the human corona virus completed genome were downloaded and organized in the previous Table, (Table1) then aligned by clustalw omega (www.ebi.ac.uk/Tools/msa/clustalo/) The RAT tool processed the multiple aligned sequences. The RAT tool was downloaded from this web site (<https://github.com/ethering/RAT>). The result can be exported to excel file included the possible recombination spots.

2.7 Assign the repeats to annotated map

The highest total length of the extracted repeats which found in the completed genome of Human corona virus (kf686343.1) were assigned to the annotated map of the following accession number (kf686343.1) by using Unipro UGENE ver. 1.16, 2015 by download the Genbank report of the following accession number (kf686343.1).

3. Results and discussion

3.1 The input regular expression and back references

To find repeated sequences in twenty nine records of human coronavirus completed genome a specific regular expression command was used, `([agct]{30})\1` through the designed tool (RFRE 1.0) which accepted that regular expression behavior. The behavior of using the regular expression, `([agct]{30})\1` found a repeated sequences in groups and in a perfect form. The meaning of every metacharacter in the previous regular expression, the metacharacter `()` was meant it matched a pattern and captured the matched pattern where the captured match can be retrieved from the resulting matched collection. Metacharacter `[agct]` was meant it matched a or g or c or t and the metacharacter `{30}` was meant it matched exactly n times but the memory parentheses and back references was used `\1` to make the regular sub expression in parentheses (r), the matched subsequence was remembered. The matched subsequence was bound to the matched variable `\1`. If there were more parentheses, their matched get bound to `\2`, `\3`, etc. so the previous regular expression found the pattern more than one period and the length of the found repeats were 60 characters in length. A simple example for the backreference system, A repeat such as ACTACT in a DNA sequence can be described by `([ACGT]+)\1` The nucleotide sequence matched `[ACGT]+` must be appeared again to be matched by `\1`. The repeats, which were found were organized and described in (Table 2). A comparison between the total of repeat length and genome size in different strains of human corona virus was

presented that the following accession numbers (kf686346.1 and kf686343.1) had the same highest repeat length(480 bp) which bolded and shaded by gray color as shown in the (Table 2 & Table 3). All 22 genomes of Coronavirus (HKU1) Genomes had tandem duplicates of a perfect 30-base acidic tandem repeat (ATR) which encodes NDDEDVVTGD [2]. The (Fig.1) displayed the comparison between the total of repeat lengths and genome size in different strains of human corona virus. There were a fluctuation among repeat lengths and genome size as shown in (Fig. 1). A correlation between the total length of repeats and genome size presented by the regression type, power of trendline as shown in (Fig. 2). The R^2 value was displayed on the chart where the ($R^2 = 0.451$) as shown in (Fig. 2). A relationship between simple sequence repeats (SSRs) and virus genome size where SSRs were accumulated in virus with large genomes, and the longer genome sequence contained the longer repeat units [9].

Table 2. The results of tandem repeats which were found by using a specific regular expression syntax ([agct]{30})¹ which written through the RFRE tool.

Genome ID	Motif	Iteration	Start	End	Repeat Unit / Position	Repeat Length	Genome Size
DQ415910.1	aatgacgatgaagatggtgttactggtgac	2	3031	3091	30	60	29797
DQ415910.1	aatgacgatgaagatggtgttactggtgac	2	3091	3151	30	60	
DQ415910.1	aatgacgatgaagatggtgttactggtgac	2	3151	3211	30	60	
DQ415910.1	aatgacgatgaagatggtgttactggtgac	2	3211	3271	30	60	
DQ415910.1	aatgacgatgaagatggtgttactggtgac	2	3271	3331	30	60	
DQ415909.1	aatgacgatgaagatggtgttactggtgac	2	3031	3091	30	60	29767
DQ415909.1	aatgacgatgaagatggtgttactggtgac	2	3091	3151	30	60	
DQ415909.1	aatgacgatgaagatggtgttactggtgac	2	3151	3211	30	60	
DQ415909.1	aatgacgatgaagatggtgttactggtgac	2	3211	3271	30	60	
DQ415908.1	aatgacgatgaagatggtgttactggtgac	2	3031	3091	30	60	29947
DQ415908.1	aatgacgatgaagatggtgttactggtgac	2	3091	3151	30	60	
DQ415908.1	aatgacgatgaagatggtgttactggtgac	2	3151	3211	30	60	
DQ415908.1	aatgacgatgaagatggtgttactggtgac	2	3211	3271	30	60	
DQ415908.1	aatgacgatgaagatggtgttactggtgac	2	3271	3331	30	60	
DQ415908.1	aatgacgatgaagatggtgttactggtgac	2	3331	3391	30	60	
DQ415908.1	aatgacgatgaagatggtgttactggtgac	2	3391	3451	30	60	

<u>DQ415906.1</u>	aatgacgatgaagatgttggttactggtgac	2	3031	3091	30	60	29857
DQ415906.1	aatgacgatgaagatgttggttactggtgac	2	3091	3151	30	60	
DQ415906.1	aatgacgatgaagatgttggttactggtgac	2	3151	3211	30	60	
DQ415906.1	aatgacgatgaagatgttggttactggtgac	2	3211	3271	30	60	
DQ415906.1	aatgacgatgaagatgttggttactggtgac	2	3271	3331	30	60	
DQ415906.1	aatgacgatgaagatgttggttactggtgac	2	3331	3391	30	60	
<u>DQ415905.1</u>	aatgacgatgaagatgttggttactggtgac	2	3031	3091	30	60	29857
DQ415905.1	aatgacgatgaagatgttggttactggtgac	2	3091	3151	30	60	
DQ415905.1	aatgacgatgaagatgttggttactggtgac	2	3151	3211	30	60	
DQ415905.1	aatgacgatgaagatgttggttactggtgac	2	3211	3271	30	60	
DQ415905.1	aatgacgatgaagatgttggttactggtgac	2	3271	3331	30	60	
DQ415905.1	aatgacgatgaagatgttggttactggtgac	2	3331	3391	30	60	
<u>DQ415904.1</u>	aatgacgatgaagatgttggttactggtgac	2	3031	3091	30	60	29295
<u>DQ415903.1</u>	aatgacgatgaagatgttggttactggtgac	2	3331	3391	30	60	29920
DQ415903.1	aatgacgatgaagatgttggttactggtgac	2	3391	3451	30	60	29920
<u>DQ415902.1</u>	aatgacgatgaagatgttggttactggtgac	2	3031	3091	30	60	29845
DQ415902.1	aatgacgatgaagatgttggttactggtgac	2	3091	3151	30	60	
DQ415902.1	aatgacgatgaagatgttggttactggtgac	2	3151	3211	30	60	
DQ415902.1	aatgacgatgaagatgttggttactggtgac	2	3211	3271	30	60	
DQ415902.1	aatgacgatgaagatgttggttactggtgac	2	3271	3331	30	60	
DQ415902.1	aatgacgatgaagatgttggttactggtgac	2	3331	3391	30	60	
<u>DQ415901.1</u>	aatgacgatgaagatgttggttactggtgac	2	3404	3464	30	60	30097
DQ415901.1	aatgacgatgaagatgttggttactggtgac	2	3464	3524	30	60	
DQ415901.1	aatgacgatgaagatgttggttactggtgac	2	3524	3584	30	60	
<u>DQ415900.1</u>	aatgacgatgaagatgttggttactggtgac	2	3031	3091	30	60	29812
DQ415900.1	aatgacgatgaagatgttggttactggtgac	2	3091	3151	30	60	
DQ415900.1	aatgacgatgaagatgttggttactggtgac	2	3151	3211	30	60	
DQ415900.1	aatgacgatgaagatgttggttactggtgac	2	3211	3271	30	60	
DQ415900.1	aatgacgatgaagatgttggttactggtgac	2	3271	3331	30	60	
<u>DQ339101.1</u>	caatgacgatgaagatgttggttactggtga	2	3030	3090	30	60	29755
DQ339101.1	caatgacgatgaagatgttggttactggtga	2	3090	3150	30	60	
DQ339101.1	caatgacgatgaagatgttggttactggtga	2	3150	3210	30	60	
DQ339101.1	acgatgaagatgttggttactggtgacaata	2	3245	3305	30	60	
<u>KF686346.1</u>	aatgacgatgaagatgttggttactggtgac	2	<u>3012</u>	<u>3072</u>	<u>30</u>	<u>60</u>	29982
<u>KF686346.1</u>	aatgacgatgaagatgttggttactggtgac	2	<u>3072</u>	<u>3132</u>	<u>30</u>	<u>60</u>	
<u>KF686346.1</u>	aatgacgatgaagatgttggttactggtgac	2	<u>3132</u>	<u>3192</u>	<u>30</u>	<u>60</u>	
<u>KF686346.1</u>	aatgacgatgaagatgttggttactggtgac	2	<u>3192</u>	<u>3252</u>	<u>30</u>	<u>60</u>	
<u>KF686346.1</u>	aatgacgatgaagatgttggttactggtgac	2	<u>3252</u>	<u>3312</u>	<u>30</u>	<u>60</u>	
<u>KF686346.1</u>	aatgacgatgaagatgttggttactggtgac	2	<u>3312</u>	<u>3372</u>	<u>30</u>	<u>60</u>	
<u>KF686346.1</u>	aatgacgatgaagatgttggttactggtgac	2	<u>3372</u>	<u>3432</u>	<u>30</u>	<u>60</u>	
<u>KF686346.1</u>	aatgacgatgaagatgttggttactggtgac	2	<u>3432</u>	<u>3492</u>	<u>30</u>	<u>60</u>	
<u>KF430201.1</u>	aatgacgatgaagatgttggttactggtgac	2	3027	3087	30	60	29934
KF430201.1	aatgacgatgaagatgttggttactggtgac	2	3087	3147	30	60	
KF430201.1	aatgacgatgaagatgttggttactggtgac	2	3147	3207	30	60	
KF430201.1	aatgacgatgaagatgttggttactggtgac	2	3207	3267	30	60	
KF430201.1	aatgacgatgaagatgttggttactggtgac	2	3267	3327	30	60	

KF430201.1	aatgacgatgaagatgttggttactggtgac	2	3327	3387	30	60	
KF430201.1	aatgacgatgaagatgttggttactggtgac	2	3387	3447	30	60	
<u>KF686344.1</u>	aatgacgatgaagatgttggttactggtgac	2	3012	3072	30	60	29695
KF686344.1	aatgacgatgaagatgttggttactggtgac	2	3072	3132	30	60	
KF686344.1	aatgacgatgaagatgttggttactggtgac	2	3132	3192	30	60	
KF686344.1	aatgacgatgaagatgttggttactggtgac	2	3192	3252	30	60	
<u>KF686343.1</u>	aatgacgatgaagatgttggttactggtgac	2	3012	3072	30	60	29983
<u>KF686343.1</u>	aatgacgatgaagatgttggttactggtgac	2	3072	3132	30	60	
<u>KF686343.1</u>	aatgacgatgaagatgttggttactggtgac	2	3132	3192	30	60	
<u>KF686343.1</u>	aatgacgatgaagatgttggttactggtgac	2	3192	3252	30	60	
<u>KF686343.1</u>	aatgacgatgaagatgttggttactggtgac	2	3252	3312	30	60	
<u>KF686343.1</u>	aatgacgatgaagatgttggttactggtgac	2	3312	3372	30	60	
<u>KF686343.1</u>	aatgacgatgaagatgttggttactggtgac	2	3372	3432	30	60	
<u>KF686343.1</u>	aatgacgatgaagatgttggttactggtgac	2	3432	3492	30	60	
<u>KF686342.1</u>	aatgacgatgaagatgttggttactggtgac	2	3012	3072	30	60	29742
KF686342.1	aatgacgatgaagatgttggttactggtgac	2	3072	3132	30	60	
KF686342.1	aatgacgatgaagatgttggttactggtgac	2	3132	3192	30	60	
KF686342.1	aatgacgatgaagatgttggttactggtgac	2	3192	3252	30	60	
<u>KF686341.1</u>	aatgacgatgaagatgttggttactggtgac	2	3012	3072	30	60	29832
KF686341.1	aatgacgatgaagatgttggttactggtgac	2	3072	3132	30	60	
KF686341.1	aatgacgatgaagatgttggttactggtgac	2	3132	3192	30	60	
KF686341.1	aatgacgatgaagatgttggttactggtgac	2	3192	3252	30	60	
KF686341.1	aatgacgatgaagatgttggttactggtgac	2	3252	3312	30	60	
KF686341.1	aatgacgatgaagatgttggttactggtgac	2	3312	3372	30	60	
<u>KF686340.1</u>	aatgacgatgaagatgttggttactggtgac	2	3012	3072	30	60	29923
KF686340.1	aatgacgatgaagatgttggttactggtgac	2	3072	3132	30	60	
KF686340.1	aatgacgatgaagatgttggttactggtgac	2	3132	3192	30	60	
KF686340.1	aatgacgatgaagatgttggttactggtgac	2	3192	3252	30	60	
KF686340.1	aatgacgatgaagatgttggttactggtgac	2	3252	3312	30	60	
KF686340.1	aatgacgatgaagatgttggttactggtgac	2	3312	3372	30	60	
KF686340.1	aatgacgatgaagatgttggttactggtgac	2	3372	3432	30	60	
<u>NC_006577.2</u>	aatgacgatgaagatgttggttactggtgac	2	3037	3097	30	60	29926
NC_006577.2	aatgacgatgaagatgttggttactggtgac	2	3097	3157	30	60	
NC_006577.2	aatgacgatgaagatgttggttactggtgac	2	3157	3217	30	60	
NC_006577.2	aatgacgatgaagatgttggttactggtgac	2	3217	3277	30	60	
NC_006577.2	aatgacgatgaagatgttggttactggtgac	2	3277	3337	30	60	
NC_006577.2	aatgacgatgaagatgttggttactggtgac	2	3337	3397	30	60	
NC_006577.2	aatgacgatgaagatgttggttactggtgac	2	3397	3457	30	60	
<u>AY597011.2</u>	aatgacgatgaagatgttggttactggtgac	2	3037	3097	30	60	29926
AY597011.2	aatgacgatgaagatgttggttactggtgac	2	3097	3157	30	60	
AY597011.2	aatgacgatgaagatgttggttactggtgac	2	3157	3217	30	60	
AY597011.2	aatgacgatgaagatgttggttactggtgac	2	3217	3277	30	60	
AY597011.2	aatgacgatgaagatgttggttactggtgac	2	3277	3337	30	60	
AY597011.2	aatgacgatgaagatgttggttactggtgac	2	3337	3397	30	60	
AY597011.2	aatgacgatgaagatgttggttactggtgac	2	3397	3457	30	60	
<u>HM034837</u>	aatgacgatgaagatgttggttactggtgac	2	3001	3061	30	60	29862

HM034837	aatgacgatgaagatgttggttactggtgac	2	3061	3121	30	60	
HM034837	aatgacgatgaagatgttggttactggtgac	2	3121	3181	30	60	
HM034837	aatgacgatgaagatgttggttactggtgac	2	3181	3241	30	60	
HM034837	aatgacgatgaagatgttggttactggtgac	2	3241	3301	30	60	
HM034837	aatgacgatgaagatgttggttactggtgac	2	3301	3361	30	60	
HM034837	aatgacgatgaagatgttggttactggtgac	2	3361	3421	30	60	
DQ415914.1	aatgacgatgaagatgttggttactggtgac	2	3031	3091	30	60	29887
DQ415914.1	aatgacgatgaagatgttggttactggtgac	2	3091	3151	30	60	
DQ415914.1	aatgacgatgaagatgttggttactggtgac	2	3151	3211	30	60	
DQ415914.1	aatgacgatgaagatgttggttactggtgac	2	3211	3271	30	60	
DQ415914.1	aatgacgatgaagatgttggttactggtgac	2	3271	3331	30	60	
DQ415914.1	aatgacgatgaagatgttggttactggtgac	2	3331	3391	30	60	
DQ415913.1	aatgacgatgaagatgttggttactggtgac	2	3031	3091	30	60	29785
DQ415913.1	aatgacgatgaagatgttggttactggtgac	2	3091	3151	30	60	
DQ415913.1	aatgacgatgaagatgttggttactggtgac	2	3151	3211	30	60	
DQ415913.1	aatgacgatgaagatgttggttactggtgac	2	3211	3271	30	60	
DQ415912.1	aatgacgatgaagatgttggttactggtgac	2	3031	3091	30	60	29845
DQ415912.1	aatgacgatgaagatgttggttactggtgac	2	3097	3157	30	60	
DQ415912.1	aatgacgatgaagatgttggttactggtgac	2	3217	3277	30	60	
DQ415912.1	aatgacgatgaagatgttggttactggtgac	2	3305	3365	30	60	
DQ415911.1	aatgacgatgaagatgttggttactggtgac	2	3031	3091	30	60	29902
DQ415911.1	aatgacgatgaagatgttggttactggtgac	2	3091	3151	30	60	
DQ415911.1	aatgacgatgaagatgttggttactggtgac	2	3151	3211	30	60	
DQ415911.1	aatgacgatgaagatgttggttactggtgac	2	3211	3271	30	60	
DQ415911.1	aatgacgatgaagatgttggttactggtgac	2	3271	3331	30	60	
DQ415911.1	aatgacgatgaagatgttggttactggtgac	2	3331	3391	30	60	
DQ415911.1	aatgacgatgaagatgttggttactggtgac	2	3391	3451	30	60	
AY884001.1	gatgaagatgttggttactggtgacaatgac	2	3037	3097	30	60	
AY884001.1	gatgaagatgttggttactggtgacaatgac	2	3097	3157	30	60	
AY884001.1	gatgaagatgttggttactggtgacaatgac	2	3181	3241	30	60	
AY884001.1	gatgaagatgttggttactggtgacaatgac	2	3241	3301	30	60	
AY884001.1	gatgaagatgttggttactggtgacaatgac	2	3301	3361	30	60	
DQ415896.1	aatgacgatgaagatgttggttactggtgac	2	3031	3091	30	60	29860
DQ415896.1	aatgacgatgaagatgttggttactggtgac	2	3091	3151	30	60	
DQ415896.1	aatgacgatgaagatgttggttactggtgac	2	3151	3211	30	60	
DQ415896.1	aatgacgatgaagatgttggttactggtgac	2	3211	3271	30	60	
DQ415896.1	aatgacgatgaagatgttggttactggtgac	2	3271	3331	30	60	
DQ415897.1	aatgacgatgaagatgttggttactggtgac	2	3031	3091	30	60	29845
DQ415897.1	aatgacgatgaagatgttggttactggtgac	2	3091	3151	30	60	
DQ415897.1	aatgacgatgaagatgttggttactggtgac	2	3151	3211	30	60	
DQ415897.1	aatgacgatgaagatgttggttactggtgac	2	3217	3277	30	60	
DQ415897.1	aatgacgatgaagatgttggttactggtgac	2	3305	3365	30	60	
DQ415898.1	aatgacgatgaagatgttggttactggtgac	2	3031	3091	30	60	29815
DQ415898.1	aatgacgatgaagatgttggttactggtgac	2	3091	3151	30	60	
DQ415898.1	aatgacgatgaagatgttggttactggtgac	2	3151	3211	30	60	
DQ415898.1	aatgacgatgaagatgttggttactggtgac	2	3211	3271	30	60	

DQ415898.1	aatgacgatgaagatgttggtactggtgac	2	3271	3331	30	60	29905
DQ415899.1	aatgacgatgaagatgttggtactggtgac	2	3031	3091	30	60	
DQ415899.1	aatgacgatgaagatgttggtactggtgac	2	3091	3151	30	60	
DQ415899.1	aatgacgatgaagatgttggtactggtgac	2	3151	3211	30	60	
DQ415899.1	aatgacgatgaagatgttggtactggtgac	2	3211	3271	30	60	
DQ415899.1	aatgacgatgaagatgttggtactggtgac	2	3271	3331	30	60	
DQ415899.1	aatgacgatgaagatgttggtactggtgac	2	3331	3391	30	60	
DQ415899.1	aatgacgatgaagatgttggtactggtgac	2	3395	3455	30	60	

Table 3.0 Conclusion Table for the total length of repeats and Genome Size.

Accession Number	Genome Size	Total Length of Repeat
DQ415904.1	29295	60
kf686344.1	29695	240
KF686342.1	29742	240
DQ415909.1	29767	240
DQ415913.1	29785	240
DQ415910.1	29797	300
DQ415900	29812	300
DQ415902.1	29845	360
DQ415912.1	29845	240
DQ415905.1	29857	360
DQ415906	29857	360
HM034837	29862	420
DQ415914.1	29887	360
DQ415911.1	29902	420
DQ415903.1	29920	120
KF686340.1	29923	420
AY597011.2	29926	420
NC_006577.2	29926	420
kf430201.1	29934	420
DQ415908.1	29947	420
kf686346.1	29982	480
kf686343.1	29983	480
DQ415901	30097	180
DQ339101.1	29755	240
KF686341.1	29832	360
DQ415896.1	29860	300
DQ415897.1	29845	300
DQ415898.1	29815	300
DQ415899.1	29905	420
AY884001.1	29815	300

(Fig. 1) The comparison between the total length of repeats and genome size in different completed genomes of (CoV(HKU1)).

All the previous extracted perfect repeats of twenty nine records of CoV(HKU1) were aligned with MAFFT tool which was a built module in the package of Unipro UGENE VERSION 1.16 and produced a highly conserved block which was ranged from position (181 – 240) as shown in (Fig. 3). ORF finder tool of NCBI gave the six forward frames of the submitted query sequence (conserved position, 181-240) and the selected frame which was used was (ORF:1..240 Frame +1) where the length of the translated repeat was sixty nine amino acid (DDEDVVTGDNDDEDVVTGDNDDEDVVTGDNDDEDVVTGDNDDEDVVTGDNDDEDVVTGDNDDEDVVTGDNDDEDVVT). The selected frame of translation (ORF:1..240 Frame +1)

was used as a seed to hit the protein sequence database by using PSI-BLAST of Primary database of Genbank- European bioinformatics institute (EBI) (<http://www.ebi.ac.uk/Tools/sss/psiblast/>) to produce significant pair wise alignment with thirty nine targeted sequences [Human coronavirus (HKU1)]. The aligned sequences were downloaded as a fasta format file and pasted in the input textbox of the PRATT tool (<http://www.ebi.ac.uk/Tools/pfa/pratt/>) which produced the best pattern (V-V-T-G-D-N-D-D-E-D-V-V-T-G-D-N-D-D-E-D). The expected pattern had a fitness score (83.4010). The shaded alignment of hits presented in clustalw format of alignment was produced by scanprosite search engine (prosite.expasy.org/scanprosite) as shown in Table 4. The shaded alignment of hits gave fifty-four hits (by the best pattern) on six sequences of human coronavirus as shown in Table 4.

(Fig 3.) The consensus repeated sequences which detected by the RFRE tool. The consensus position (181 – 240) which located in 29 records of completed genome of human coronavirus.

Table 4.0 Shown 54 hits (by 1 pattern) on 6 sequences.

Shaded Alignment Motif , (VVTGDNDDDEDVVTGDNDDDE)			
sp P0C6U5 R1A CVHN5	VVTGDNDDDEDVVTGDNDDDED	sp P0C6X3 R1AB CVHN2 32	sp P0C6U3 R1A CVHN1 47
VVTGDNDDDEDVVTGDNDDDED	sp P0C6X2 R1AB CVHN1 17	VVTGDNDDDEDVVTGDNDDDED	VVTGDNDDDEDVVTGDNDDDED
sp P0C6U5 R1A CVHN5 2	VVTGDNDDDEDVVTGDNDDDED	sp P0C6X3 R1AB CVHN2 33	sp P0C6U3 R1A CVHN1 48
VVTGDNDDDEDVVTGDNDDDED	sp P0C6X2 R1AB CVHN1 18	VVTGDNDDDEDVVTGDNDDDED	VVTGDNDDDEDVVTGDNDDDED
sp P0C6U5 R1A CVHN5 3	VVTGDNDDDEDVVTGDNDDDED	sp P0C6U4 R1A CVHN2 34	sp P0C6U3 R1A CVHN1 49
VVTGDNDDDEDVVTGDNDDDED	sp P0C6X2 R1AB CVHN1 19	VVTGDNDDDEDVVTGDNDDDED	VVTGDNDDDEDVVTGDNDDDED
sp P0C6U5 R1A CVHN5 4	VVTGDNDDDEDVVTGDNDDDED	sp P0C6U4 R1A CVHN2 35	sp P0C6U3 R1A CVHN1 50
VVTGDNDDDEDVVTGDNDDDED	sp P0C6X2 R1AB CVHN1 20	VVTGDNDDDEDVVTGDNDDDED	VVTGDNDDDEDVVTGDNDDDED
sp P0C6U5 R1A CVHN5 5	VVTGDNDDDEDVVTGDNDDDED	sp P0C6U4 R1A CVHN2 36	sp P0C6U3 R1A CVHN1 51
VVTGDNDDDEDVVTGDNDDDED	sp P0C6X2 R1AB CVHN1 21	VVTGDNDDDEDVVTGDNDDDED	VVTGDNDDDEDVVTGDNDDDED
sp P0C6U5 R1A CVHN5 6	VVTGDNDDDEDVVTGDNDDDED	sp P0C6U4 R1A CVHN2 37	sp P0C6U3 R1A CVHN1 52
VVTGDNDDDEDVVTGDNDDDED	sp P0C6X2 R1AB CVHN1 22	VVTGDNDDDEDVVTGDNDDDED	VVTGDNDDDEDVVTGDNDDDED
sp P0C6X4 R1AB CVHN5 7	VVTGDNDDDEDVVTGDNDDDED	sp P0C6U4 R1A CVHN2 38	sp P0C6U3 R1A CVHN1 53
VVTGDNDDDEDVVTGDNDDDED	sp P0C6X2 R1AB CVHN1 23	VVTGDNDDDEDVVTGDNDDDED	VVTGDNDDDEDVVTGDNDDDED
sp P0C6X4 R1AB CVHN5 8	VVTGDNDDDEDVVTGDNDDDED	sp P0C6U4 R1A CVHN2 39	sp P0C6U3 R1A CVHN1 54
VVTGDNDDDEDVVTGDNDDDED	sp P0C6X2 R1AB CVHN1 24	VVTGDNDDDEDVVTGDNDDDED	VVTGDNDDDEDVVTGDNDDDED
sp P0C6X4 R1AB CVHN5 9	VVTGDNDDDEDVVTGDNDDDED	sp P0C6U4 R1A CVHN2 40	
VVTGDNDDDEDVVTGDNDDDED	sp P0C6X3 R1AB CVHN2 25	VVTGDNDDDEDVVTGDNDDDED	
sp P0C6X4 R1AB CVHN5 10	VVTGDNDDDEDVVTGDNDDDED	sp P0C6U4 R1A CVHN2 41	
VVTGDNDDDEDVVTGDNDDDED	sp P0C6X3 R1AB CVHN2 26	VVTGDNDDDEDVVTGDNDDDED	
sp P0C6X4 R1AB CVHN5 11	VVTGDNDDDEDVVTGDNDDDED	sp P0C6U4 R1A CVHN2 42	
VVTGDNDDDEDVVTGDNDDDED	sp P0C6X3 R1AB CVHN2 27	VVTGDNDDDEDVVTGDNDDDED	
sp P0C6X4 R1AB CVHN5 12	VVTGDNDDDEDVVTGDNDDDED	sp P0C6U3 R1A CVHN1 43	
VVTGDNDDDEDVVTGDNDDDED	sp P0C6X3 R1AB CVHN2 28	VVTGDNDDDEDVVTGDNDDDED	
sp P0C6X2 R1AB CVHN1 13	VVTGDNDDDEDVVTGDNDDDED	sp P0C6U3 R1A CVHN1 44	
VVTGDNDDDEDVVTGDNDDDED	sp P0C6X3 R1AB CVHN2 29	VVTGDNDDDEDVVTGDNDDDED	
sp P0C6X2 R1AB CVHN1 14	VVTGDNDDDEDVVTGDNDDDED	sp P0C6U3 R1A CVHN1 45	
VVTGDNDDDEDVVTGDNDDDED	sp P0C6X3 R1AB CVHN2 30	VVTGDNDDDEDVVTGDNDDDED	
sp P0C6X2 R1AB CVHN1 15	VVTGDNDDDEDVVTGDNDDDED	sp P0C6U3 R1A CVHN1 46	
VVTGDNDDDEDVVTGDNDDDED	sp P0C6X3 R1AB CVHN2 31	VVTGDNDDDEDVVTGDNDDDED	
sp P0C6X2 R1AB CVHN1 16	VVTGDNDDDEDVVTGDNDDDED		

3.3 Searching for the repeats by using YASS tool

YASS uses seeds to detect potential similarity regions, and then tried to extend them to local alignments, this genomic search tool used multiple transition constrained spaced seeds that enable to search more fuzzy repeats, as non-coding DNA/RNA. The result of using YASS tool gave a dot plot graph then the zooming option was used to focus on the region of repeats. It took a diagonal repeats which was an indication for a repeated regions in the accession number (KF430201.1) of Human corona virus genome which aligned to itself as shown in the (Fig. 4a) and (Fig. 4b). The Zoom mode showed the repeated sequences as a Small Square (multiple diagonal lines paralleled to the main diagonal line of similarity) as shown in the (Fig. 4b).

(Fig 4. a)

(Fig 4. b)

(Fig. 5 a) and (Fig. 5 b) shown the repeated sequences as a small square (multiple diagonal lines paralleled to the main diagonal line of similarity of the accession number (KF430201.1) that accession number was used as a model to ensure the sensitivity of RFRE tool to find repeat .

3.4 The Possible recombination spot

The RAT tool searched and expected the possible recombination spot, which occurred at nucleotide position 3012. This position shared and crossed among the twenty nine records of human corona virus as shown in (Fig. 5). and detected also as a start position of repeated sequences in the following accession numbers (KF686346.1, KF686344.1, KF686343.1, KF686342.1, KF686341.1 and KF686340.1) The possible recombination sites were produced and organized by the RAT tool in tabulated format as shown in the (Table 5). Deletion of part of the repeat region during the viral replication as a result of inexact replication by the recombination between the repeat regions of different Human coronavirus (HKU1) strains[2]

(Fig 5.) Shown the possible recombination spot occurs at position 3012.

Table 5.0 Shown the possible recombination spot between every two accession numbers which occurred at position 3012 among 29 records of human corona virus

Accession numbers	Position 3012 and probabilities
gi 530291047 gb KF430201.1	0.9917
gi 545299236 gb KF686341.1	0.9917
gi 545299246 gb KF686342.1	0.991368
gi 545299276 gb KF686346.1	0.991368
gi 545299256 gb KF686343.1	0.991368
gi 306569684 gb HM034837.1	0.986056
gi 85667876 ref NC_006577.2	0.991036
gi 85068130 gb AY597011.2	0.991036
gi 89515469 gb DQ415903.1	0.990704
gi 89515406 gb DQ415896.1	0.990704
gi 89515442 gb DQ415900.1	0.990704
gi 545299266 gb KF686344.1	0.989376
gi 89515532 gb DQ415910.1	0.99004
gi 89515451 gb DQ415901.1	0.990704
gi 89515514 gb DQ415908.1	0.990372
gi 89515496 gb DQ415906.1	0.990372
gi 89515487 gb DQ415905.1	0.990704
gi 89515478 gb DQ415904.1	0.990372
gi 89515523 gb DQ415909.1	0.990372
gi 89515568 gb DQ415914.1	0.990372
gi 89515505 gb DQ415907.1	0.990704
gi 89515424 gb DQ415898.1	0.98506
gi 85541020 gb DQ339101.1	0.986056
gi 89515550 gb DQ415912.1	0.985724
gi 89515415 gb DQ415897.1	0.986056
gi 89515559 gb DQ415913.1	0.98506
gi 89515433 gb DQ415899.1	0.985392
gi 85372431 gb AY884001.1	0.985392
gi 89515541 gb DQ415911.1	0.986056
gi 89515460 gb DQ415902.1	0.986056

3.5 Assigning the detected repeats of Human corona virus (kf686343.1) on a graphical map

The highest total length of the repeats (480 bp) which found inside the completed genome of Human corona virus (kf686343.1) were assigned to the annotated map of accession number, (kf686343.1) by using Unipro UGENE ver. 1.16, 2015. The assigned repeats were located inside the pb1a gene (replicase polyprotien 1a) from the nucleotide position (3013-3492) as shown in (Fig 7.).

(Fig 7.) Shown the location of the extracted repeats from position (3012-3492) which annotated and added to genbank file of the accession number (kf686343.1) by using the UGENE ver. 1.16, annotation module.

4. Conclusions

In conclusion, this study focused on the importance of identifying the tandem repeats in of human coronavirus (hku1) and make a relation between the genome size of (hku1) types and tandem repeats. The repeats which were found converted to amino acid sequence and used to build a conserved block of multiple sequence alignment to produce a specific motif for six sequences of CoV (HKU1) strains. Recombination between the repeated regions in the complete genomes of different CoV (HKU1) strains were observed in the recombination position (3012) which was also detected as a repeated position among the human corona virus and expected to be a key role of virus evolution. Maybe viruses combined partial genome sequences of hosts in infecting by using the repeats sequences as a hot spot region for recombination.

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References

- [1] Zhao, Zhixin, et al. "Genome-wide analysis of tandem repeats in plants and green algae." *G3: Genes/ Genomes/ Genetics* 4.1 (2014): 67-78.
- [2] Woo, Patrick CY, et al. "Coronavirus diversity, phylogeny and interspecies jumping." *Experimental Biology and Medicine* 234.10 (2009): 1117-1127.

- [3] Lau, Susanna KP, et al. "Molecular epidemiology of human coronavirus OC43 reveals evolution of different genotypes over time and recent emergence of a novel genotype due to natural recombination." *Journal of virology* 85.21 (2011): 11325-11337.
- [4] Aaziz, Rachid, and Mark Tepfer. "Recombination in RNA viruses and in virus-resistant transgenic plants." *Journal of General Virology* 80.6 (1999): 1339-1346.
- [5] Jeffreys, Alec J., John Murray, and Rita Neumann. "High-resolution mapping of crossovers in human sperm defines a minisatellite-associated recombination hotspot." *Molecular cell* 2.2 (1998): 267-273.
- [6] Bierne, Hélène, et al. "Isolation of a dnaE mutation which enhances RecA-independent homologous recombination in the Escherichia coli chromosome." *Molecular microbiology* 24.6 (1997): 1225-1234.
- [7] Osterrieder, Nikolaus, Nina Wallaschek, and Benedikt B. Kaufer. "Herpesvirus genome integration into telomeric repeats of host cell chromosomes." *Annual Review of Virology* 1 (2014): 215-235.
- [8] Biet, E., J-S. Sun, and M. Dutreix. "Conserved sequence preference in DNA binding among recombination proteins: an effect of ssDNA secondary structure." *Nucleic acids research* 27.2 (1999): 596-600.
- [9] Zhao, Xiangyan, Yonglei Tian, Ronghua Yang, Haiping Feng, Qingjian Ouyang, You Tian, Zhongyang Tan et al. "Coevolution between simple sequence repeats (SSRs) and virus genome size." *BMC genomics* 13, no. 1 (2012): 435.