Finding all amino acid mutations in SARS-CoV-2

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Background

Groups studying the SARS-CoV-2 virus are using genomes from GISAID to keep track of variants that could have functional effects on the virus phenotype. Some of them may be positively or negatively selected for. These genomes are updated daily from sequencing results across the world. There are at present ~8000 sequences for the human virus present. This data is available with free registration on the GISAID website. One way of using these sequences is to calculate the number of non-synonymous mutations arising in the genome since the first identified isolate from Wuhan was sequenced. There is more than one way to do this as usual. Typically a phylogenetic approach is used using the nucleotide sequence. An alternate way is to annotate the genomes and extract the corresponding protein sequences across all non-redundant genomes (non-identical). Then these can all be aligned against the reference to see the amino acid substitutions. Here I use some code from a Python package I made called `pygenefinder` to do the annotation. Notice that annotating all the genomes like this is somewhat inefficient since they are practically identical. However since they're viral genomes it's very quick and it lets us extract the sequence for any protein of interest. This approach would work for more distant sequences too.

Method

There were 7428 complete nucleotide sequences for SARS-CoV-2 sampled from humans present in the GISAID database at time of writing. These were reduced to 6408 by removing exactly identical sequences. They are annotated and the function returns a pandas dataframe of all proteins from all samples. From these we extract the orthologs by name (we could do it by sequence similarity too) and then collapse these to the unique set of protein sequences which will be much less. Finally we can compare each one to the reference to see the mutation(s). This is done by pairwise comparisons to the reference.

Annotations

First let's check that our annotation works ok against the reference genome which is NC_045512. Here are the proteins in the NCBI RefSeq annotation:

gene	locus_tag	length
orf1ab	GU280_gp01	7096
S	GU280_gp02	1273
ORF3a	GU280_gp03	275
E	GU280_gp04	75
M	GU280_gp05	222
ORF6	GU280_gp06	61
ORF7a	GU280_gp07	121
ORF7b	GU280_gp08	43
ORF8	GU280_gp09	121
N	GU280_gp10	419
ORF10	GU280_gp11	38

Next we can annotate with my own routine as follows:

```
from pygenefinder import app,tools
sc2,sc2recs = app.run_annotation('NC_045512.fa', kingdom='viruses')
```

Here is my annotation below. You can see there are some differences. We are missing the E protein and ORF10. ORF8 is there but without the gene name. My method probably needs improvement! Even automatic annotations will not be the same. However it's good enough that we can use this for the reference proteins, since most of the proteins are present and the sequences should be identical.

gene	product	length
1a	Replicase polyprotein 1a	4388
rep	Replicase polyprotein 1ab	2595
S	Spike glycoprotein	1273
3a	Protein 3a	275
M	Membrane protein	222
7a	Protein 7a	121
N	Nucleoprotein	419
-	hypothetical protein	43
-	hypothetical protein	121
-	hypothetical protein	51

Some code

Imports:

```
import pandas as pd
from Bio import SeqIO,AlignIO
from Bio.Seq import Seq
from Bio.SeqRecord import SeqRecord
import pylab as plt
from pygenefinder import app,tools
```

This method allows us to load sequences from a fasta with duplicate sequences:

```
def load_deduplicated_sequences(filename):
    """Load a fasta file of sequences and ignore duplicates"""

    newrecs = {}
    unique_seqs=defaultdict(list)
    with open(filename, 'r') as in_handle:
        for rec in SeqIO.parse(in_handle, "fasta"):
            if rec.seq in unique_seqs:
                continue
            if not rec.id in gisrecs:
                try:
```

```
                    id = rec.id.split('|')[1]
                    newrecs[id] = rec
                    #seqs.append(rec.seq)
                    unique_seqs[str(rec.seq)] = id
                except:
                    pass
    return newrecs
```

Here's the method used to get the mutations. Pairwise comparisons are used so the record can be stored with the mutation name.

```
def get_mutations(recs, ref):
    mutations = {}
    positions = []
    for rec in recs:
        aln = tools.clustal_alignment(seqs=[ref, rec])
        #print (aln)
        for pos in range(len(aln[0])):
            refaa = aln[0,pos]
            aa = aln[1,pos]
            if aa != refaa:
                #print (refaa, aln[:,pos], aa)
                mut = refaa+str(pos+1)+aa
                mutations[rec.seq] = mut
```

```
                positions.append(pos)
    return mutations
```

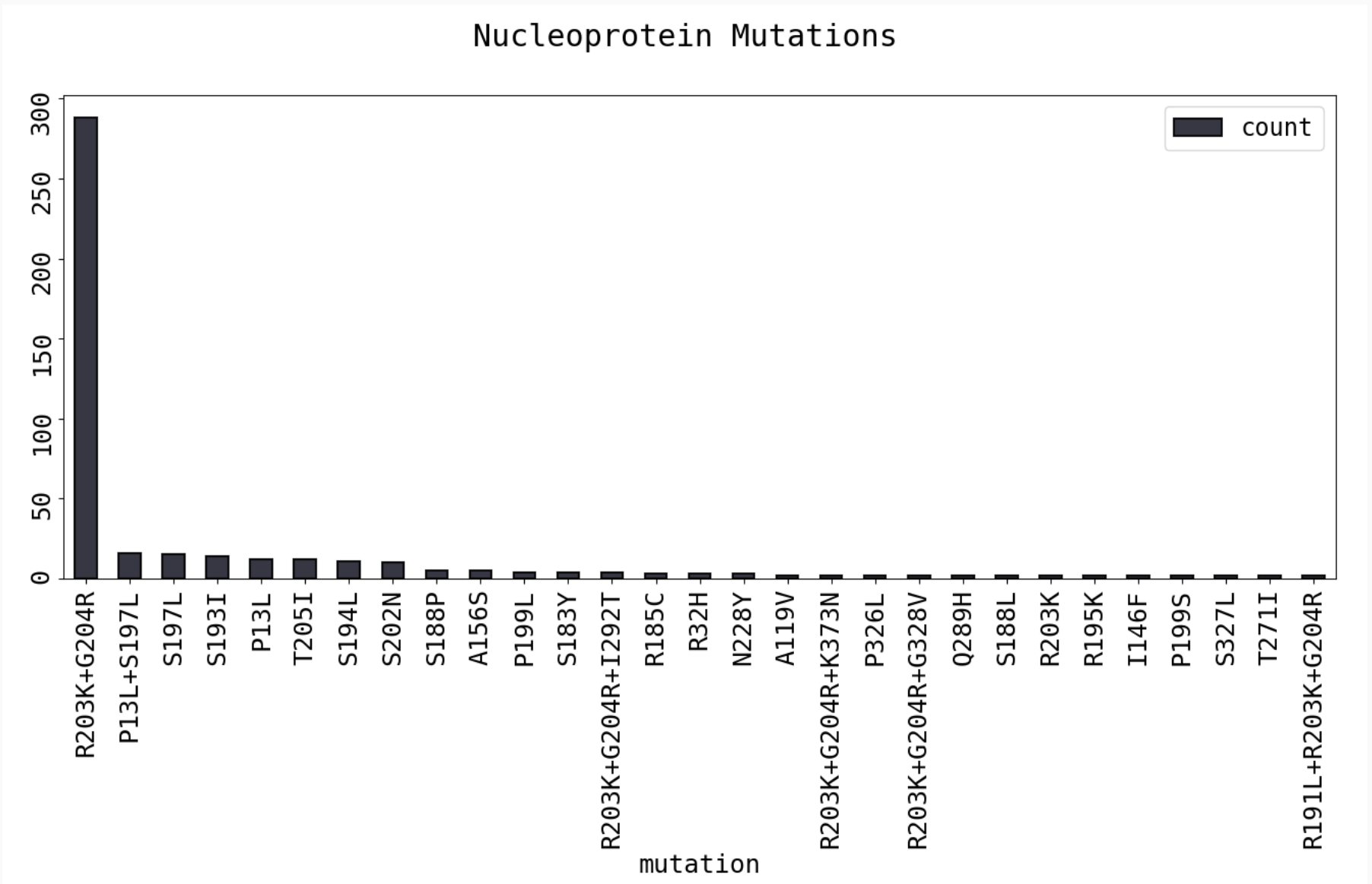
This is the code that runs the whole process. We can run this for each protein by providing the reference version and all the records (with nucleotide sequences). Note that the annotation is run once and the result passed to the function. The other methods used here are not shown in detail. The code for those methods can be found on the github repo for pygenefinder here.

```
#run the annotation
gisrecs = load_deduplicated_sequences('gisaid_cov2020_sequences.fasta')
annot = app.annotate_files(gisrecs, outdir='gisaid_annot', kingdom='viruses')

names = ['Protein 7a', 'Protein 3a', 'Spike glycoprotein','Membrane protein',
         'Nucleoprotein','Replicase polyprotein 1a','Replicase polyprotein 1ab']
res=[]
mutant_seqs = {}
for protname in names:
    refprot = sc2[sc2['product']==protname].iloc[0]
    refrec = SeqRecord(Seq(refprot.translation),id='ref')
    print (protname)
    annot_seqs = app.get_similar_sequences(protein, annot)
    unique_seqs, counts = tools.collapse_sequences(annot_seqs, refrec)
    print ('%s unique sequences' %len(unique_seqs))
    mutations = get_mutations(unique_seqs, refrec)
    #convert mutations to string and count the frequency
```

```
    muts = {seq:'+'.join(mutations[seq]) for seq in mutations}
    #save the sequences for later use
    mutant_seqs[protname] = muts
    freqs = [(muts[seq], counts[seq]) for seq in muts]
    #convert freq table to dataframe
    fdf = pd.DataFrame(freqs,columns=['mutation','count']).sort_values('count',ascending=False)
    fdf['protein'] = protname
    print (fdf[:10])
    res.append(fdf)

res = pd.concat(res).sort_values('count',ascending=False)
```

Results

The output, as would be expected, is a large number of rare substitutions that appear only once or several times and a very few common mutations that have been fixed in the population. The rare mutations could be more recent, random for the particular isolate or possibly even due to sequencing error. This is indicated in the plot of the top mutations in the N protein:



The top 15 most frequent mutations are below. `D614G` in the **Spike glycoprotein** is by now a well known mutation. As is `R203K+G204R` in the **Nucleoprotein**, which [is present in one third of reported sequences from Europe]. This polymorphism is predicted to reduce the

binding of an enclosing putative HLA-C*07-restricted epitope, an allele common in Caucasians.

mutation	count	protein
D614G	792	Spike glycoprotein
P214L	769	Replicase polyprotein 1ab
Q57H	386	Protein 3a
R203K+G204R	288	Nucleoprotein
G251V	225	Protein 3a
P1327L+Y1364C	222	Replicase polyprotein 1ab
T248I	165	Replicase polyprotein 1a
L3589F	61	Replicase polyprotein 1a
T175M	46	Membrane protein
I722V+P748S+L3589F	44	Replicase polyprotein 1a
D431N	38	Replicase polyprotein 1a
D3G	33	Membrane protein
G196V	28	Protein 3a
F3054Y	27	Replicase polyprotein 1a

The file with all mutations is found here. As you can see this is a non-phylogenetic method and has limitations as such. We can't easily see from this which clade the mutation exists in for instance or when it initially appeared. It hopefully illustrates that there are multiple alternative methods to achieve similar goals in genomic analysis.

Links

- Notebook with this code
- Exploring the genomic and proteomic variations of SARS-CoV-2 spike glycoprotein
- First report of COVID-19 in Scotland
- Selection analysis of gisaid sars-cov-2 data
- GISAID
- covid19dataportal.org

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