Annex C15

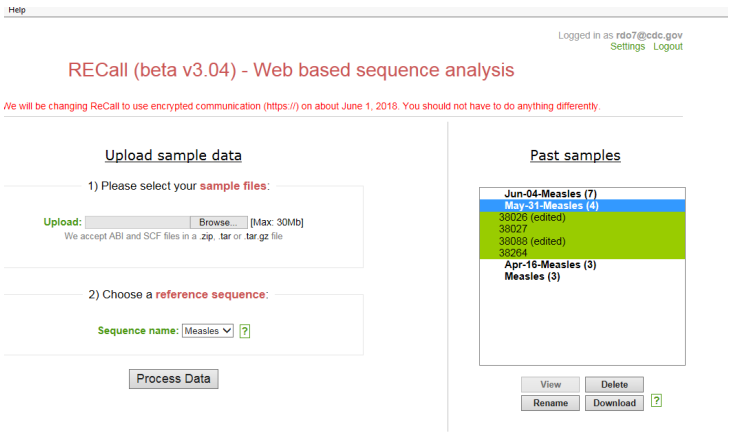
Using the RECall program for on-line sequence analysis of measles and rubella

Changes from version 5 is website domain.

1. Before beginning, a request MUST be made to set up an account. Please email rdo7@cdc.gov to set up an account on the site.
2. For measles and rubella the RECall website is: **https://recall.bccfe.ca/account/login**
   Preferred browsers are Chrome or Firefox.
3. After user account is set up, go to the website and login. If login is successful, a sample entry page should be visible as seen in figure below (your Past samples window will be empty).
4. To set your email as the location to send final Fasta file, go to settings. Under file formats, enter your email and check the box. You can choose Fasta format, or text, or both, if you prefer. Click the Back button (if you use the back arrow, the changes will not be saved).
5. For **Measles** (skip to step 14 for rubella):

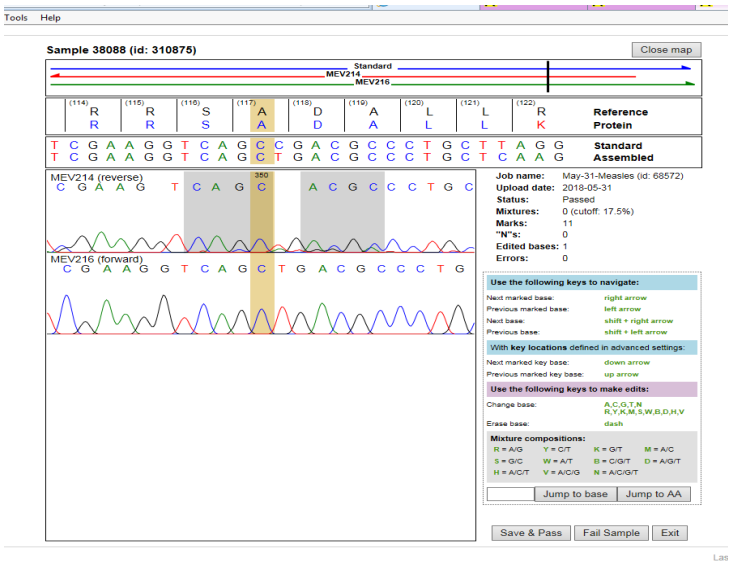
   In order to upload your .abi files they all need to be named in a very specific way so the program can tell which chromatograms come from which sample and how to align the samples based on the primers. The current scheme for **Measles** is:

   a. For each abi file: Sample ID first, then an underscore (_), then the primer number either 216 or 214.
   b. The sample ID for all sequence files from one sample need to be **exactly** the same (case sensitive); examples MT1_216 and MT1_214.
6. Next, a zip file containing the sequence files needs to be created. To do this: select the .abi files named as above for the viruses to submit (multiple viruses can be included in one zip file); right click and choose Send to: compressed (zipped) folder. This folder is what is uploaded to the Recall website.
7. On the RECall sample entry page, under Upload sample data, browse for your .zip folder and select.
8. Under Choose a reference sequence, select **Measles**.
9. Click on Process Data. It may take a few minutes.
10. If the upload is successful, a submission date-Measles line should appear in the Past samples box. The number indicates the number of viruses in the submission.
11. If there was a problem with the naming, an error message will appear.
12. **Using the website to analyze samples**
    a. Click on one submission in the Past samples box. The list of sample names it contains will appear.

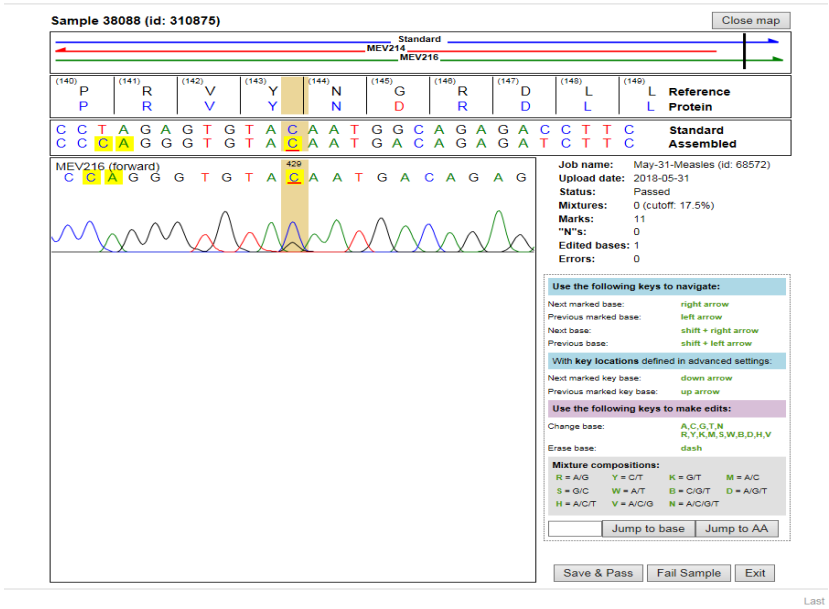


b. Click on one virus name. Click on View at the bottom.
c. The analysis page will open.
d. Click on Open Map at the top to ensure that all files for that virus are there and aligned properly.

e. The chromatogram peaks will be aligned with the corresponding nucleotide in the text bar at the top. The top text line is a reference virus and the bottom text line is the new sequence being analyzed.
f. The sequence starts with the normal GTC of the sequencing window.
g. Hold the shift key and the arrow keys to click through the sequence one nucleotide at a time. The highlighted brown box will move from nucleotide to nucleotide as you click the arrow.
h. Problem areas will be highlighted in grey.



i. Problem nucleotides will be highlighted in yellow.



j. Bases can be edited by selecting a nucleotide in the highlighted brown box. Type over the incorrect nucleotide with the correct one.

k. After you finish correcting any errors, click on Save & Pass.

l. Click on Download to download the Fasta file and a summary excel file.

m. A zip file will also be mailed to you if you set it up in Settings. It will contain a consensus Fasta file and a summary excel file.

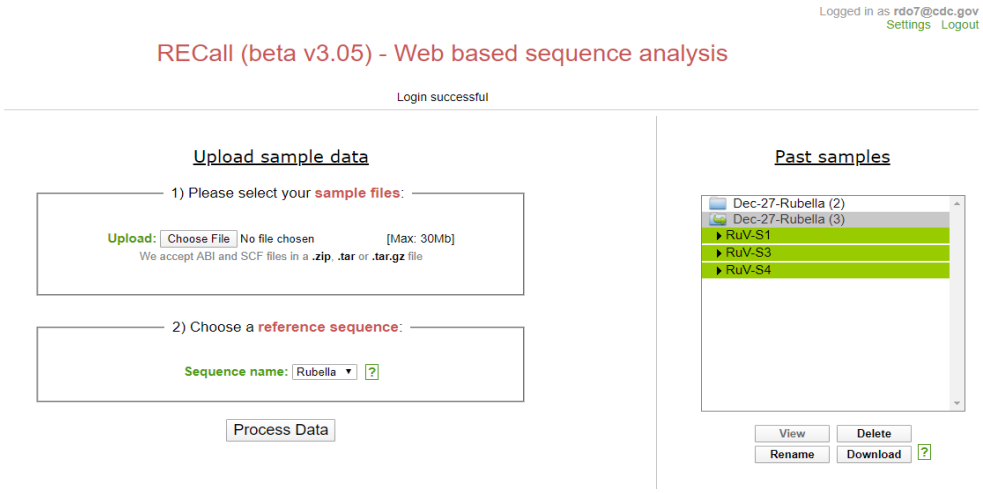
13. For **Rubella**:

In order to upload your .abi files they all need to be named in a very specific way so the program can tell which chromatograms come from which sample and how to align the samples based on the primers. The current scheme for **Rubella** is:

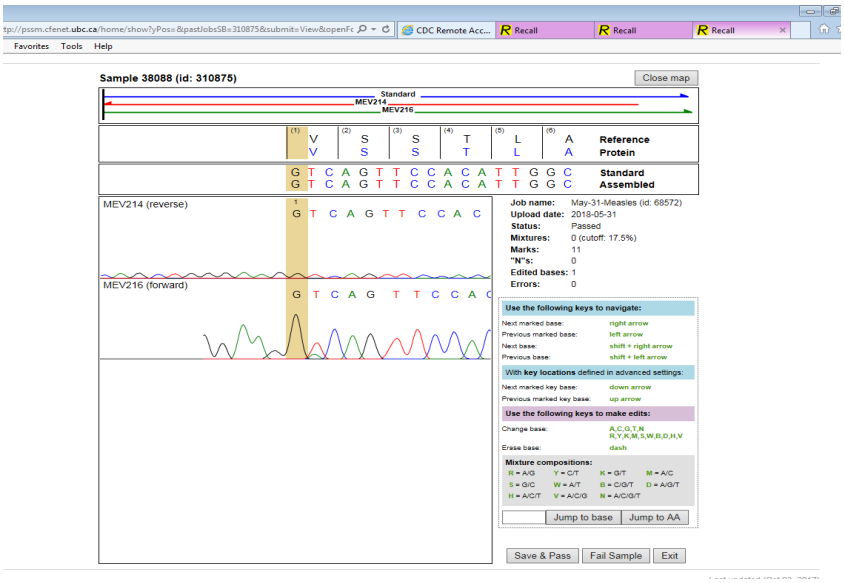
a. For each abi file: Sample ID first, then an underscore (_), then the primer number either 8633 or 9112 for fragment 1 and 8945 or 9577 for fragment 2.

b. The sample ID for all sequence files from one sample need to be **exactly** the same (case sensitive); examples GA1_8633, GA1_9112, GA1_8945, and GA1_9577.

14. Next, a zip file containing the sequence files needs to be created. To do this: select the .abi files named as above for the viruses to submit (multiple viruses can be included in one zip file); right click and choose Send to: compressed (zipped) folder. This folder is what is uploaded to the Recall website.
15. On the RECall sample entry page, under Upload sample data, browse for your .zip folder and select.
16. Under Choose a reference sequence, select **Rubella**.
17. Click on Process Data. It may take a few minutes.
18. If the upload is successful, a submission date-Rubella line should appear in the Past samples box. The number indicates the number of viruses in the submission.
19. If there was a problem with the naming, an error message will appear.
20. **Using the website to analyze samples**
    a. Click on one submission in the Past samples box. The list of sample names it contains will appear.



    b. Click on one virus name. Click on View at the bottom.
    c. The analysis page will open.
    d. Click on Open Map at the top to ensure that all files for that virus are there and aligned properly.



e. The chromatogram peaks will be aligned with the corresponding nucleotide in the text bar at the top. The top text line is a reference virus and the bottom text line is the new sequence being analyzed.

f. The sequence starts with AA not the normal GTT of the sequencing window because the 2 nucleotides before the 739 start of the sequencing window had to be added to maintain the proper open reading frame. These will need to be removed later when you receive the final output file.

g. Hold the shift key and the arrow keys to click through the sequence one nucleotide at a time. The highlighted brown box will move from nucleotide to nucleotide as you click the arrow.

h. Problem areas will be highlighted in grey.

i. Problem nucleotides will be highlighted in yellow.

j. Bases can be edited by selecting a nucleotide in the highlighted brown box. Type over the incorrect nucleotide with the correct one.

k. After you finish correcting any errors, click on Save & Pass.

l. Click on Download to download the Fasta file and a summary excel file.

m. A zip file will also be mailed to you if you set it up in Settings. It will contain a consensus Fasta file and a summary excel file.

n. Remember, the Fasta file that is returned for rubella will be 741 nucleotides. It is necessary to remove the first 2 nucleotides (AA) from this file before phylogenetic analysis.

21. More details on the program can be found at: http://webrecall.wikia.com/wiki/BCCFE_Web_RECall_Wiki.