Annex C15

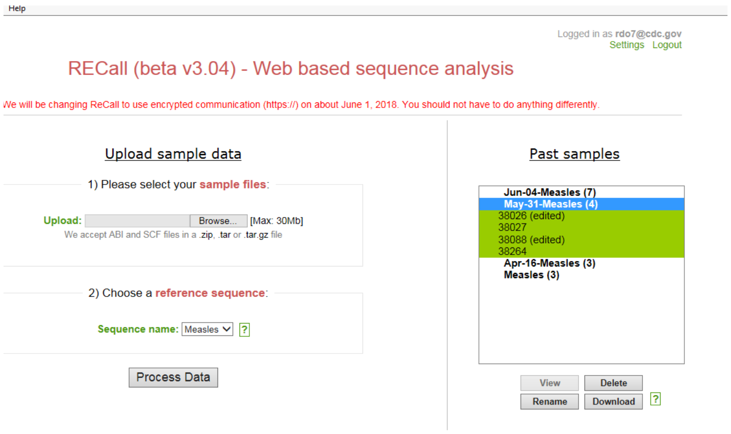
Using the RECall program for on-line sequence analysis of measles and rubella

Changes from version 6 is new feature to check for mixed bases, updating the method of receiving the edited files, updated information regarding the QC section.

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. Go to settings and verify your email is correct. Under file formats, UN-check the box next to email address. Select preference for data format: Fasta, text, or both. This file format will be used when downloading the final data from the 'passed' samples. Click Save, then click the Back button (if you use the back arrow before hitting save, 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 name (examples: 216 and 214 or F and R or Forward and Reverse-name of primer does not matter as long as it follows the underscore and is recognizable for analysis).
   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 NOT 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.



Logged in as rdo7@cdc.gov

RECall (beta v3.04) - Web based sequence analysis

We will be changing ReCall to use encrypted communication (https://) on about June 1, 2018. You should not have to do anything differently.

Upload sample data

1) Please select your sample files:

Upload: [Max: 30Mb]
We accept ABI and SCF files in a .zip, .tar or .tar.gz file

2) Choose a reference sequence:

Sequence name: Measles

Past samples

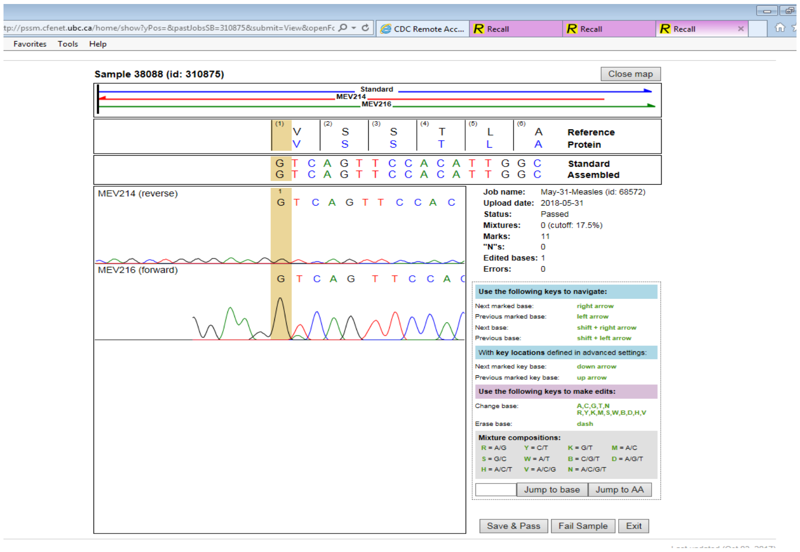
Jun-04-Measles (7)
May-31-Measles (4)
38026 (edited)
38027
38088 (edited)
38264
Apr-16-Measles (3)
Measles (3)

b. If any of the sample names appear in red or black, they have failed. Do not waste time analyzing them.

c. Click on one virus name. Click on View at the bottom.

d. The analysis page will open.

e. Click on Open Map at the top to ensure that all files for that virus are there and aligned properly.



Sample 38088 (id: 310875)

Job name: May-31-Measles (id: 68572)
Upload date: 2018-05-31
Status: Passed
Mixtures: 0 (cutoff: 17.5%)
Marks: 11
"N"s: 0
Edited bases: 1
Errors: 0

Use the following keys to navigate:

Next marked base:	right arrow
Previous marked base:	left arrow
Next base:	shift + right arrow
Previous base:	shift + left arrow

With key locations defined in advanced settings:

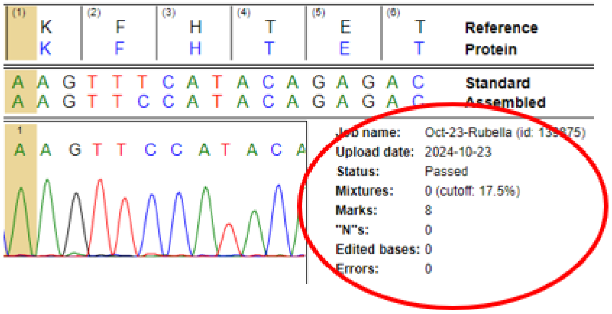
Next marked key base:	down arrow
Previous marked key base:	up arrow

Use the following keys to make edits:

Change base:	A,C,G,T,N R,Y,K,M,S,W,B,D,H,V
Erase base:	dash

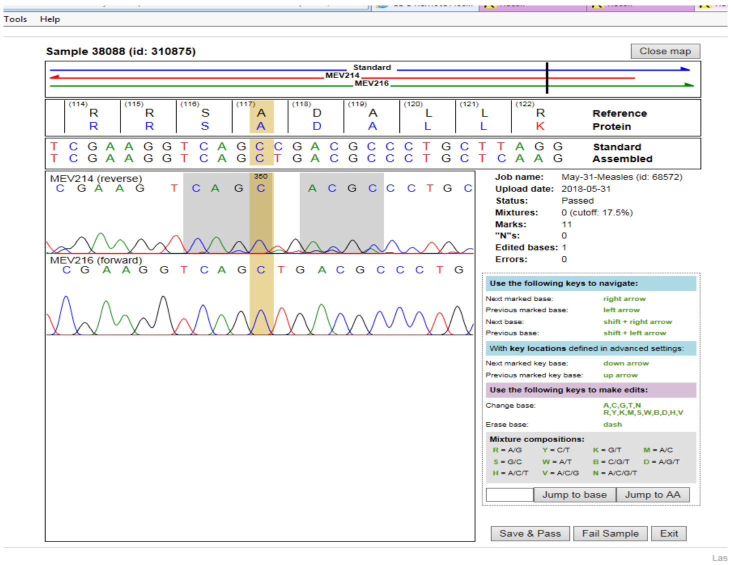
Mixture compositions:
R = A/G Y = C/T K = G/T M = A/C
S = G/C W = A/T B = C/G/T D = A/G/T
H = A/C/T V = A/C/G N = A/C/G/T

f. Note any messages in the QC section which would indicate any identified mixtures, marks, ambiguous bases, and errors. It will also list the errors the software has identified.



Job name: Oct-23-Rubella (id: 13[illegible]875)
Upload date: 2024-10-23
Status: Passed
Mixtures: 0 (cutoff: 17.5%)
Marks: 8
"N"s: 0
Edited bases: 0
Errors: 0

g. 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.
h. The sequence starts with the normal GTC of the sequencing window.
i. 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.
j. Problem areas will be highlighted in grey.

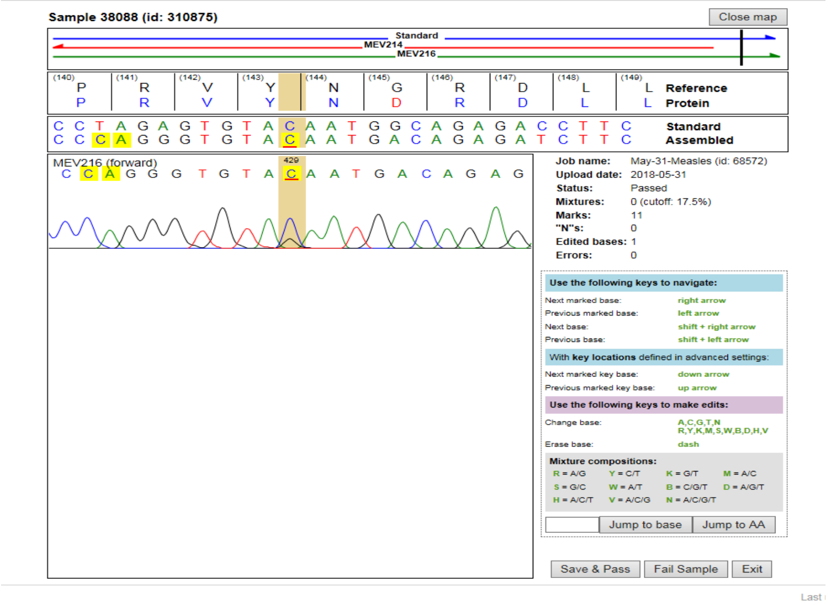


Sample 38088 (id: 310875)

Job name: May-31-Measles (id: 68572)
Upload date: 2018-05-31
Status: Passed
Mixtures: 0 (cutoff: 17.5%)
Marks: 11
"N"s: 0
Edited bases: 1
Errors: 0

Use the following keys to navigate:
Next marked base: right arrow
Previous marked base: left arrow
Next base: shift + right arrow
Previous base: shift + left arrow
With key locations defined in advanced settings:
Next marked key base: down arrow
Previous marked key base: up arrow
Use the following keys to make edits:
Change base: A,C,G,T,N R,Y,K,M,S,W,B,D,H,V
Erase base: dash
Mixture compositions:
R = A/G Y = C/T K = G/T M = A/C
S = G/C W = A/T B = C/G/T D = A/G/T
H = A/C/T V = A/C/G N = A/C/G/T

k. Problem nucleotides will be highlighted in yellow.



Sample 38088 (id: 310875)

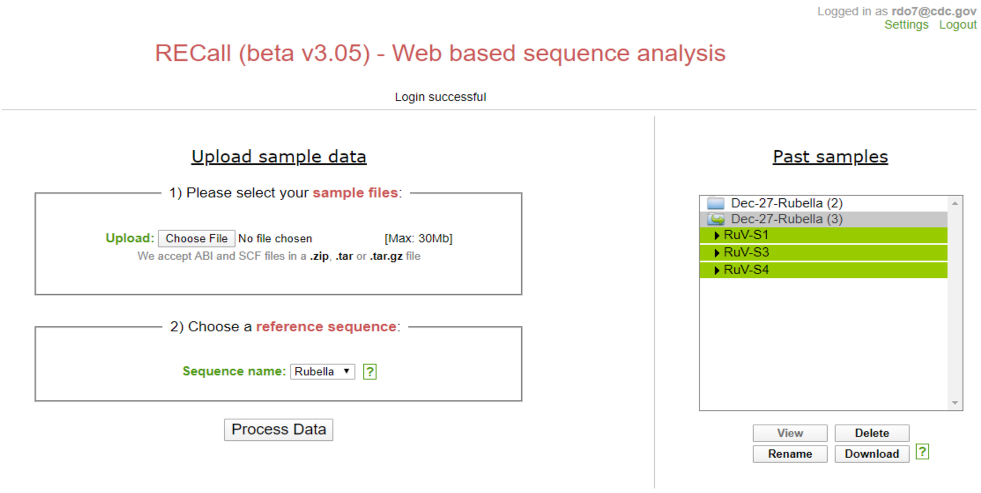
Job name: May-31-Measles (id: 68572)
Upload date: 2018-05-31
Status: Passed
Mixtures: 0 (cutoff: 17.5%)
Marks: 11
"N"s: 0
Edited bases: 1
Errors: 0

Use the following keys to navigate:
Next marked base: right arrow
Previous marked base: left arrow
Next base: shift + right arrow
Previous base: shift + left arrow
With key locations defined in advanced settings:
Next marked key base: down arrow
Previous marked key base: up arrow
Use the following keys to make edits:
Change base: A,C,G,T,N R,Y,K,M,S,W,B,D,H,V
Erase base: dash
Mixture compositions:
R = A/G Y = C/T K = G/T M = A/C
S = G/C W = A/T B = C/G/T D = A/G/T
H = A/C/T V = A/C/G N = A/C/G/T

l. Bases can be edited by selecting a nucleotide in the highlighted brown box. Type over the incorrect nucleotide with the correct one.
m. After you finish analyzing and correcting any errors, click on Save & Pass.

    n. Click on Download.
    o. A zip file will be downloaded which contain a consensus Fasta/text file of all passing samples 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. This can be the primer position numbers (8633 or 9112 for fragment 1 and 8945 or 9577 for fragment 2) or just indicate which fragment and primer (F1F, F1R, F2F, F2R).
    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.


Logged in as **rdo7@cdc.gov**

RECall (beta v3.05) - Web based sequence analysis

Login successful

Upload sample data

1) Please select your **sample files**:

Upload: Choose File No file chosen [Max: 30Mb]

We accept ABI and SCF files in a **.zip**, **.tar** or **.tar.gz** file

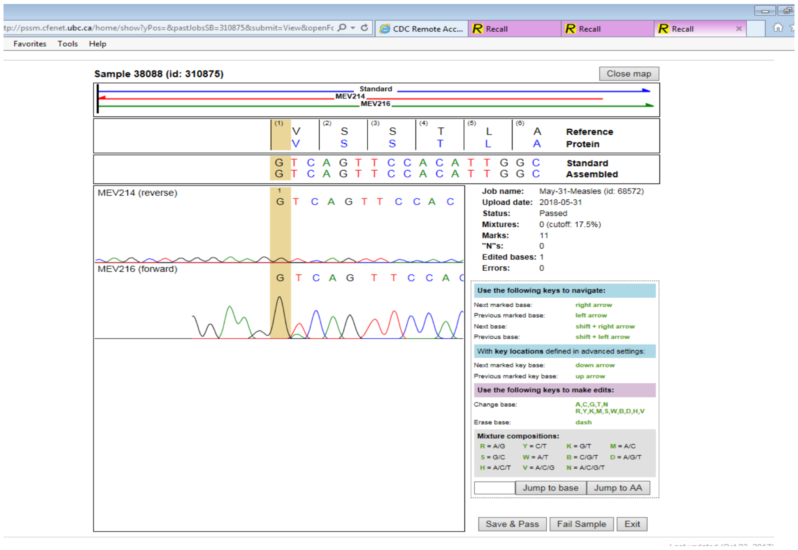
2) Choose a **reference sequence**:

Sequence name: Rubella

Past samples

Dec-27-Rubella (2)
Dec-27-Rubella (3)
RuV-S1
RuV-S3
RuV-S4

    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.



Sample 38088 (id: 310875)

Job name: May-31-Measles (id: 68572)
Upload date: 2018-05-31
Status: Passed
Mixtures: 0 (cutoff: 17.5%)
Marks: 11
"N"s: 0
Edited bases: 1
Errors: 0

Use the following keys to navigate:

Next marked base:	right arrow
Previous marked base:	left arrow
Next base:	shift + right arrow
Previous base:	shift + left arrow

With **key locations** defined in advanced settings:

Next marked key base:	down arrow
Previous marked key base:	up arrow

Use the following keys to make edits:

Change base:	A,C,G,T,N R,Y,K,M,S,W,B,D,H,V
Erase base:	dash

Mixture compositions:

R = A/G
Y = C/T
K = G/T
M = A/C
S = G/C
W = A/T
B = C/G/T
D = A/G/T
H = A/C/T
V = A/C/G
N = A/C/G/T

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.

Sample RuV 1 (id: 426418)

Job name: Dec-27-Rubella (id: 93636)
Upload date: 2019-12-27
Status: Passed
Mixtures: 0 (cutoff: 17.5%)
Marks: 1
"N"s: 0
Edited bases: 0
Errors: 0

Use the following keys to navigate:

Next marked base:	right arrow
Previous marked base:	left arrow
Next base:	shift + right arrow
Previous base:	shift + left arrow

With **key locations** defined in advanced settings:

Next marked key base:	down arrow
Previous marked key base:	up arrow

Use the following keys to make edits:

Change base:	A,C,G,T,N R,Y,K,M,S,W,B,D,H,V
Erase base:	dash

Mixture compositions:

R = A/G
Y = C/T
K = G/T
M = A/C
S = G/C
W = A/T
B = C/G/T
D = A/G/T
H = A/C/T
V = A/C/G
N = A/C/G/T

i. Problem nucleotides will be highlighted in yellow.

Sample RuV 1 (id: 426418)

Job name: Dec-27-Rubella (id: 93636)
Upload date: 2019-12-27
Status: Passed
Mixtures: 0 (cutoff: 17.5%)
Marks: 1
"N"s: 0
Edited bases: 0
Errors: 0

Use the following keys to navigate:

Next marked base: right arrow
Previous marked base: left arrow
Next base: shift + right arrow
Previous base: shift + left arrow

With **key locations** defined in advanced settings:

Next marked key base: down arrow
Previous marked key base: up arrow

Use the following keys to make edits:

Change base: A,C,G,T,N R,Y,K,M,S,W,B,D,H,V
Erase base: dash

Mixture compositions:

R = A/G
Y = C/T
K = G/T
M = A/C
S = G/C
W = A/T
B = C/G/T
D = A/G/T
H = A/C/T
V = A/C/G
N = A/C/G/T

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 reviewing the entire sequencing window, be sure to review for any mixed bases. To do this hold down the **ctrl + shift + right arrow** to skip to mixed bases. This step is important to verify the primers inside the sequencing window have not influenced the outcome of the sequence.

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

m. Click on Download.

n. A zip file will be downloaded which contain a consensus Fasta/text file of all passing samples and a summary excel file.

o. Remember, the Fasta/text 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.