charles river

Summary

Occasionally samples don't yield sufficiently high-quality sequence data to produce an identification. Samples that do not initially meet our data quality criteria are processed a second time, following scientific review. If this still results in poor-quality data, a Sample Notification Report is sent. Once this report is generated, processing has ended.

MICROBIAL SOLUTIONS

Sample Notification Report: Reasons for No Identification

AccuGENX-ID®/AccuGENX-ST® Reports

Reason: DNA sequence consisted of low or no signal

Figure 1 shows raw data with low signal levels; the actual sequence peaks are not of sufficient intensity for the software to interpret the data and make accurate base determinations.

As shown in Figure 2, no signal indicates there was no usable sequence data detectable above background. The most likely causes for low- or no-signal DNA sequence data are PCR inhibitors in the sample, poor primer annealing, failure to extract genomic DNA or poor PCR amplification.

For comparison purposes, Figure 3 illustrates high-quality sequence data.

Additionally, our proprietary methodologies are developed to minimize the effects of any inhibitors, but sometimes sequence failures do occur. Some of the compounds that can inhibit PCR include blood agar from plates, pigments and high DNA concentrations.

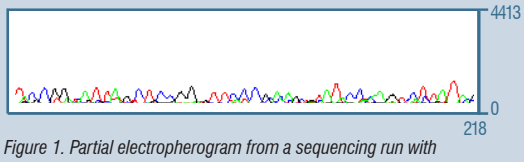


Figure 1. Partial electropherogram from a sequencing run with low signal which results in the inability to accurately make base calls.

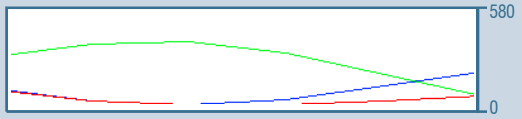


Figure 2. Partial electropherogram from a sequencing run with no signal. There are no detectable peaks and thus no useable sequence data.

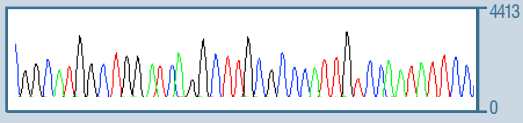


Figure 3. Partial electropherogram from a sequencing run with high-quality signal data for analysis.

Certain genera are also known to have sequence changes within the 16S/ITS ribosomal primer regions, and these changes affect the ability of the PCR primers to anneal to the template. This may also be true for some primers and isolates involved in sequence typing. As a result, PCR amplification of the target DNA is not efficient and can result in low or no sequence data.

Additionally, certain bacteria and fungi can be more difficult to lyse and effectively extract DNA to be used as a template for sequencing, and thus the result is low or no signal. The 16S rDNA, ITS2 rDNA or MLST target genes of certain microorganisms have areas that are GC-rich or regions that form stable secondary structures. These inherent features can interfere with DNA synthesis during PCR amplification, leading to poor accumulation of the target DNA and low or no sequencing signal product.

If a sample was submitted as an ethanol preparation and resulted in a Sample Notification Report, it is most frequently due to inaccurate sample preparation; e.g., one with a low DNA or high protein concentration. The highest frequency of failure is observed on samples for which we cannot see the original colonies, such as with ethanol preparations. Our preferred source of material, for quality control, is colony submission on agar plates.

Reason: DNA sequence consisted of mixed sequence data or complex insertion/deletion events

Submission of mixed population cultures can result in mixed sequence data, particularly if the organisms are not morphologically distinct or are growing within the same colony. Multiple peaks of equal or different intensities become apparent throughout the sequencing electropherogram, making the sequence data unresolvable, as shown in Figure 4.

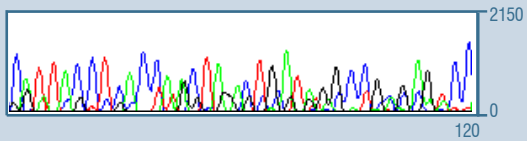


Figure 4. Partial electropherogram showing mixed sequence data. It is not possible to differentiate the bases, which results in a significant number of "N" or "any base" calls.

The 16S and ITS2 ribosomal regions we sequence for microbial identification can be multicopy. Each copy is not always the same sequence or same length due to insertions or deletions within one or more copies of the sequencing target. The primers we use will amplify all targets, thus generating multiple different sets of peaks which, when analyzed, can appear to be derived from a mixed culture. Most times, we are able to reconcile these events, but there are instances when the composition of the sequence data is too complex to resolve. Figure 5 is a partial electropherogram showing an example of an insertion event where our data analysis experts were able to resolve the data to determine the identification of an unknown isolate.

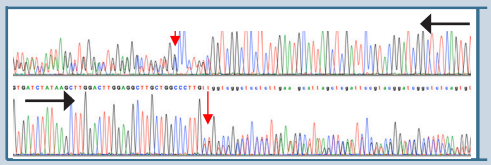


Figure 5. Partial electropherogram showing a simple "insertion/deletion event" (red arrows). Black arrows indicate the direction of the sequence; top: right to left; bottom: left to right. The raw data before the insertion or deletion of a base show clear, single peaks, whereas the data after the event show multiple peaks at each position. In all but the most complex situations, these data can be reconciled and an identification report generated.

Reason: Nonspecific DNA amplification

A microorganism may have another region within its genome with a small level of homology to the ends of the primers used for identification or sequence typing. Under certain circumstances, the primers may anneal and result in the amplification of a nonspecific PCR product. The data that results from this event can be of a quality sufficient to pass QC, but the sequence would not be from the target 16S, ITS2 or MLST target genes regions and thus would provide no phylogenetic information. In this case, no ID report can be generated from this genetic information.

Reason: Unable to provide a Strain Typing Comparison Report (applies Only to AccuGENX-ST®)

We are unable to provide AccuGENX-ST® comparison reports if the samples you submit are 1) not the same species, or 2) there is no validated AccuGENX-ST® service for the species for which you wish to compare strains.

To minimize disruption to the service, you should contact us prior to sample submission to inquire whether your species of interest is already validated for AccuGENX-ST®. Alternatively, you can view our up-to-date, validated AccuGENX-ST® species list here.

To determine the appropriate gene targets and primer sets, our process begins with identification analysis to confirm the species of your isolate(s). If the species you indicated for AccuGENX-ST® is different from the samples submitted, our Technical and Customer Support Group will contact you to discuss options for continuing with AccuGENX-ST®. If AccuGENX-ST® is not available for the samples submitted, you will be charged for and receive BacSeq/FunITS reports indicating the species of the organisms you have submitted.

2) In the case of a method not being validated, our research and development team can evaluate the organism for multilocus sequence typing (MLST) development and, if suitable, develop an assay for that organism. There is a 3- to 4-week lead time for MLST development and no additional charge for the method development work being performed.

DuPont RiboPrinter®

RiboPrint™ patterns characterize environmental isolates. When the RiboPrinter® is unable to produce a pattern, it is generally due to one of the following reasons:

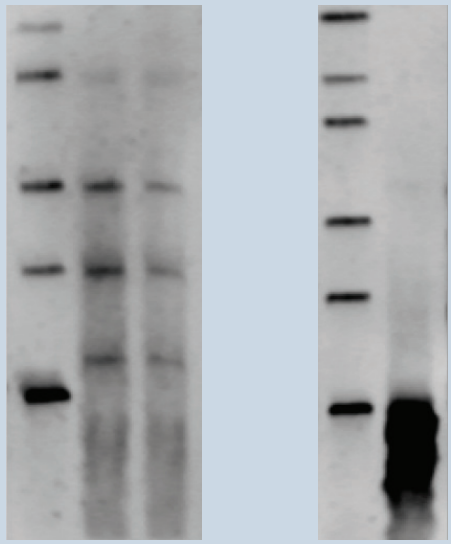
1. Low signal-to-background ratio

2. Uncut DNA

The RiboPrinter® performs most steps in the ribotyping process automatically. Bacterial colonies are picked, re-suspended in a sample buffer and transferred to a sample carrier. The samples are processed and analyzed robotically through numerous enzymatic, electrophoretic, hybridization and detection steps.

Successful hybridization is detected with a CCD camera, which converts the light patterns to a digital image. Software normalizes the image and corrects for the background. However, at times this automated process may not be successful at one of the automated processing stages (lysis, capture on the nylon membrane, washing, etc.). This results in a low intensity of the banding pattern when compared to the background, or the overall background is too high. The low signal-to-background ratio can make it difficult for the software to interpret the banding patterns and assign a RiboPrint™ group.

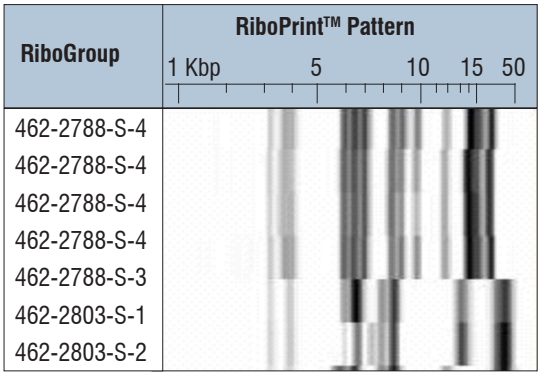
Alternatively, the culture may be lysed effectively, but the enzymes are not able to cut the DNA. Uncut DNA can be due to no restriction sites in the ribosomal region or a salt imbalance, for example, and there are no banding patterns for assignment to a RiboPrint™ group. (See Figure 6 illustrations for examples of RiboPrinter® banding patterns.)



Low signal-to-background ratio *Uncut DNA*

Figure 6. Examples of RiboPrinter® banding patterns.

High-Quality Banding Pattern



AccuPRO-ID®

There are three instances whereby you will receive a Sample Notification Report for AccuPRO-ID® services:

1) The submitted sample did not generate any spectra or the spectra didn't match any spectral files in our Accugenix® reference library. In this instance, the sample will automatically transfer to our AccuGENX-ID® service for further testing.

2) You request AccuPRO-ID® and the sample either references as filamentous fungi on MALDI-TOF or if the expert opinion of our microbiology technologists determines the morphology to be a filamentous fungi. In this case, the sample will need to be transferred to our AccuGENX-ID® (FunITS) service, as we currently do not maintain a validated AccuPRO-ID® library for filamentous fungi. This transfer is not an automatic transfer, and a Technical and Customer Support Specialist will contact you to determine if you would like to proceed with the service. Note that you will incur the fees associated with FunITS service should you choose to proceed.

3) The culture or test streak provided has no growth for us to provide you with an identification.