

Overview

At MicrobesNG we sequence according to coverage, which is the average depth of sequencing achieved for your target organism.

Our target coverage varies by service, and depends on whether you have paid for additional coverage or not. As standard, our Short Read service has a target coverage of 30X Illumina data, and our Long Read service has a target coverage of 50X Nanopore data. Our hybrid service is a combination of short and long, targeting 30 and 50X respectively.

Sometimes your sample won't reach the target coverage. If the sample appears to be pure then, as per our terms and conditions of service, we will attempt to sequence the sample one more time at no additional cost.

This document is designed to help you understand why your samples might have failed to meet coverage and to help guide you through what is the best option for your specific samples. We want to give you the opportunity to get the best sequencing results possible.

How our resequencing QC works

Once your samples have been sequenced and passed through our bioinformatics QC an internal 'action code' is assigned to each sample. Below is a table explaining these in more detail:

Action Code	Sample type	DNA conc.	Cell count	MicrobesNG action	What we need from you
AC1	DNA or Strain	Pass	N/A	Sample will be sequenced again	Nothing! - samples will be placed on the next available sequencing run, you can track the progress of your samples on your project portal and you will receive an email update when the data is ready / sequencing is complete
AC2	Strain	Fail	Pass	Sample will be sequenced again	Nothing! - we will requeue your sample for a new DNA extraction and your samples will be placed on the next available sequencing run. You can track the progress of your samples on your project portal and you will receive an email when the data is ready / sequencing is complete.
AC3	Strain	Fail	Fail	We are waiting for a response from you.	We cannot sequence again with the Strain/DNA that you gave us, we need you to send us new material.
AC4	DNA	Fail	N/A	We are waiting for a response from you.	Please see below for more information on why we are asking for new samples.

Common Questions

How do I know if you require a resubmission?

If we require additional material from you, we will notify you by email. Please do not send us additional materials unless we have asked you to.

Okay, I want to send you a new sample - what now?

Please contact us via email (info@microbesng.com) to notify us of your decision to send new samples. A reply to the email you received from us would be ideal and if that is not possible then please ensure that you include the project number and sample ids that you are referring to in the body of the email.

We'll need to set up a new project for your resubmitted samples before going any further. The names of resubmission projects will end in the letter "R", and you will be issued with new sample ids for your resubmitted samples. Because your samples have already been sequenced once, resubmission samples will only be sequenced once more before they are completed.

If you have submitted a strain sample that requires a resubmission, we may ask you to send us DNA instead. Our extraction methodology is extremely robust, and extraction failures are rare when our submission protocols are followed appropriately. However for some strains with specific extraction conditions, we may ask that you perform the DNA extraction and follow our [DNA submission protocol instead](#), to ensure you have the best chance of obtaining data.

It's important that you follow our submission protocols correctly, failure to do so may lead to your samples being destroyed. You can find our [strain submission protocol here](#), and our [DNA submission protocol here](#).

What is your postal address? / Can I have more return labels?

You'll find a link to your personal return label in your project portal. Please print this label and attach it to the outside of your parcel.

The project number must be clearly visible on the label, and include the words "REPLACEMENT SAMPLES" on it. This really helps the lab identify projects and progress your samples as quickly as possible.

When sending us any samples we strongly recommend that you use a courier service to ensure full track and trace on your consignment. Our contact information below can be provided to courier services:

Laboratory Services - MicrobesNG
Units 1-2 The BioHub, Birmingham Research Park
97 Vincent Drive
Birmingham, B15 2SQ
UK
Tel : (+44) 0121 295 1910

Why do I need to resubmit my samples?

If we have requested for you to resubmit your samples, this is because it did not meet our target coverage on the first sequencing run, and your sample fell below our QC thresholds.

We want to give you the greatest chance of getting the best quality data possible. We are requesting a new sample because the existing sample is likely to fail to meet coverage again.

Why didn't you take my samples off the pipeline when it failed QC?

We are a high throughput laboratory, and once a sample is in our pipeline, it is not feasible to remove it. This is why we give you the opportunity to send us more DNA should your samples not meet the target coverage.

Also, in our experience, some samples that show low concentrations do actually meet our target coverage during sequencing.

My samples didn't meet the target coverage but they haven't been requeued

There are a couple of reasons this could happen:

The sample contains more than one species

If this is the case you will see other QC information supporting this (such as the taxonomy or genome size).

The sample has already been sequenced twice.

We will not incur the cost of sequencing for a third time unless there was an issue on our part. As standard we will not sequence samples for a third time and in the unlikely event that this is required it will be communicated on a case by case basis directly with our customers.

Who should I contact if I have any questions about my resequencing?

We hope that this guidance document explains the process and options clearly to you, but if you have any questions please feel free to email us at info@microbesng.com. Please include your project number in all communications with us.