

Re: ITS & 16S full dataset for analysis

Cheeke, Tanya E <tanya.cheeke@wsu.edu>

Sun 8/6/2023 2:02 PM

To: Porter, Stephanie <stephanie.porter@wsu.edu>; Geoffrey Zahn <GZahn@uvu.edu>

Cc: Letendre, Brittany Nicole <brittany.letendre@wsu.edu>

 1 attachments (50 KB)

IMR-SampleSubmissionSheet_v19(Cheeke_July182023).xlsx;

Thanks, Stephanie.

Geoff—for the AMF samples, please use the IMR submission sheet I just sent you today that includes the extra controls and soybean samples.

It's a bit of a long story, but our AMF DNA extracts didn't run well the first time at IMR, so IMR sent the plates back to Brittany at WSU Vancouver, and then she sent them to my lab at WSUTC for in-house PCR with WANDA/AML2 primers. My technician (Gunnar) did all of the AMF PCRs, plus a few extra soybean samples from Stephanie, and some additional in-house controls.

So, for the AMF dataset, please use the most current submission sheet that I sent (attached), rather than the original from Brittany, as the order in the new AMF plates may have changed (but sample # should be the same), and the new set for AMF re-dos has more total samples than the one Brittany had originally submitted.

I have attached it here again for everyone's records – the submission form that Gunnar put together for the AMF samples he re-did, plus the soybeans (x6) and the PCR controls. The six soybean samples should not be included in Brittany's analysis, but at some point, Stephanie would like the data analyzed for a separate study.

Thanks,
-Tanya

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From: Stephanie Porter <stephanie.porter@wsu.edu>

Date: Sunday, August 6, 2023 at 12:49 PM

To: Geoffrey Zahn <GZahn@uvu.edu>

Cc: Cheeke, Tanya E <tanya.cheeke@wsu.edu>, Letendre, Brittany Nicole <brittany.letendre@wsu.edu>

Subject: Re: ITS & 16S full dataset for analysis

Also, regarding metadata, attached are the last sheets we sent to the IMR sequencing center. Below is an exchange regarding a mistake in coloring the sequencing plate. I believe that now all the labels

generated by IMR are correct and reflect a correction of this labelling error. Brittany, do you agree?

Andre at IMR sequencing center: "As for moving forward with the 16S V6V8 and ITS2 (btw: how was the contamination level in the V6V8 without the PNA?) - I have modified the sample numbers in the queue and you are keeping your queue position from when they arrived (Dec.14th) and not starting at the bottom again. We're a bit behind and currently working on Oct/Nov right now, so there are a couple of months to go before we get to them, which will give you plenty of time to do the AML and then send back to us for all to be done together...unless you for-sure want the AML results first, in which case we can do them first and wait until you've looked at them to decide on the 16S+ITS.

As mentioned above, I've adjusted the numbers in the queue according to your new sheet you sent, but I just want to confirm something on Plate2: you edited the label below the plate and I think there is a mistake - the sample coloring on the plate shows (as it was before) column 8 is ITS2-only and column 9 is AMF-only, however your label has these switched which would not match the cell coloring from before. I assume it should say the one sample at A8 is ITS2 (yellow) and the 3 samples in A9-C9 are AMF (grey)? If so, final numbers I count remaining to do on the sheet are: 108 for AMF, then 190 for V6V8 and 190 for ITS2."

Brittany: "Oh, I think I'm seeing what he's talking about. The notes section wasn't updated below plate 2.

So yes, his assumption is correct:

"I assume it should say the one sample at A8 is ITS2 (yellow) and the 3 samples in A9-C9 are AMF (grey)? If so, final numbers I count remaining to do on the sheet are: 108 for AMF, then 190 for V6V8 and 190 for ITS2."

He was clearly more observant than I was. That was a good catch.

The change is correct on the new spreadsheet. There should be 108 AMF samples and, 190 V6V8 & 190 ITS samples. I think in my haste at the end of last semester, I swapped the AMF controls color label with the ITS control.

You can also inform André that chloroplast contamination was 0.9%, so we will move forward without the PNA clamps."

Stephanie

Stephanie S. Porter
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On Sun, Aug 6, 2023 at 12:38 PM Stephanie Porter

<stephanie.porter@wsu.edu> wrote:

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> So sorry about the mix-up. Here are the additional data for ITS & 16S:

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> Here is(are) the download link(s) for your data for the samples

> discussed in the email below:

>

> https://nam12.safelinks.protection.outlook.com/?url=https%3A%2F%2Furldefense.com%2Fv3%2F__https%3A%2F%2Fwww.dropbox.com%2Fs%2F5u6heshz0s2jtky%2FLeTendrelTS2.zip%3Fdl%3D1__%3B!!JmPEgBY0HMsZNaDT!ukSCYLe7joXD9IBF9yFs-1pnznGHlpE0B4luhubOEtW96iVI8OQ4aQaSKKJaSh8qXvlexyc2-Umie8w8MZcei83Q8r6ys0%24&data=05%7C01%7Ctanya.cheeke%40wsu.edu%7C9f843fbae93042a966e908db96b63049%7Cb52be471f7f147b4a8790c799bb53db5%7C0%7C0%7C638269481503196960%7CUnknown%7CTWFpbGZsb3d8eyJWIjojMC4wLjAwMDAiLCJQIjoiV2luMzliLCJBTiI6IklhaWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=MBes2ghouQjhb9ALWQVkrXRJPoLGH9GY8n5nVCaoOY%3D&reserved=0

> https://nam12.safelinks.protection.outlook.com/?url=https%3A%2F%2Furldefense.com%2Fv3%2F__https%3A%2F%2Fwww.dropbox.com%2Fs%2Ffhqtt8vhu8rzoX8c%2FLeTendreV6V8.zip%3Fdl%3D1__%3B!!JmPEgBY0HMsZNaDT!ukSCYLe7joXD9IBF9yFs-1pnznGHlpE0B4luhubOEtW96iVI8OQ4aQaSKKJaSh8qXvlexyc2-Umie8w8MZcei83xaDiWao%24&data=05%7C01%7Ctanya.cheeke%40wsu.edu%7C9f843fbae93042a966e908db96b63049%7Cb52be471f7f147b4a8790c799bb53db5%7C0%7C0%7C638269481503196960%7CUnknown%7CTWFpbGZsb3d8eyJWIjojMC4wLjAwMDAiLCJQIjoiV2luMzliLCJBTiI6IklhaWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=ZuGv4KactaVcp%2FWCPlykDdCUviVOU8RYfnPK22uv0q8%3D&reserved=0

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> Please have all interested parties download the data promptly as the

> files will be removed from our temporary storage after two (2) weeks

> (archiving is the client's responsibility).

>

> Almost all the samples worked - just a handful for each target (mostly

> same, most with "N" so maybe negatives?) are weak/failed at the PCR

> step (in the folder of that same name in the ZIP), hence sequenced

> poorly or failed...you may still be able to recover some usable data

> from some of them, they just might not be enough to remain after the

> typical "sample normalization" (equal # of reads/sample) step.

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> Stephanie

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> Stephanie S. Porter

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> <https://nam12.safelinks.protection.outlook.com/?url=https%3A%2F%2Fflabs.wsu.edu%2Fstephanie->

[porter%2F&](#)

[data=05%7C01%7Ctanya.cheeke%40wsu.edu%7C9f843fbae93042a966e908db96b63049%7Cb52be471f7f147b4a8790c799bb53db5%7C0%7C0%7C638269481503196960%7CUnknown%7CTWFpbGZsb3d8eyJWljojMC4wLjAwMDAilCJQljojV2luMzliLCJBTiI6lk1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=KK9LmDQJcuC65QJnOYI4s0Rp%2FowNsPFvuXevqQWCYoc%3D&reserved=0](#)

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> On Sun, Aug 6, 2023 at 12:05 PM Geoffrey Zahn <GZahn@uvu.edu> wrote:

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>> The full data I have is (with equivalent ITS reads from the same samples):

>>

>> unique_id sample_number sample_id block host_id drought inoculum

>> B-R2-059 Q059 1 B-R2-059 3 GrandFir ND 1Burn_W4

>> B-R2-063 Q063 2 B-R2-063 3 GrandFir ND 3Burn_W6

>> B-R2-076 Q076 3 B-R2-076 3 GrandFir D 3Burn_W5

>> B-R2-078 Q078 4 B-R2-078 3 Snowbrush ND 0Burn_W1

>> B-R2-083 Q083 5 B-R2-083 3 Snowbrush ND 3Burn_W5

>> B-R2-084 Q084 6 B-R2-084 3 Snowbrush D 0Burn_W1

>> B-R2-154 Q154 7 B-R2-154 6 GrandFir D 1Burn_W3

>> B-R2-165 Q165 8 B-R2-165 6 Snowbrush D 1Burn_W4

>> B-S2-083 Q083 9 B-S2-083 3 Snowbrush ND 3Burn_W5

>> B-S2-165 Q165 10 B-S2-165 6 Snowbrush D 1Burn_W4

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>> Geoffrey Zahn, PhD

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>> https://nam12.safelinks.protection.outlook.com/?url=https%3A%2F%2Furldefense.com%2Fv3%2F_https%3A%2F%2Fgzahn.github.io%2F__%3B!!JmPEgBY0HMsZNaDT!ukSCYLe7joXD9IBF9yFs-1pnznGHlpE0B4luhubOEtW96iVI8OQ4aQaSKKJaSh8gXvlexxyc2-Umie8w8MZcei83kz4LL9k%24&data=05%7C01%7Ctanya.cheeke%40wsu.edu%7C9f843fbae93042a966e908db96b63049%7Cb52be471f7f147b4a8790c799bb53db5%7C0%7C0%7C638269481503196960%7CUnknown%7CTWFpbGZsb3d8eyJWljojMC4wLjAwMDAilCJQljojV2luMzliLCJBTiI6lk1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=ndkDntC%2Fh7DUsgVjlyfKmR6FnLjOCdj3Ln6ncT%2BxI0%3D&reserved=0

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>> From: Cheeke, Tanya E <tanya.cheeke@wsu.edu>

>> Sent: Sunday, August 6, 2023 12:43 PM

>> To: Letendre, Brittany Nicole <brittany.letendre@wsu.edu>; Geoffrey Zahn <GZahn@uvu.edu>; Porter, Stephanie <stephanie.porter@wsu.edu>

>> Subject: ITS & 16S full dataset for analysis

>>

>> Hi Brittany,

>>

>> Geoff Zahn from Utah Valley University is presently trying to analyze your sequence data but only seems to have data for 10 samples for the ITS and 16S data. Could you please direct him to the file that contains all of your sequence data, as well as the metadata, for all primer sets? He will need to know what sequences go with what sample/plant/treatment, etc.

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>> He was hoping to get us some preliminary figures for the grant proposal by Monday, so any help you could

provide at your earliest convenience would be super helpful.

> >

> > Thanks!

> > Tanya

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> > Sent from my iPhone