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ITS1 Amplicon Prep

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ABSTRACT

This protocol was provided by IDT in the the xGen ITS1 Amplicon Panel manual.

ATTACHMENTS

xgen-16s-amplicon-panel-v2-and-xgen-its1-amplicon-panel-protocol.pdf

Protocol Citation: aglazer 2023. ITS1 Amplicon Prep. **protocols.io** https://protocols.io/view/its1-amplicon-prep-cxyfxptn

MANUSCRIPT CITATION: *xGen 16S Amplicon Panel v2 xGen ITS1 Amplicon Panel*. (n.d.). Retrieved July 30, 2023, from https://sfvideo.blob.core.windows.net/sitefinity/docs/default-source/protocol/xgen-16s-amplicon-panel-v2-and-xgen-its1-amplicon-panel-protocol.pdf?sfvrsn=acace007_16

Protocol status: In development

We are still developing and optimizing this protocol

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Prepare panel-specific Multiplex PCR Reaction Mix

1 Before mixing reagents, calculate the total volume of the Master Mix based on the number of reactions required, with 10% overage for pipetting.

2 Make the Multiplex PCR Reaction Mix according to the table below and **pipette-mix** the Master Mix thoroughly.

> Note
>
> Vortex components G1 (ITS1 Amplicon Panel Primer) and G2 and pulse-spin tubes to collect contents before use. Gently shake Enzyme G3 at room temperature for 00:05:00 minutes or until all solutes appear to be in solution. Place back on ice for the remainder of the protocol.

> Note
>
> Keep prepared Master Mix and all components **ON ICE** until ready to use.

	Reagents	Volume per sample (uL)
	Reagent G1 - ITS1 Amplicon Panel Primer	2
	Reagent G2	3
	Enzyme G3	15
	Total Volume	20

Multiplex PCR Master Mix Recipe

3 Use a multichannel to pipette out 20 µL the PCR Mastermix into each well of a mid-skirt plate as needed and keep ON ICE until template addition.

Perform Multiplex PCR

4 Pre-program the thermal cycler for multiplex PCR according to the table below and **allow the block to reach 98°C before loading samples**

> Note
>
> Confirm that lid heating is turned **ON** and is set to 105°C

	A	B	C
		98C	30 sec
	4 cycles	98C	10 sec
		63C	5 min
		65C	1 min
	14 cycles	98C	10 sec
		64C	1 min
		65C	1 min
		4C	Hold

Multiplex PCR Thermal Cycler Program

5 Use a multichannel to add 10 µL of template to the 20 µL Multiplex PCR Reaction Mix and pipette-mix thoroughly.

6 Place the mid-skirt plate containing template and Master Mix in the thermal cycler and run the

Multiplex PCR Program.

> **Note**
>
> Near the completion of the thermal cycler run, prepare the Indexing Reaction Mix in the post-PCR area. **Assemble this reaction mix on ice and keep cold until adding it to samples in the indexing step**. All components except indexes may be added to the Master Mix when running multiple samples in parallel.

Perform Post-Multiplex PCR Cleanup

10m 30s

7 Make sure that beads and samples are at room temperature. Spin down the sample plate and briefly vortex beads to homogenize before use.

8 Add 30 µL of magnetic beads (ratio: 1.0x) to each 30 uL sample after thermal cycling and mix by **vortexing**. Pulse-spin the samples in a microcentrifuge tube to collect contents.

> **Note**
>
> Make sure no bead-sample suspension droplets are left on the sides of the tube. If droplets are visible, pulse-spin the samples in a microcentrifuge to collect contents.

9 Incubate the samples for 00:05:00 minutes at room temperature, off the magnet. After incubation, place the sample tubes on a magnetic rack until the solution clears and a pellet is formed (about 00:05:00 minutes).

10m

10 While the sample is on the magnet, remove and discard the supernatant without disturbing the pellet (about 5 µL may be left behind) using a clean pipette tip. Leave the plate on the magnet.

11 Add 180 µL of **freshly prepared** 80% ethanol solution to the pellet while it is still on the magnet. Use care not to disturb the pellet. Incubate for 00:00:30 seconds, then carefully remove the ethanol solution.

30s

12 Repeat the previous step, for a second wash with the ethanol solution.

13 Pulse-spin the samples in a microcentrifuge, place back onto the magnet, and remove any residual ethanol solution from the bottom of the tube with a small volume tip.

14 Resuspend each bead pellet in 17.4 µL post-PCR TE Buffer.

> **Note**
>
> If an "off-bead" PCR is preferred, place tubes back on the magnet and transfer 17.4 µL of eluate to a fresh plate.

> **Note**
>
> Keep samples at room temperature. At no time should "with bead" samples be stored on ice, as this could affect binding to magnetic beads.

Prepare Indexing PCR Master Mix

15 Before mixing reagents, calculate the total volume of master mix based on the number of reactions required, with 10% overage for pipetting. It is recommended to prepare at least 10 reactions at any one time to maintain a volume of Enzyme I3 that can be accurately pipetted.

16 Prepare the Indexing Master Mix **ON ICE** according to the following table

> **Note**
>
> All components **except indexes** may be added to the Master Mix when running multiple samples in parallel.

Component	Volume per Sample (uL)

	Component	Volume per Sample (uL)
	Reagent I1	3.3
	Enzyme I2	0.5
	Enzyme I3	0.1
	Enzyme I4	25
	Total Volume	28.9

Indexing Master Mix Recipe

17 Set aside the Indexing MMX **on ice** until template addition.

Perform Indexing PCR

18 Program the thermal cycler with the Indexing PCR program described in the table below and **allow the block to reach 37^{o}C before loading samples.**

> **Note**
>
> Confirm lid heating is turned **ON** and set to reach 105^{o}C.

> **Note**
>
> The PCR cycle number can be increased for samples that may give low yields.

	A	B	C
		37C	20 min
		98C	30 sec
	7 cycles	98C	10 sec
		60C	30 sec
		66C	1 min
		4C	Hold

Indexing PCR Program

19 Add 3.7 µL of the pre-mixed xGen Amplicon UDI primer pair to each sample (17.4 µL).

20 Add 28.9 µL of the cold indexing PCR reaction mix, stored on ice, to each sample. Mix thoroughly by **pipetting** (total volume will be 50 µL).

21 Spin down the sample plate, place in the thermal cycler, and run the Indexing PCR program.

Perform Post-Indexing PCR Cleanup

12m 30s

22 Make sure the PEG NaCl solution is at room temperature. Spin down the sample plate and briefly vortex the PEG NaCl solution to homogenize before use.

23 Add 42.5 µL of PEG NaCl solution (ratio: 0.85x) to each 50 uL sample after thermal cycling and mix by **vortexing**.

> Note
>
> Make sure no bead-sample suspension droplets are left on the sides of the tube. If droplets are visible, pulse-spin the samples in a microcentrifuge to collect contents.

> Note
>
> If performing an "off-bead PCR," use 42.5 uL (ratio: 0.85x) of fresh magnetic beads.

24 Incubate the samples for 00:05:00 minutes at room temperature, off the magnet. Pulse-spin the samples in a microcentrifuge. Afterwards, place the sample tubes on a magnetic rack 10m

until the solution clears and a pellet is formed (about 00:05:00 minutes).

25 While the sample is on the magnet, remove and discard the supernatant without disturbing the pellet (about 5 µL may be left behind) using a clean pipette tip. Leave the plate on the magnet.

26 Add 180 µL of **freshly prepared** 80% ethanol solution to the pellet while it is still on the magnet. Use care not to disturb the pellet. Incubate for 00:00:30 seconds, then carefully remove the ethanol solution. 30s

27 Repeat the previous step, for a second wash with the ethanol solution.

28 Pulse-spin the samples in a microcentrifuge, place back onto the magnet, and remove any residual ethanol solution from the bottom of the tube with a small volume tip.

29 Immediately add 20 µL of post-PCR TE buffer and resuspend the pellet by pipetting up and down until homogenous. Incubate for 00:02:00 minutes off the magnet. 2m

30 Place the samples back on the magnet and transfer the clean 20 µL library eluates to a fresh plate. Make sure that the eluate does not contain magnetic beads (indicated by brown color in eluate). If magnetic beads are present, place the plate on the magnet, wait for pellets to re-form, then transfer eluate again.

31 **SAFE STOP:** Store freshly prepared libraries at -20^{O}C.