Genomic DNA Preamplification Using the Advanta CFTR NGS Preamp Reagent Kit

IMPORTANT Before using this quick reference, read and understand the detailed instructions and safety guidelines in the Advanta™ CFTR NGS Library Preparation protocol for LP 48.48 IFC with Juno™ (PN 101-6270), LP 192.24 IFC with Juno (PN 101-6212), or LP 48.48 IFC with Access Array™ (PN 101-6957).

Prepare the Preamplification Reactions

IMPORTANT

- Centrifuge the enzymes before use.
- Vortex and centrifuge all other buffers and reagents before use.

☐ **1** In a DNA-free hood, prepare the preamplification pre-mix in a 1.5-mL tube in the order shown in Table 1.

Table 1. Preamplification pre-mix

	Component	Vol. per Reaction (µL)	Vol. for 96 Reactions (µL)*
1	PCR Water (Fluidigm PN 100-5941)	1.8	216
2	4X TSP Master Mix (Fluidigm PN 101-3055)	1.25	150
3	TSP Sample Loading Reagent V2 (Fluidigm PN 101-7634)	0.25	30
4	TSP DNA Polymerase (Fluidigm PN 101-0995)	0.2	24
5	Advanta CFTR NGS Preamp Pool (Fluidigm PN 101-7279)	0.5	60
Total		**4.0**	**480**

*Includes overage.

☐ **2** Mix the preamplification pre-mix by briefly vortexing, and then centrifuging to bring down the contents.

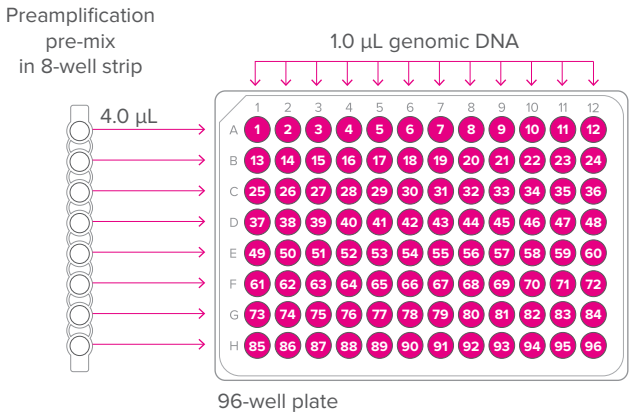

Figure 1. Preparation of preamplification reaction mix

☐ **3** Prepare the preamplification reaction mixes per sample as shown in Table 2 and Figure 1.

Table 2. Preamplification reaction mix

Component	Vol. per Reaction (µL)
Preamplification pre-mix (see Table 1)	4.0
Genomic DNA (gDNA), 5–30 ng/µL	1.0
Total	**5.0**

a Using an 8-channel pipette, transfer preamplification pre-mix into the wells of a 96-well PCR plate.

b Remove the plate from the DNA-free hood and add DNA samples to each well containing pre-mix, as shown in Figure 1.

☐ **4** Seal the plate using an adhesive seal.

☐ **5** Mix the reactions by briefly vortexing, and then centrifuge.

☐ **6** Place the plate in the thermal cycler and cycle using the following thermal protocol:

Temperature	Time	Cycles	Description
95 °C	15 min		Hold
95 °C	15 sec	14	Denaturation
60 °C	8 min		Annealing/extension
4 °C	∞		Hold

Cycling time is approximately 2 hours and 45 min.

☐ **7** After cycling, dilute the product as shown in Table 3.

Table 3. Preamplification product dilution

Component	Vol. per Reaction (µL)
Preamplification product	2
DNA Dilution Reagent (LP 48.48: Fluidigm PN 100-9167; LP 192.24: Fluidigm PN 100-8730)	38
Total	**40**

☐ **8** Seal the plate using an adhesive seal.

☐ **9** Mix the diluted products by briefly vortexing, and then centrifuge.

☐ **10** Use the diluted preamplified gDNA as a template for preparing the sample mixes as directed in the protocol.

STOPPING POINT The diluted preamplification products can be stored at 2–8 °C if used within 1 week, or stored at −20 °C for later use.

For technical support visit fluidigm.com/support.

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