# 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 on the LP 48.48 IFC with Juno™ Protocol (PN 101-6270) or the Advanta CFTR Targeted Library Preparation on the Juno LP 192.24 IFC Protocol (PN 101-6212).

## Prepare the Preamplification Reactions

**IMPORTANT** Preamplified and non-preamplified samples can be run on the same IFC. However, libraries for each must be generated separately and normalized before combining and sequencing. Failure to independently generate and normalize the libraries will results in unequal sequencing read distributions between preamplified and non-preamplified samples.

☐ **1** Manually flick the bottom of the TSP DNA Polymerase tube with your forefinger to mix the enzyme (**do not vortex**). Vortex all other reagents for 20 sec, and then briefly centrifuge all reagents before use.

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

Table 1. Preamplification pre-mix. Combine and mix in order shown.

| | Component | | Volume per Reaction (µL) | Volume for 96 Reactions (µL)* |
|---|---|---|---|---|
| 1 | PCR Water (PN 100-5941) | ⚪ | 1.8 | 216 |
| 2 | 4X TSP Master Mix (PN 101-3055) | 🟡 | 1.25 | 150 |
| 3 | TSP Sample Loading Reagent v2 (PN 101-7634) | 🟣 | 0.25 | 30 |
| 4 | TSP DNA Polymerase (PN 101-0995) | 🟠 | 0.2 | 24 |
| 5 | Advanta CFTR NGS Preamp Pool (PN 101-7279) | 🟢 | 0.5 | 60 |
| **Total** | | | **4.0** | **480** |

*Includes overage.

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

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

**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.

Table 2. Preamplification reaction mix

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

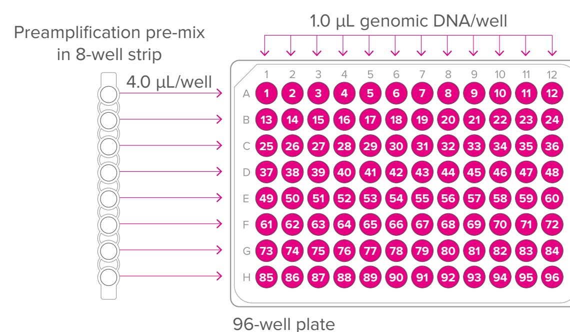


Figure 1. Preparation of preamplification reaction mix

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

☐ **6** Mix the reactions by briefly vortexing, and then centrifuge at 3,000 x *g* for 1 min to bring down contents.

☐ **7** Place the plate in a standard 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 hr and 15 min.

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

Table 3. Preamplification product dilution

| Component | | Volume per Reaction (µL) |
|---|---|---|
| Preamplification product | | 2 |
| DNA Dilution Reagent (PN 100-9167) | 🟢 | 38 |
| **Total** | | **40** |

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

☐ **10** Mix the diluted products by briefly vortexing, and then centrifuge at 3,000 x *g* for 1 min to bring down contents.

☐ **11** Use 2.5 µL of the diluted preamplified gDNA as a template for preparing the sample mixes.

**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.**

**North America** +1 650 266 6100 | Toll-free: 866 358 4354 in the US | techsupport@fluidigm.com **Latin America** +1 650 266 6100 | techsupportlatam@fluidigm.com
**Europe/Middle East/Africa/Russia** +44 1223 598100 | techsupporteurope@fluidigm.com **China (excluding Hong Kong)** +86 21 3255 8368 | techsupportchina@fluidigm.com
**Japan** +81 3 3662 2150 | techsupportjapan@fluidigm.com **All other Asian countries/India/Australia** +1 650 266 6100 | techsupportasia@fluidigm.com

**For Research Use Only. Not for use in diagnostic procedures.**

**Limited Use License to Perform Preamplification with Fluidigm IFCs:** A license to use Thermo Fisher Scientific's patented preamplification method workflows involving a Fluidigm integrated fluidic circuit (IFC) can be obtained (i) with purchase of a Fluidigm IFC from Fluidigm Corporation or (ii) by a separate license from Thermo Fisher Scientific. For licensing information, outlicensing@lifetech.com.

Information in this publication is subject to change without notice. **Patent and license information:** fluidigm.com/legalnotices. **Trademarks:** Fluidigm, the Fluidigm logo, Advanta, and Juno are trademarks or registered trademarks of Fluidigm Corporation in the United States and/or other countries. All other trademarks are the sole property of their respective owners. © 2018 Fluidigm Corporation. All rights reserved. 07/2018