applied biosystems
life technologies

TaqMan® OpenArray® Genotyping

Publication Number MAN0011116 **Revision** A.0

SUBJECT: Sample Preamplification Guide

Revision history

Revision	Date	Description
A.0	February 2015	New publication

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Guidelines for sample preamplification

Preamplification in OpenArray® experiments helps to ensure sufficient template copies in the 33-nL qPCR reaction. There is typically no need to preamplify samples if template in the qPCR reaction is present at >100 copies human gDNA, but at low template copy numbers, stochastic effects can dominate the reaction because the random events at each template molecule represent a large portion of the potential extension events. Stochastic events in qPCR reactions are generally dominant at levels of <10 template copies and negligible at levels of >100 template copies. Thus, the standard OpenArray® protocol for human SNP assays recommends starting with 50 ng/µL of genomic DNA. When diluted 50% in master mix and loaded into a 33-nL reaction chamber, the final template amount is 825 pg. This converts to 250 genomic copies (125 genomic copies for each allele in a heterozygote). Preamplification can also be used to effectively dilute PCR inhibitors from sample preparations that are of sufficient DNA concentration, but contain impurities and do not amplify well with the TaqMan® OpenArray® Genotyping Master Mix.

This protocol provides guidelines for performing a targeted multiplex preamplification of 1 to 256 SNP loci located within human genomic DNA samples. A multiplex pool of TaqMan® SNP Genotyping Assays is used to simultaneously preamplify up to 256 target polymorphisms in a single reaction using a reduced amount of input DNA sample. The preamplification product can be used as the sample template input for SNP genotyping reactions with any of the individual TaqMan® SNP Genotyping Assays included in the multiplex preamplification assay pool. For specific applications, a custom OpenArray® Preamp Pool specific for your TaqMan® SNP assay panel can be ordered along with your OpenArray® plate order (please contact your sales representative for details). Perform genotyping using the individual TaqMan® SNP Genotyping Assays following the standard protocol with the exception of substituting preamplified product for genomic DNA sample. It is not necessary to quantify or normalize preamplified product. The preamplified sample can be input directly into the reaction plate or further diluted in 1X TE Buffer to the desired concentration.

Note: The protocol is compatible for use with TaqMan® SNP Genotyping Assays, but is not recommended for use with TaqMan® Copy Number Assays.

Guidelines for human genomic DNA sample concentration

DNA sample concentration	Preamplification	Recommendation
<0.4 ng/µL	Not recommended	Preamplification of DNA at concentrations <0.4 ng/µL is not recommended, due to the potential for stochastic events arising from low target number in the early rounds of the preamplification and qPCR reactions.
0.4 ng/µL to 4.0 ng/µL	Ideal preamplification range	The preamplification protocol was developed using genomic DNA samples within a starting concentration range of 0.4 ng/µL to 4.0 ng/µL and a total of 0.5 ng to 5 ng in the preamplification reaction. Optimal performance will be achieved if the starting concentration of genomic DNA samples is near the middle of the working range (2.5 ng/µL). Our recommended protocol for accurate quantification of human gDNA samples using the RNase P Detection Reagents Kit can be found in Appendix B of the *Pharmacogenomics Experiments User Guide*.
4 ng/µL to 25 ng/µL	User discretion	Samples at these concentrations may be preamplified without dilution, but these are out of the optimal range.
>25 ng/µL	Not recommended	Samples with DNA sample concentration >25 ng/µL do not need to be preamplified. If samples at these concentrations do not amplify well with SNP assays, they likely contain PCR inhibitors. If this is the case, high concentration DNA samples may yield good results after dilution, and preamplification of these samples may be unnecessary.

Note: 1 ng of human genomic DNA = 300 genomic copies or 150 copies of each allele in a heterozygote.

User supplied materials

Reagent	Catalog no.
TaqMan® PreAmp Master Mix	4391128
OpenArray® PreAmp Pool	4485255[1]
Genomic DNA Samples	Supplied by user
1X TE Buffer, pH 8.0	12090015
Nuclease-free Water	Major laboratory supplier

[1] This product cannot be ordered directly from the Life Technologies website. For information on ordering an OpenArray® Preamp Pool, please contact your sales representative.

Perform the preamplification

1. Prepare the individual preamplification reactions in a 96-well reaction plate. For each individual genomic DNA sample, combine the reaction reagents as described in the table below. PreAmp Master Mix and OpenArray® PreAmp Pool can be prepared as a single cocktail and distributed to the plate in 3.75-µL aliquots. These volumes can be increased 2-fold if more preamplified product is needed. It is convenient to use plates with well volumes that can accommodate a 20X larger volume than the reaction for the 20-fold dilution step after preamplification.

Reagent	Stock conc.	Final conc.	Volume
TaqMan® PreAmp Master Mix	2X	1X	2.5 µL
OpenArray® PreAmp Pool	4X (0.20 each assay)	1X (0.05X each assay)	1.25 µL
Genomic DNA Sample	0.4 ng/µL to 4 ng/µL	0.1 ng/µL to 1 ng/µL (versus 150-1500 copies per µL)	1.25 µL
Total	**—**	**—**	**5.0 µL**

2. Firmly seal the reaction plate with a MicroAmp® Clear Adhesive Film (PN 4306311).

3. Vortex the reaction plate for 10 seconds and spin briefly.

4. Run the preamplification cycling program on a GeneAmp® PCR System 9700 (silver or gold block) or Veriti® Thermal Cycler using the following temperature and time settings. The number of cycles can range from 10 to 14.

Stage	Step	Temp	Time
Hold	Activate	95°C	10 min
Cycling (10-14 cycles)	Denature	95°C	15 sec
	Anneal/Extend	60°C	4 min
Hold	Inactivate	99.9°C	10 min
Hold	—	4°C	up to 1 h or overnight

5. Transfer the reaction plate from the thermal cycler to a container with ice. Keep the plate on ice until you are ready to dilute the preamplified product.

Dilute and store the preamplified product

1. Spin the reaction plate briefly prior to removing the film.
2. Remove film and add 95 µL of 1X TE Buffer to each well containing a preamplified product to create a 1:20 dilution.
3. Firmly seal the reaction plate with a new MicroAmp® Clear Adhesive Film.
4. Vortex the reaction plate for 10 seconds and spin briefly.
5. Store the preamplified product at −25°C to −15°C.

Documentation and support

Related documentation

Portable document format (PDF) versions of this guide and the following publication is also available on **www.lifetechnologies.com**.

Document	Publication number
Applied Biosystems® QuantStudio® 12K Flex Real-Time PCR System OpenArray® Experiments User Guide	4470935
Pharmacogenomics Experiments User Guide	MAN0009612

Note: To open the user documentation, use the Adobe® Reader® software available from **www.adobe.com**

Note: For additional documentation, see "Customer and technical support" on page 5.

Customer and technical support

Visit **www.lifetechnologies.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
- Order and web support

- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

The information in this guide is subject to change without notice.

DISCLAIMER

LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

Important Licensing Information

These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Trademarks

All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. Adobe and Reader are trademarks of Adobe Systems, Inc. TaqMan® is a registered trademark of Roche Molecular Systems, Inc., used under permission and license.

For support visit **lifetechnologies.com/support** or email **techsupport@lifetech.com**

lifetechnologies.com

13 February 2015

life technologies

A Thermo Fisher Scientific Brand