# NūPCR™ Assay Protocol Guide

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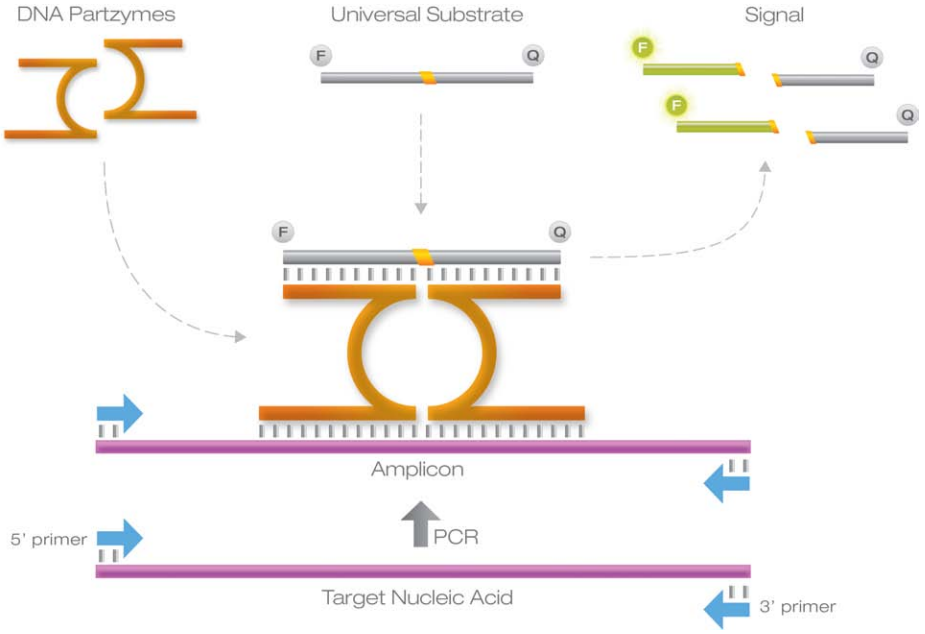
# Introduction

This document describes the NuPCR™ Assay protocol used to amplify and detect any DNA target (genomic DNA (gDNA), complementary DNA (cDNA) or plasmid DNA). NuPCR Gene Expression assays are designed using Illumina® DesignStudio™ software and contain all the oligonucleotides required for performing nucleic acid enzyme, NuZyme™, based quantitative PCR (qPCR). The NuPCR Assay can be run on any real-time PCR instrument.

Figure 1 NuPCR Assay Concept




NuZymes are nucleic acid enzymes that recognize and assemble on target DNA sequences forming a catalytic complex. The enzymatic activity of the NuZyme cleaves a fluorescently-labeled universal substrate, producing a signal that can be detected by a real-time PCR instrument.

NuZymes are composed of two oligonucleotides that are partial enzymes or PartZymes™. Each PartZyme contains part of the catalytic core sequence of the NuZyme, flanked by a substrate arm sequence and a sensor arm sequence. Each

sensor arm binds with high specificity to its target sequence, while the substrate arms bind the fluorescently-labeled, quenched universal substrate.

PartZymes contain no catalytic activity individually or in the absence of the target sequence. The target sequence serves to bring the two partzymes in close proximity, which facilitates the formation of the catalytically active NuZyme. The active NuZyme can now cleave the universal substrate oligo, however the target sequence remains intact. NuZymes are multiple-turnover enzymes, cleaving multiple substrate oligos in succession.

During a NuPCR reaction, target sequences are amplified using 5′ and 3′ primers and a DNA polymerase. The sensor arms of the partzymes bind to their corresponding sequences in the target amplicon, forming the catalytic NuZyme. The universal substrate binds the NuZyme substrate arms and is cleaved, releasing the fluorophore from the quencher, resulting in increased fluorescence. The fluorescent signal allows for detection and quantification of target sequences in real time.

NuPCR assays are supplied as a 20X solution containing all the oligonucleotides required for performing NuZyme based qPCR. The NuPCR master mix is provided as a 2X formulation containing all the components required for real-time PCR except primers, universal substrate, and DNA template. The ROX passive reference dye can be used for instruments that require it.

NOTE

NuPCR uses a three-step cycling protocol. Please read the *Assay Protocol* on page 10 carefully to properly setup your PCR instrument with the correct cycling conditions and fluorescence data acquisition step.

NOTE

- The NuPCR Assay can be run on any real-time PCR instrument. It has been shown to work on the following:
- Eco™ Real-Time PCR System
- Applied Biosystems 7900HT
- Applied Biosystems 7500
- Applied Biosystems ViiA 7
- Bio-Rad CFX 384

NOTE

You can access DesignStudio on the Illumina website at http://www.illumina.com/applications/designstudio.ilmn. A MyIllumina account is required.

### Multiplex PCR with NuPCR

Two-color multiplex real-time PCR can be performed with NuPCR. DesignStudio can design optimal primer and NuZyme sets for single tube multiplex assays for up to two targets. All sets are analyzed for oligo interactions and homology to other targets for specific and efficient amplification.

## What's New

The following changes have been made in this guide revision:

- Added a note in the Introduction to emphasize the importance of proper programming of the PCR instrument.
- Moved the workflow diagram to directly precede the protocol.
- Added a PCR instrument program diagram to the protocol.

# Best Practices

When performing qPCR, you should always adhere to good molecular biology practices to prevent contamination, which can lead to false positive results.

- Wear a clean lab coat and clean gloves when preparing samples.
- Change gloves between handling different samples.
- Clean work surfaces thoroughly before and after the procedure.
- Maintain separate areas and segregate equipment and supplies for pre-PCR setup and post-PCR analysis.
  - Do not bring amplified PCR products into the pre-PCR area.
- Use aerosol-resistant filter tips.
- Pipette carefully to avoid spillage.
- Protect the NuPCR Gene Expression Assay mix from light. Excessive exposure to light may affect the fluorescent probe contained in the mix.
- Centrifuge the tube or plate before placing it in the real-time PCR instrument.

# Contents

Check to ensure that you have at least one assay and one master mix identified in this section before proceeding.

## Assays

Each NuPCR assay is shipped at room temperature. As soon as you receive it, store it at -15° to -25°C.

Table 1 NuPCR Assays

| Description | Reactions | Catalog # |
|---|---|---|
| NuPCR Gene Expression Assays - FAM | 250 | EC-309-1001 |
| NuPCR Gene Expression Assays - FAM | 500 | EC-309-1002 |
| NuPCR Gene Expression Assays - FAM | 2000 | EC-309-1003 |
| NuPCR Gene Expression Assays - HEX | 250 | EC-309-1011 |
| NuPCR Gene Expression Assays - HEX | 500 | EC-309-1012 |
| NuPCR Gene Expression Assays - HEX | 2000 | EC-309-1013 |
| NuPCR Gene Expression Assays - ROX | 250 | EC-309-1021 |
| NuPCR Gene Expression Assays - ROX | 500 | EC-309-1022 |
| NuPCR Gene Expression Assays - ROX | 2000 | EC-309-1023 |
| NuPCR Gene Expression Assays - Q670 | 250 | EC-309-1031 |
| NuPCR Gene Expression Assays - Q670 | 500 | EC-309-1032 |
| NuPCR Gene Expression Assays - Q670 | 2000 | EC-309-1033 |

## Master Mix

Each NuPCR master mix is shipped on dry ice. As soon as you receive it, store it at -15° to -25°C.

Table 2 NuPCR Master Mixes

| Description | Reactions | Catalog # |
|---|---|---|
| NuPCR Gene Expression Master Mix | 250 | EC-305-1001 |
| NuPCR Gene Expression Master Mix | 500 | EC-305-1002 |
| NuPCR Gene Expression Master Mix | 2000 | EC-305-1003 |

# Consumables and Equipment

Check to ensure that you have all of the necessary user-supplied consumables and equipment before proceeding to the NuPCR protocol.

Table 3 User-Supplied Consumables

| Consumable | Supplier |
|---|---|
| 2 µl barrier pipette tips | General lab supplier |
| 2 µl multichannel pipettes | General lab supplier |
| 2 µl single channel pipettes | General lab supplier |
| 10 µl barrier pipette tips | General lab supplier |
| 10 µl multichannel pipettes | General lab supplier |
| 10 µl single channel pipettes | General lab supplier |
| Aerosol-resistant filter tips | General lab supplier |
| Optically clear adhesive seals | General lab supplier |
| PCR grade water | General lab supplier |
| PCR plates | General lab supplier |
| PCR tubes | General lab supplier |
| RNase/DNase zapper (to decontaminate surfaces) | General lab supplier |

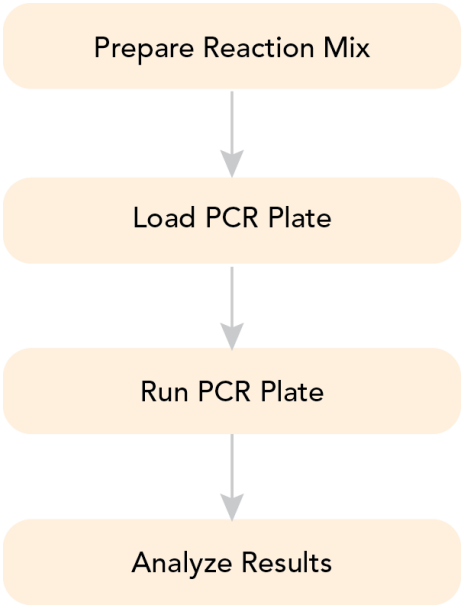
Table 4 User-Supplied Equipment

| Equipment | Supplier |
|---|---|
| Real-time qPCR System | General lab supplier |
| Microplate centrifuge | General lab supplier |
| Vortexer | General lab supplier |

# Assay Workflow

The following figure illustrates the processes of the NuPCR Assay protocol to amplify and detect any DNA target.

Figure 2 NuPCR Assay Workflow



# Assay Protocol

This protocol describes how to set up for a 20 µl reaction volume. Reaction volumes can be scaled as needed for the real-time PCR instrument being used.

## Illumina-Supplied Consumables

- NuPCR Gene Expression Assays
- NuPCR Gene Expression Master Mix

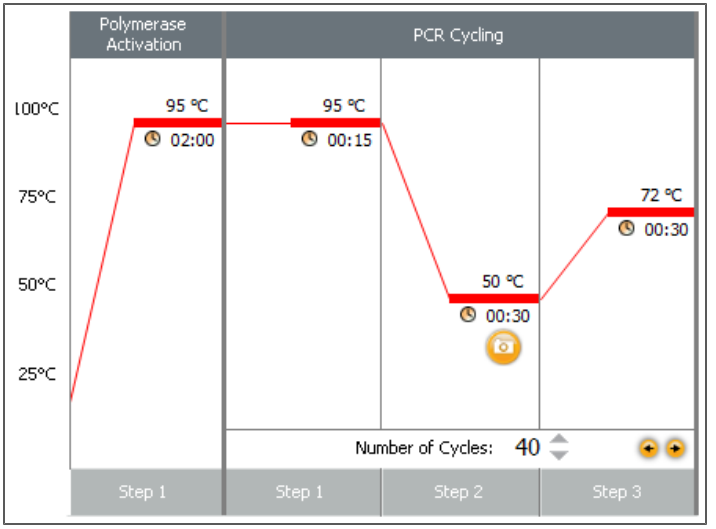
## User-Supplied Consumables

- DNA Template
- PCR Grade Water
- PCR Plate
- PCR Plate Adhesive Seal
- PCR Tube

## Preparation

- Remove one each of the NuPCR Gene Expression Assays and NuPCR Gene Expression Master Mix tubes from -15° to -25°C storage and let stand to thaw at room temperature.
- Vortex the thawed NuPCR Gene Expression Assays and NuPCR Gene Expression Master Mix tubes to mix thoroughly.
- Pre-program the real-time PCR instrument thermal cycle settings as follows:
  - 95°C for 2 minutes
  - 40 cycles of:
    - 95°C for 15 seconds
    - 50°C for 30 seconds
    - 72°C for 30 seconds
- Program the real-time PCR instrument to acquire fluorescence data on each cycle of the 50°C, 30 second annealing step.

Figure 3 PCR Instrument Settings




## Procedure

1 Do one of the following:

- **For non-multiplex PCR**, prepare the following reaction mix in a new PCR tube. Vortex the tube to mix thoroughly. Multiply each volume by the number of samples being prepared. If you are preparing multiple samples, multiply each reagent volume by 1.2.

| Reagent | Volume (µl) |
|---|---|
| NuPCR Gene Expression Master Mix | 10 |
| NuPCR Gene Expression Assays Mix | 1 |
| PCR Grade Water | enough to bring the total volume to 20 µl per sample |
| **Total Volume per Sample** | **20 - DNA volume** |

- **For multiplex PCR**, prepare the following reaction mix in a new PCR tube. Vortex the tube to mix thoroughly. Multiply each volume by the number of samples being prepared. If you are preparing multiple samples, multiply each reagent volume by 1.2.

| Reagent | Volume (µl) |
|---|---|
| NuPCR Gene Expression Master Mix | 10 |
| NuPCR Gene Expression Assays Mix 1 | 1 |
| NuPCR Gene Expression Assays Mix 2 | 1 |
| PCR Grade Water | enough to bring the total volume to 20 µl per sample |
| **Total Volume per Sample** | **20 - DNA volume** |

2 Transfer the total volume of reaction mix per sample to a new PCR tube or to each well of a new PCR plate.

3 Add the appropriate volume of DNA sample to the PCR tube or to each well of the PCR plate that contains the reaction mix. The total volume per tube or plate well should be 20 µl per sample.

4 Cap the PCR tube or seal the PCR plate with an adhesive seal.

5 Briefly centrifuge the tube or plate to remove air bubbles.

6 Place the PCR tube or plate in the pre-programmed real-time PCR instrument. Close the lid and start the amplification.

# Data Analysis

NuPCR data analysis varies depending on the qPCR instrument being used. Reference the appropriate qPCR instrument user guide for data analysis instructions.

# Notes

# Technical Assistance

For technical assistance, contact your NuPCR distributor.

Table 5 Illumina General Contact Information

| | |
|---|---|
| **Illumina Website** | http://www.illumina.com |

## MSDSs

Material safety data sheets (MSDSs) are available on the Illumina website at http://www.illumina.com/msds.

## Product Documentation

You can obtain PDFs of additional product documentation from the Illumina website. Go to http://www.illumina.com/support and select a product. To download documentation, you will be asked to log in to MyIllumina. After you log in, you can view or save the PDF. To register for a MyIllumina account, please visit https://my.illumina.com/Account/Register.

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