xxpress™
The Fastest qPCR in the World

xxpress® Demonstration PCR Assay Protocol

Originator	:	Neil Mallon
Project name	:	xxpress
Document number	:	Doc-01
Document revision	:	1.2

xxpress™ from BJS Biotechnologies Ltd
ISO9001: 2008 Certified BSI Certificate No FS 535379

65 Bideford Avenue Perivale Greenford Middlesex UB6 7PP United Kingdom
TEL: +44 (0)203 021 3750 EMAIL: info@xxpressPCR.com
Registered in England & Wales No 02959160 VAT No: GB 105 8702 29

Revision History			
Revision	**Change Description**	**Date**	**Author**
1.0a	Initial Issue	01-11-2013	Neil Mallon
1.1	Added lower reaction volume protocols to page 7	23-04-2014	Neil Mallon
1.2	Added Step by Step Instructions	04-09-2014	Neil Mallon

Glossary

Abbreviations / Acronyms

Following abbreviations / acronyms are used in this document.

PCR	Polymerase Chain Reaction
HgDNA	Human genomic DNA
NFW	Nuclease Free Water

Table Of Contents

1 Introduction

1.1 Background

The unique resistive heating technology that is utilised in the xxpress PCR instrument can allow a 40 cycle PCR assay to complete in as little time as 10 minutes. In order to demonstrate this capability a standardised PCR assay has been developed.

1.2 Overview

This particular assay tests for the 18S housekeeping gene using Human genomic DNA (HgDNA) as the input template for the reaction. The assay is run in a 20µL reaction volume using a 24 well xxpress® plate.

1.3 Applicable Documents / References

The following documents / references provide background information for this document:

Document Title	Document No.	Version	Date
N/A	N/A	N/A	N/A

2 Materials & Reagents

24 Well xxpress® Plate.
P10 Pipette + Tips.
P100 Pipette + Tips.
P1000 Pipette + Tips.
PCR Cold Block Rack.
1.5mL Microfuge Tubes.
xxpress® Instrument.
xxpress® Heat Sealer Films
SYBR Fast Supermix (Kapa Biosystems, Cat: KK4601).
HgDNA (Bioline, Cat: BIO-35025).
18S Forward Primer (Sigma, AAACGGCTACCACATCCAAG, 100µM).
18S Reverse Primer (Sigma, CCTCCAATGGATCCTCGTTA, 100µM).
Nuclease Free Water (Ambion, Cat: AM9914G).

3 Protocol

3.1 Reaction Preparation

For a 20µL reaction:
10µL Kapa SYBR Fast Supermix.
7µL Nuclease Free Water (NFW).
1µL 18S Forward Primer (100µM Stock).
1µL 18S Reverse Primer (10µM Stock).
1µL HgDNA (10ng/µL Stock).

1. Aliquot 40µL KAPA SYBR Fast Supermix in to an empty microcentrifuge tube.
2. Aliquot 28µL of nuclease free water in to the same tube and mix well.
3. Aliquot 4µL of 18S Forward Primer (100µM Stock) in to the tube and mix well.
4. Aliquot 4µL of 18S Reverse Primer (100µM Stock) in to the tube and mix well.
5. Aliquot 4µL of HgDNA (10ng/µL) in to the tube and mix well.
6. Aliquot 20µL of the reaction mixture in to three separate wells on a 24 well xxpress® plate.
7. Place the xxpress® plate on to the heat sealer and place a heat film on top of the plate. Seal the heat film on to the xxpress® plate.
8. Place the xxpress® plate with the reaction mixture in to the xxpress® centrifuge and centrifuge at 1,000rpm for 1 minute.
9. The xxpress® plate is now ready to be run on the xxpress® instrument.

For a 10µL reaction:
Dilute the Forward Primer Stock, Reverse Primer Stock 1 in 2:
5µL Kapa SYBR Fast Supermix.
2µL Nuclease Free Water (NFW).
1µL 18S Forward Primer (50µM Stock).
1µL 18S Reverse Primer (50µM Stock).
1µL HgDNA (5ng/µL Stock).

1. Aliquot 20µL KAPA SYBR Fast Supermix in to an empty microcentrifuge tube.
2. Aliquot 14µL of nuclease free water in to the same tube and mix well.
3. Aliquot 2µL of 18S Forward Primer (100µM Stock) in to the tube and mix well.
4. Aliquot 2µL of 18S Reverse Primer (100µM Stock) in to the tube and mix well.
5. Aliquot 2µL of HgDNA (5ng/µL) in to the tube and mix well.
6. Aliquot 10µL of the reaction mixture in to three separate wells on a 54 well xxpress® plate.
7. Place the xxpress® plate on to the heat sealer and place a heat film on top of the plate. Seal the heat film on to the xxpress® plate.
8. Place the xxpress® plate with the reaction mixture in to the xxpress® centrifuge and centrifuge at 1,000rpm for 1 minute.
9. The xxpress® plate is now ready to be run on the xxpress® instrument.

For a 5µL reaction:
Dilute the Forward Primer Stock, Reverse Primer Stock 1 in 4:
2µL Kapa SYBR Fast Supermix.
1µL 18S Forward Primer (25µM Stock).
1µL 18S Reverse Primer (25µM Stock).

1µL HgDNA (2.5ng/µL Stock).

1. Aliquot 10µL KAPA SYBR Fast Supermix in to an empty microcentrifuge tube.
2. Aliquot 7µL of nuclease free water in to the same tube and mix well.
3. Aliquot 1µL of 18S Forward Primer (100µM Stock) in to the tube and mix well.
4. Aliquot 1µL of 18S Reverse Primer (100µM Stock) in to the tube and mix well.
5. Aliquot 1µL of HgDNA (2.5ng/µL) in to the tube and mix well.
6. Aliquot 5µL of the reaction mixture in to three separate wells on a 96 well xxpress® plate.
7. Place the xxpress® plate on to the heat sealer and place a heat film on top of the plate. Seal the heat film on to the xxpress® plate.
8. Place the xxpress® plate with the reaction mixture in to the xxpress® centrifuge and centrifuge at 1,000rpm for 1 minute.
9. The xxpress® plate is now ready to be run on the xxpress® instrument.

Thermal cycling Protocol:
Initial Denaturation of 95°C for 20 seconds followed by:
40 cycles of:
95°C for 1 second.
60°C for 10 seconds. Collect a read using channel 1.