YOUSEQ

BEEF SPECIATION

qPCR TEST HANDBOOK

For Research Use Only. Not intended for diagnostic use.

CAT NO.: YS-qPS-IC-Beef-100

100 reactions
with Internal Extraction Control and Frozen MasterMix

VERSION 1.3

YOUSEQ

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INTENDED USE

This YouSeq Speciation test offers an efficient, multiplex solution for the detection of both Bos taurus (Beef) and total Universal Animal DNA (UM) in the same reaction. Comparing the species-specific signal with the universal animal signal allows for the percentage of target species DNA in the sample to be estimated while conserving the sample material. It is intended for use by trained professionals in a suitable molecular biology laboratory.

SPECIFICITY AND SENSITIVITY

Specificity

The YouSeq qPCR test for Beef Speciation is designed to specifically detect species relevant to the food industry. The primers and probes typically have a ≥95% homology with all reference data used, from relevant, publicly available databases at the time of design.

For further information on the detection profile of the product, please do not hesitate to contact our team: support@youseq.com

Sensitivity

The qPCR test is suitable for the detection of these targets across a wide dynamic range. Under ideal PCR conditions the assay can detect less than 100 copies of the target in the PCR reaction.

CONTENTS

Component	Cap Colour	Volume
Multiplex primer/probe mix: Beef (FAM) UM (VIC/HEX) Internal Control (CY5)		100 µL
Tetra™ 2X qPCR MasterMix		1 mL
Beef Positive Control (PTC)		500 µL*
Internal Control DNA Template		500 µL*
Template Resuspension Buffer (TRB)		1.5 mL
DNase/RNase Free Water		1.5 mL

* Supplied dried – requires resuspension. See instructions in resuspension section.

RECOMMENDED ADDITIONAL REAGENTS AND MATERIALS

Nucleic Acid extraction kit – Internal Control DNA is to be included in the sample extraction. See 'Use of Internal Control DNA' section below.

General laboratory equipment (pipettes, pipette tips, (micro)centrifuge tubes, compatible strip tubes/plates, plate seals, etc.).

qPCR instrument with channels to detect FAM, VIC/HEX and CY5.

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BEST PRACTICE

Decontamination:

Before beginning laboratory work, thoroughly decontaminate any work surfaces and pipettes being used, to eliminate potential contamination.

The supplied target-species DNA acts as both a run control and a calibration reference. Because this material contains target DNA, it should be treated as a contamination risk. Handle it separately from the main reaction setup, keep it away from other reagents and samples, and add it only after sample and negative-control wells have been closed or sealed.

General use and set-up:

All components should be fully defrosted with contents at the bottom of the tube before opening. To ensure contents are at the bottom, centrifuge or gently tap the tube. After use, reagents should be returned to the freezer.

Once any reagents are resuspended, mark the tick box on the tube for future reference. After this, or after combining reagents, the tube should be pulse vortexed to ensure it is mixed well.

It is advised to set up the tubes/plate and reaction mix on ice to minimise artefact formation, which may reduce sensitivity.

When preparing the qPCR reaction mix, it is recommended to incorporate an overage when calculating the total number of reactions to compensate for potential volume losses incurred during pipetting.

Set-up environments:

It is best practice to set up qPCR tubes/plates in two different environments - a clean (no template) lab and PCR (template) lab.

No Template Control(s) (NTC) and Positive Control(s) (PTC) should be included in every run. To reduce contamination, NTCs and samples can be set up and sealed in a clean lab before moving to the PCR lab.

BENCH SIDE PROTOCOL

RESUSPENSION

Before first use, resuspend the designated component with the correct reagent and specified volume, as per the table below:

1. Add the resuspension reagent and pulse vortex the tube to ensure each is mixed well.

Component	Reagent	Volume	Location
Internal Control DNA Template	TRB	500 µL	Extraction lab
Beef Positive Control (PTC)	TRB	500 µL	PCR lab

USE OF INTERNAL CONTROL DNA

1. At the relevant step of the extraction protocol, pause and add 5 µL of the resuspended Internal Control DNA into the extraction/lysis buffer for each sample that is to be extracted.

Please note: Do not add this Internal Control DNA directly into the biological sample as this may cause degradation of the control DNA.

2. Continue nucleic acid extraction as per the manufacturer's instructions.

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qPCR REACTION SET-UP

Please note: During each assay, The positive control is used to establish the expected relationship between the species-specific signal and the universal animal signal, creating a benchmark for a sample made entirely from the target species. **A positive control must be run with every plate to confirm that both detection systems are performing as expected.** If the control does not amplify correctly, the results from that run should not be accepted and the assay should be repeated.

1. Retrieve the required components and appropriate plasticware for qPCR reaction set-up.

2. In an appropriately sized (micro)centrifuge tube, combine the following reagents to create a reaction mix that will cover all required tube/wells (e.g. samples, NTC and PTC).

Please note: When calculating required reactions, include an overage to allow for volume loss during pipetting

Component	Volume (per reaction)
Tetra™ 2X qPCR MasterMix	10 µL
Beef Multiplex specific primer/probe mix	1 µL
Reaction mix volume	11 µL

3. Mix the combined reagents by briefly vortexing or inverting.

4. Dispense 11 µL of the reaction mix into all required tubes/wells.

5. For the NTC(s), add 9 µL of DNase/RNase Free Water into required tube/well(s).

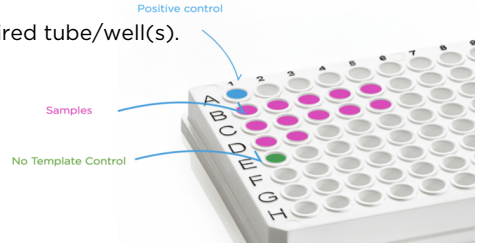
6. For each extracted sample, add 9 µL into required tube/well(s).

Please note: It is best practice to seal the NTC(s) and sample tube/wells before proceeding to the positive control step.

7. For the positive control(s), add 9 µL of the resuspended PTC into the required tube/well(s).

8. Seal the tube(s)/plate.

9. Briefly vortex the sealed tube(s)/plate, then spin in a centrifuge to ensure all reagents are fully resuspended and at the bottom of the tubes/wells before proceeding.



qPCR AMPLIFICATION PROTOCOL

1. Load the tubes/plate onto the qPCR instrument and set up the qPCR protocol following the table below.

2. Set the total reaction volume to 20 µL.

Please note: If using a qPCR instrument that uses ROX as a passive reference, ensure the passive reference is turned off or set to "none" before starting the run.

Temperature	Time	Number of Cycles
95°C	3 minutes	-
95°C	15 seconds	x 45
60°C	60 seconds	

Collect fluorogenic data through FAM, VIC/HEX, and CY5 channels during this step.

3. Start the run.

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INTERPRETATION OF RESULTS – OVERVIEW

If using single threshold analysis – YouSeq recommends setting the threshold at 10% of the End Point Fluorescence (EPF) for each channel.

- For the Target channel, use the PTC EPF to set the threshold.
- For the Internal Control, use the average EPF from samples to set the threshold.

Results interpretation:

Reaction Type		qPCR Signal (Cq)		
Positive control:		18 ± 2	18 ± 2	18 ± 2
No template control:		-	-	-
Sample	Internal Control: (CY5)	≤ 31	≤ 31	≤ 31
	Bos taurus: (FAM)	+	+	-
	Universal Meat: (VIC/HEX)	+	-	-
Result		**Positive result** Calculate species % below	**Negative result** No target species detected	**Inconclusive** No animal DNA detected

INTERPRETATION OF RESULTS: CONTROLS EXPLAINED

Positive control

The PTC should amplify in a Cq range of 18 ± 2 for each target. If this Cq range is not achieved, the sample test result for the associated target is invalid and should be repeated.

Please note: The positive control is a sequence representative of the target regions and does not contain the organism's entire genome. The positive control does not include the Internal Control sequence and should not be expected to amplify in the CY5 channel.

No template control

The NTC well(s) should be negative, with no amplification.

Please note: Background laboratory contamination can result in a very late signal in NTC wells. If the NTC has amplification, comparison to the sample test result is necessary:

- If the NTC is ≥5 Cq later than the sample signal, it can be considered negative, and the sample test result is valid.
- If the NTC is <5 Cq later, the sample test result is invalid – the test should be repeated after potential sources of contamination have been removed.

Internal Control

Detection of the Internal Control is through the CY5 channel. This gives information about the efficiency of the nucleic acid extraction. Cq values ≤ 31 indicate a successful extraction has taken place. If the signal is later than this, repeating the nucleic acid extraction is advised.

Please note: If the sample delivers a strong positive result for the target(s) of interest, then amplification of the Internal Control may be affected and may appear later. In this case, lack of Internal Control amplification is acceptable, and the sample test result is valid.

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INTERPRETATION OF RESULTS: CALCULATING SPECIES PERCENTAGE

The proportion of target-species DNA is calculated by comparing the target-species Cq shift with the universal animal Cq shift, using the positive control as the 100% reference point.

Formula

Species % = 2^-ΔΔCq × 100

where:

ΔΔCq =

[(Target species Cq sample − Target species Cq positive control) − (Universal Animal Cq sample − Universal Animal Cq positive control)]

Worked Example

Assume the assay gives the following Cq values:

Target species assay on sample: 26.7

Target species assay on positive control: 24.9

Universal animal assay on sample: 23.8

Universal animal assay on positive control: 24.0

Calculation:

ΔCq target species = 26.7 − 24.9 = 1.8

ΔCq universal animal = 23.8 − 24.0 = −0.2

ΔΔCq = 1.8 − (−0.2) = 2.0

Species % = 2^-2.0 × 100

Species % = 0.25 × 100

Species % = 25.0%

In this example, the sample contains an estimated 25.0% target-species DNA.

Results above 100% may occur in some inhibited samples and should generally be reported as 100%. Very high calculated values, such as results above 400%, indicate that inhibition may be too severe for reliable quantification, and the sample should be re-extracted with additional purification steps before repeat testing.

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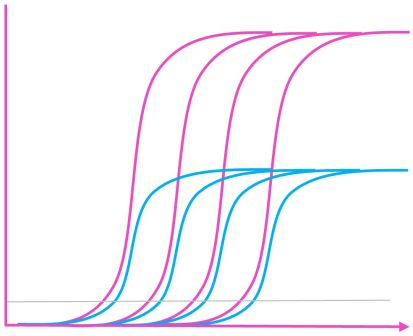
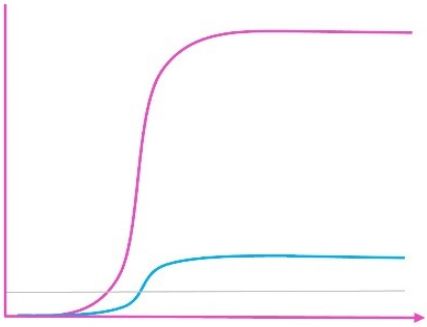
MULTIPLEX TROUBLESHOOTING

	1	2
Trace		
What can you see?	Different fluorescence levels between Targets	Unexpected low level of fluorescence, very similar to amplification from a different channel within the same well
Cause	Fluorescence depends on the assay design and the channel e.g. FAM channel is typically the brightest	'Bleed-through' between channels; Amplification from one channel has been incorrectly assigned to a different channel e.g. fluorescence in FAM 'bleeding-through' to VIC/HEX
Action	Analyse each channel individually so the Y-axis is appropriate for that assay	Ensure instrument is compatible with the dye (s) used in this test and/or recalibrate qPCR instrument

PRODUCT SPECIFICATIONS

Storage

Store at -20°C from arrival. The product's shelf life is outlined as an expiry date on the pouch label.

Suitable input material

This qPCR test will work well with any source of good quality nucleic acid. Good quality is defined as nucleic acid with high integrity (not degraded). Poor quality input nucleic acid is the leading cause of test failure.

Regulatory status

This product has been developed for Research Use Only and is not intended for diagnostic use. It should not be used for diagnosis of disease or infection unless specifically approved by the regulatory authorities in the country of use.

Quality Control

In accordance with the YouSeq Ltd ISO EN 13485-certified Quality Management System, each lot of Bos taurus Speciation qPCR Test Kit is tested against predetermined specifications to ensure consistent product quality. The primers/probe(s) typically demonstrate ≥95% in silico specificity to their intended target and are periodically checked against newly available sequence information to maintain their detection profile.

Technical Assistance

For customer support, please contact:

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