Microsynth

User Manual

Triplex Real-Time PCR AllVegi

Principle

Quantitative detection of DNA of the below mentioned elements of transgenic plants by Real-Time polymerase chain reaction (Real-Time PCR).
The amplified products are detected simultaneously via fluorescent dyes, each dye is characteristic for one species. DNA of the following species can be detected by exciting the corresponding fluorescence dye (ex = max. excitation wavelength [nm]; em = max. emission wavelength [nm]):

Fish: **FAM (ex 494 / em 520)**
Myostatin (Mammals/Birds):
HEX (ex 535 / em 556)
Plants: **Cy5 (ex 646 / em 662)**

The cycle at which the fluorescence from a dye crosses a given threshold yields the cycle threshold, Ct. Quantification of the amount of specific DNA contained in a sample can be achieved through comparison of the measured Ct to known standards.

Contents and Storage

5 tubes of primer-probe mix, lyophilized, for 5x20 reactions. Shipped at ambient temperature, store at -20°C, do not expose to light.

Reagents to be Supplied by User

5x HOT FIREPol® Multiplex qPCR Mix from Solis Biodyne (Cat.no. 08-01-00020) or similar product.

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Protocol

1. Add 150 µl water (PCR grade) per tube of primer-probe mix, vortex vigorously and incubate for 5 min at 60°C (store solution at 4°C, do not expose to light, stable for 1 week).

2. Add 250 µl HOT FIREPol® Probe qPCR Mix Plus or respective amount of similar product and mix well.
Yields 400 µl ready-to-use mastermix for 20 reactions à 20 µl reaction volume.

3. Mix 20 µl ready-to-use mastermix with 5 µl sample solution (recommended amount of DNA: 100 ng) in a suitable PCR reaction vessel.

4. Set up your Real-Time PCR machine according to the manufacturer in order to be able to measure the used fluorescence dyes.

5. Use the following thermal cycling profile:

1. 15 min, 95°C
2. 5 s, 95°C
3. 60 s, 60°C
4. 15 s, 72°C
5. Repeat steps 2 to 4 **45 times in total**

6. Analyze the fluorescence traces according to the manufacturer of your Real-Time PCR machine and determine the Ct-values and the amount of target DNA in each sample by comparing to known standards. However, quantification is only possible if the reference material corresponds to the sample in terms of species. Especially between poultry and mammals the copy numbers of the myostatin vary considerably.

Contact

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Further Information

The Fish System captures all common fish, the Plant System captures all plants and the Myostatin captures animal DNA from mammals and poultry. Insects are not recorded.

https://www.microsynth.com/food-testing-assays.html

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