

Assay Type: PCR – can distinguish between transgene negative and transgene positive tissues.

DNA Extraction: DNA from cells was extracted using Qiagen’s DNeasy Blood and Tissue kit (Cat# 69506). Kit directions for cells were performed with a few minor modifications as follows: repeat AW1 and AW2 wash steps one time, elute in 200ul of AE buffer once.

Primer Information:

- 1) Name: DsRed F Sequence: 5'-TGT CCC CCC AGT TCC AGT AC-3'
- 2) Name: DsRed R Sequence: 5'-GTC CAC GTA GTA GTA GCC GG-3'

Primer location: Both primers are located in the DsRed gene.

Assay name: DsRed PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25 mM	3.2
DsRed F	Sigma	25 µM	0.3
DsRed R	Sigma	25 µM	0.3
FastStart <i>Taq</i>	Roche (Cat#12032953001)	5 U/µl	0.2
Sterile Water			13

PCR Setup:

Final Reaction: 19µl master mix & 1µl DNA template (10-20ng/ul)

All reactions were performed in 200µl thin walled PCR tubes and were run in an Applied Biosystems 2700 thermocycler.

Cycle Parameters:

- 1) 94°C 3 minutes
- 2) 94°C 30 seconds
- 3) 58.8°C 30 seconds
- 4) 72°C 1 minute
- 5) Repeat steps 2-4 34 times for a total of 35 cycles
- 6) 72°C 10 minutes
- 7) 4°C hold until refrigerate product

Product Analysis:

All products were analyzed on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).

Alignment Marker: QX Alignment Marker 15bp/3Kb (Cat# 929522)

Size Marker: QX DNA Size Marker 100-3Kb (Cat# 929553)

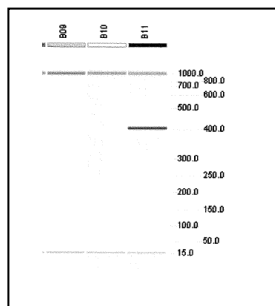
Method: AM320 Injection: 10s at 5KV

Separation: 320s at 6KV

Expected Products:

Transgene Positive: 407bp
 Transgene negative: no product

Example Gel:



Lanes B09 and B10 display transgene negative samples (no product)
 Lane B11 displays a transgene positive sample (407bp product)

Please note: the 15bp and 3kb bands are reference markers specific to the QIAxcel method and do not represent expected products.