

## Assay Transfer for the Gene of Interest Nr1i3<tm1142 Arte> (A.1561)

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<u>Taconic Line #:</u> 8222, 9103	

A PCR assay was optimized for the gene of interest and final PCR conditions, thermalcycling parameters and expected product sizes are shown below.

Table 1. Optimal PCR Conditions per Reaction

Reagent	Initial Concentration	Volume per reaction (μL)	Final Concentration
Distilled H <sub>2</sub> O, DNase, RNase free	N/A	Q.S. to 25	N/A
PCR Buffer w/ MgCl <sub>2</sub> (Qiagen #203205)	10X, 15mM	2.5	1X, 1.5mM
MgCl <sub>2</sub> (Qiagen #203205)	25mM	1.0	2.5mM (final)
Q-solution (Qiagen #203205)	5X	5.0	1X
Deoxynucleotide Mix (dNTPs) (Sigma #D-7295)	10mM	0.5	0.2mM
1062_2 primer (MWG Biotech)	25μM	0.5	0.5μM
1063_1 primer (MWG Biotech)	25μM	0.5	0.5μM
1064_2 primer (MWG Biotech)	25μM	0.5	0.5μM
HotStarTaq™ DNA Polymerase (Qiagen #203205)	5U/μL	0.25	0.05U/μL
Genomic DNA template	10ng/μL	5.0	2ng/μL
Total reaction volume	N/A	25	N/A

Table 2. Thermalcycling Template: Taconic Standard Template 3

Step	Temperature (°C)	Time (min)	Number of Cycles
Hot Start	95	15:00	1
Denature	94	0:45	35
Anneal	60	1:00	
Extension	72	1:00	
Final Extension	72	5:00	1

Table 3. Primer Sequences

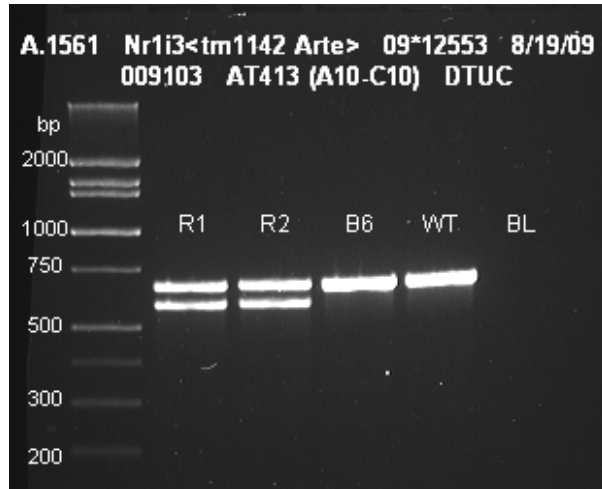
Primer Name	Primer Sequence (5' → 3')
1062_2	GCA AAC GGA CAG ATG GGA C
1063_1	CTC AAC TCC TCC CAC ATT CAG
1064_2	TCC CAT CCC CTG TGT TTC C

Table 4. Expected PCR Product Sizes

D	R	W
---	~663 bp	~663 bp
~559 bp	~559 bp	---

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Optimized assay performed on initial samples. Where applicable, “sT” is a simulated heterozygous control; “WT” is an internal wild-type control; “BL” is a mastermix blank negative control, no genomic DNA added.



Samples from line 8222 were also tested to verify backwards-compatibility of the assay (data not shown).