

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

Robert Kloc and Denver Tucker
 Molecular Analysis Department, Taconic
 5 University Place, Rensselaer, NY 12144
 Phone (518) 257-2030 X12154
 Fax (518) 257-2031
 Robert.Kloc@taconic.com

<u>Client:</u> TaconicArtemis	<u>Date of Revision:</u> 8/31/09
<u>Principal Investigator:</u> Dr. Nico Scheer	<u>Taconic Area Technical Representative:</u> N/A
<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 ₂ O, DNase, RNase free	N/A	Q.S. to 25	N/A
PCR Buffer w/ MgCl ₂ (Qiagen #203205)	10X, 15mM	2.5	1X, 1.5mM
MgCl ₂ (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

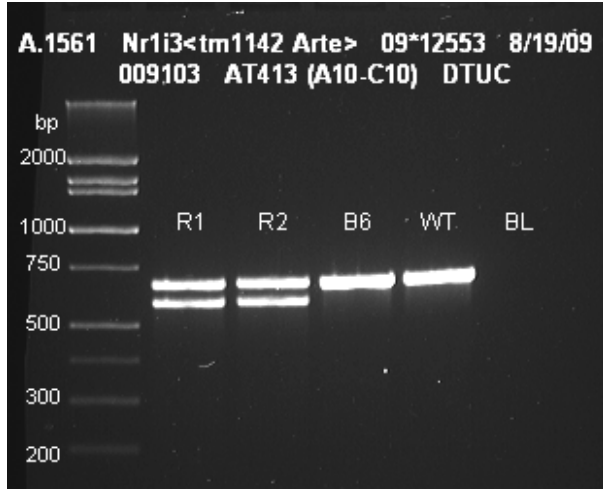
Table 3. Primer Sequences

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	---

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).