PCR Primers

Gene	Primer sequences	Species*	#cycles	Product size
Insulin	TCCTACCCCTGCTGGCCCTGC	m	18	305
	AGTTGCAGTAGTTCTCCAG			
nkx6.1	TCTTCTGGCCTGGGGTGATG	r	27	277
	GTGCTTCTTTCTCCACTTGGTCC			
nkx2.2	CATCTTGGACCTTCCGGACAC	m	27	267
	GGACTTGGAGCTCGAGTCTTG			
pdx-1	ACCACCTTCCAGCTCAGCTCC	m	30	213
	CGGGTGTAGGCAGTACGGGTC			
Pax4	ACCCTGTGACATTTCACGGAG	m	30	260
	CGATTGATAGAGGACACACTGG			
Pax6	TCACAGCGGAGTGAATCAGCT	m	27	204
	TACTCACACAACCGTTGGATAC			
BETA2/NeuroD	CTTGGCCAAGAACTACATCTGG	m	25	228
	GGAGTAGGGATGCACCGGGAA			
glucokinase	TGAGCCCAGAGAAGAAAGCT	m	30	479
	CTGGAACTCTGCCAGGAT			
GLUT2	CAAGATCACCGGAACCTTGG	m	30	311
	ATTCCGCCCACTGCAAAGCT			
SUR1	CAGTCATGGAGAGGAAAGCCCC	m	27	493
	GGATGATGCGATTGAGGAGGCT			
α-tubulin	GCGTGAGTGTATCTCTATCCAC	m	18	247
	GGTAGGTTCCGGTGCGTACTT			
G6PD	ACCTGCAGAGCTCCAATCAAC	m	30	172
	CACACAAGCAATGTTGTCTCG			
SV40 T antigen	ATCCTGGTGTTGATGCAATG		30	272
	CAGAGAGGAATCTTTGCAGC			
Isl-1	AGCAAGAACGACTTCGTGATG	r/h	27	187
	GACTGAGAGGGTCTCCAGCTC			
HNF3β	CGCTCGGGACCCCAAGACATACC	m	30	240
	GGCGAGCGGGGCACCTTGAGAAA			
HNF6	TCCCGCGCACCCGCGTTCCATG	m	30	193
	CGCATAAGTGTGAAACTACCGC			

* m=mouse; r=rat; h=hamster

TOUCHDOWN RT-PCR PROTOCOL

This protocol uses the **Titan™ One Tube RT-PCR System** from Boehringer Mannheim & a Perkin Elmer 9700 PCR System. Primers are at 10 uM.

In two separate tubes, prepare the following:

1 ul top primer
1 ul bottom primer
1 ul a-tubulin top (internal control)
1 ul a-tubulin bottom (internal control)
1 ul 10 mM dATP (USB)
0.5 ul 10 mM dCTP (USB)
1 ul 10 mM dGTP (USB)
1 ul 10 mM dTTP (USB)
0.25 ul [α-^{32}P] dCTP
2.5 ul DTT solution (kit)
0.25 ul Rnase Inhibitors (Promega)
50 ng RNA template
water to 25 ul V_1

14 ul water
10 ul 5X RT-PCR buffer (kit)
1 ul enzyme mix (kit)
water to 25 ul V_2

Pre-warm PCR machine to 50°C. Combine tubes 1 & 2 in a 0.2 ul PCR reaction tube and cycle as follows:

Step 1	50° x 30 min; 94° x 2 min	(1 cycle)
Step 2	94° x 1 min; 63° x 1 min; 72° x 1 min	(2 cycles)
Step 3	94° x 1 min; 62° x 1 min; 72° x 1 min	(2 cycles)
Step 4	94° x 1 min; 61° x 1 min; 72° x 1 min	(2 cycles)
Step 5	94° x 1 min; 60° x 1 min; 72° x 1 min	(2 cycles)
Step 6	94° x 1 min; 59° x 1 min; 72° x 1 min	(34 cycles)
Step 7	72° x 7 min; 4° hold	

Remove samples at appropriate time points (i.e., cycles) and add to an equal volume of formamide loading dye.

Load samples in a 6% sequencing gel and run until first dye band runs off bottom of gel. Dry gel and expose to film overnight.