

MUTANT MOUSE REGIONAL RESOURCE CENTER NODE  
UNIVERSITY OF CALIFORNIA, DAVIS

ANIMAL RESOURCE SERVICES  
OLD DAVIS ROAD, J1 BLDG. ROOM 1S9  
DAVIS, CA 95616  
(530) 754-8686  
FAX (530) 752-2993

---

FACSIMILE TRANSMITTAL SHEET

---

TO:	Rick Palazola	FROM:	Renee Araiza
COMPANY:	Jackson Laboratories	DATE:	1/14/02
FAX NUMBER:	(207) 288-6687	TOTAL NO. OF PAGES INCLUDING COVER:	4
PHONE NUMBER:		SENDER'S REFERENCE NUMBER:	
RE:	PCR for B6;129S-Slc12a1<tm1Unc>	YOUR REFERENCE NUMBER:	MMRRC strain # 17

---

URGENT     FOR REVIEW     PLEASE COMMENT     PLEASE REPLY     PLEASE RECYCLE

---

Hi Rick,

Here is the PCR for Dr. Takahashi's NKCC2 strain.

Renee

---



University of North Carolina at Chapel Hill  
Department of Pathology and Laboratory Medicine  
703 Brinkhous Bullitt, CB 7525  
Chapel Hill, NC 27599



Laboratories of Nobuyo Maeda & Oliver Smithies

Phone: 919-966-6912

Fax: 919-966-8800

To: Renee Araiza From: Nobuyuki Takahashi  
Fax: 530-752-2992 Pages: 3  
Phone: \_\_\_\_\_ Date: 12/20/01  
Re: NKCC2, MHRRC CC: \_\_\_\_\_

Dear Renee,

I fax the PCR protocol to genotype  
NKCC2 mice.

We use NaOH to prepare DNA

from toes. The protocol for it is also

faxed. (Biotechniques 29: 52-54, 2000)

Sincerely,

Nobuyuki Takahashi

## GENOTYPING BY PCR PROTOCOL FORM

### MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

Investigator/PI: Oliver Smithies Address: 701 BBB, UNC-CH Contact: Nobuyuki Takahashi

Telephone: 919-966-6912 FAX: 919-966-8800 email: ntakaha@med.unc.edu Recharge #: \_\_\_\_\_

Protocol: B6.129S-Slc12a1<tm1Unc>  
 NAME OF PCR: NKCC2

Reagent/ Constituent	Volume (uL)
DNA Sample	1
10x Buffer (contains 15mM MgCl2)	2.5
dNTPs (stock concentration is 2mM) <u>10mM</u>	2.5
Primer 1 (stock concentration is 20 uM) Name: <u>NeoR2</u>	1
Primer 2 (stock concentration is 20 uM) Name: <u>F16-2</u>	1
Primer 3 (stock concentration is 20 uM) Name: <u>F74-B</u>	1
Water	8.25
Taq Polymerase	0.25
Other? <u>DMSO</u>	2
<b>TOTAL VOLUME OF REACTION:</b>	<b>25.50 ul</b>

Comments on protocol (e.g., different concentration of MgCl2, etc): 10x PCR Buffer contains 67mM EDTA, 166mM ammonium sulfate, 670mM Tris (pH 8.5), 67mM MgCl2, 50mM beta-mercaptoethanol

**Strategy:**

Steps	Temp (°C)	Time (min)	# of Cycles
1. Initiation/Melting <u>HOT START?..CHECK HERE</u>	—	—	1
2. Denaturation	<u>94°C</u>	<u>30 sec</u>	) <u>40</u>
3. Annealing	<u>60°C</u>	<u>2 min</u>	
4. Elongation	—	—	
5. Amplification (i.e., 72°C, 10 min)	—	—	1
6. Finish (i.e., 4°C, indefinite)	—	n/a	(n/a)

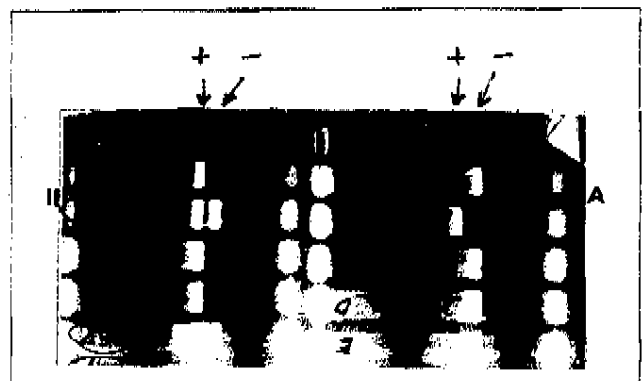
**Primers:**

Primer Name	Nucleotide sequence (5' - 3')																																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	
1: <u>NeoR2</u>	C	T	T	C	T	A	T	C	G	C	C	T	T	C	T	T	G	A	C	G														
2: <u>F16-2</u>	C	A	A	T	A	G	G	C	T	G	C	T	G	A	G	A	T	G	A	G														
3: <u>F74-B</u>	G	C	A	T	C	T	T	A	C	T	C	T	T	G	G	G	A	G	C															

**Electrophoresis Protocol:**

% Agarose: 1.2% mV: 120  
 Estimated Running Time (min): 1 hr

Number	Band (kb)	genotype
1	<u>620</u>	<u>+/+</u>
2	<u>620/490</u>	<u>+/-</u>
3	<u>490</u>	<u>-/-</u>



July 2000

Protocol for NaOH method  
for extraction of DNA from mouse toe

add 600  $\mu$ l of 50 mM NaOH to toe



heat it to 95°C for 30 min



vortex



neutralize w/ 50  $\mu$ l of 1 M Tris (pH 8)



centrifuge 5 min



transfer supernatant to a new tube

Use 1  $\mu$ l for PCR

\* You can get better signal than  
usual proteinase K preps

\* You do not need proteinase K.