

PCR genotyping of mouse strains in Nam and Capecchi, 2020

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Gently cut ear clips from mice. Put 1 or 2 ear clips in 1.7 mL Eppendorf tubes. Store @ -20 deg C until ready to process.

Process with HotSHOT buffers:

- (1) Add 77 μ L of HotSHOT buffer 1.
- (2) Spin down the buffer and the ear clip(s), so they are at the bottom of the tube.
- (3) Incubate for ~1 hour at ~95-99 deg C oven.
- (4) Let cool down a little. Add 77 μ L of HotSHOT buffer 2.
- (5) Shake up and down vigorously about 15 times. See if the ear clips have disintegrated. If not, shake a little more.
- (6) Spin down the insoluble matter. Centrifuge 3 min @ 14k rpm.
- (7) Ready to set up PCR reactions. Store @ 4 deg C or -20 deg C. "Goes bad" if stored at room temperature overnight - appearance of non-specific products in the PCR reactions.

HotSHOT buffers:

Buffer 1

NaOH	10 N	25 μ L	25 mM final
EDTA	0.5 M	4 μ L	0.2 mM final
Water		to 10 mL	

Buffer 2

Tris-HCl	solid	325 mg	40 mM final
Water		to 50 mL	

HotSHOT buffer 1 "goes bad" over some months. I now make it fresh every time. What happens when the buffer 1 "goes bad" is the ear clips don't disintegrate anymore. In that case, spin down the left over ear clip, aspirate away the "no good no more" buffer, then add fresh buffer to repeat.

Set up the PCR reactions:

- (1) Make a mix with water, 2x Taq mix, and primers as noted on page 2. The primer stock is at 10 μ M of each in TE. Store @ -20 deg C.
- (2) Vortex and spin down. Keep on ice.
- (3) Aliquot 11.5 μ L to 8-strip PCR tubes. Keep on ice.
- (4) Add 2 μ L of the HotSHOT DNA. Mix and spin down. Keep on ice.
- (5) Set up the PCR cycling program as noted on page 3 (same as in Nam and Capecchi, 2020).
- (6) Start the cycling program. Keep the tubes with the PCR reactions on ice until the cyler's bottom plate has reached ~85-90 deg C. Once the cyler's bottom plate is hot (i.e., before the first step at 94-95 deg C), then put on the tubes with the PCR reactions.
- (7) Let the PCR cycling program go.
- (8) Run 5-10 μ L of the PCR reaction on 2% agarose gel in TBE.

The 2x Taq mix was purchased from multiple sources. Initially, we purchased from Takara. More recently, we purchased from Azura Genomics. They both worked beautifully. At one point, the lab was using home-made Taq "TAAZ TAQ," and that was little bit trickier to set up initially.

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Nam and Capecchi, 2020 PCR genotyping reaction setup

	x1	x2	x3	x4	x5
Total	13.5	27	40.5	54	67.5
2x mix	6.25	12.5	18.75	25	31.25
Primers	1	2	3	4	5
Water	4.25	8.5	12.75	17	21.25
DNA	2	4	6	8	10
	x6	x7	x8	x9	x10
Total	81	94.5	108	121.5	135
2x mix	37.5	43.75	50	56.25	62.5
Primers	6	7	8	9	10
Water	25.5	29.75	34	38.25	42.5
DNA	12	14	16	18	20
	x11	x12	x13	x14	x15
Total	148.5	162	175.5	189	202.5
2x mix	68.75	75	81.25	87.5	93.75
Primers	11	12	13	14	15
Water	46.75	51	55.25	59.5	63.75
DNA	22	24	26	28	30
	x16	x17	x18	x19	x20
Total	216	229.5	243	256.5	270
2x mix	100	106.25	112.5	118.75	125
Primers	16	17	18	19	20
Water	68	72.25	76.5	80.75	85
DNA	32	34	36	38	40

A standardized PCR cycling program

95 °C 3 min, (95 °C 20 s, 60 °C 30 s, 72 °C 1 min) × 30 cycles, 72 °C 2 min

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