Elchrom Scientific

Technical Manual for GenoMouse-Speed Kit (Speed Congenics, Accelerating background crossing)

(P/N 5001, 5002, 5003, 5004, 5005)

1 Index

2 Product Description

During accelerated background crossing (speed congenics), GenoMouse-Speed Kits allow the fast identification of the mice that should preferable be chosen for further backcrossing. The aim of an accelerated background crossing is to obtain a mouse showing a specific genetic characteristic in a defined genetic background (Figure 1).

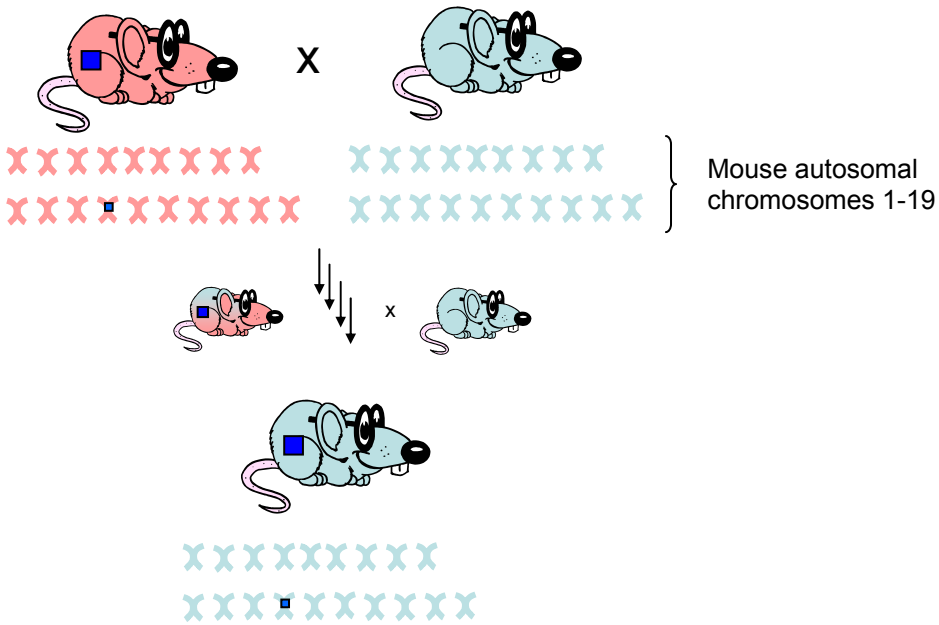


Figure 1. Principle of Accelerated Background Crossing. A starter mouse (pink) showing a specific change in the genome (such as transgen, knock out or other genetic changes) (blue square) is crossed with a mouse presenting a defined genetic background (light blue).

The GenoMouse-Speed Kits are based on the detection of 96 microsatellite markers that are variable for the two paternal mice crossed. For each of the 19 autosomal chromosomes, 5 (6 on chromosome 1) microsatellite markers that are spread along the chromosome have been selected. This is important to monitor any deletions or recombinations in the genomic background during crossing. The microsatellites used are tetranucleotide repeats and the length of the PCR product varies between 150 and 260 bp. The primer pairs are named according to their relative position on the chromosome as shown in Figure 2.

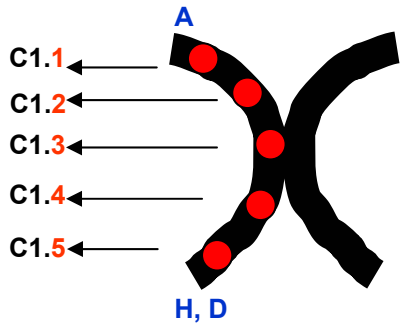


Figure 2. Primer pair names. The five detected microsatellite markers (red spots) on each chromosome are located on each mouse chromosome. The name of the primer pair detecting a given microsatellite is built by the number of the chromosome (C) (black) and the ascending number of the marker on this chromosome (red). Number 1 is located near the beginning (A region) of the chromosome and number 5 is located near the end (H region for the biggest and D region for the smallest) of the chromosome.

3 Storage Conditions

- Store PCR plates at 4°C horizontally, top side up
- Store Spreadex hydrogels at 4°C
- Do not freeze any of the components of the kit!

4 Kit Components

4.1 Overview

Table 1. GenoMouse-Speed Kit Components

Component description		Amount
96 well plates containing the PCR primers for 96 microsatellites		6 plates
Spreadex® EL 500, Wide Mini S-4x25		6 (4x25) hydrogels
M1 DNA size marker (for 26 loadings)		120µl

4.2 Content of the plates

The dried blue pellet in the 96 well plates includes the primer pairs (Table 2) and the PCR blue dye[1]. The order of the dried primers within the plate is listed in Figure 3.

Table 2. Primer content of the PCR plates

Plate No and No of holes		Primer pairs		
1		C1.1	-	C3.5
2		C4.1	-	C7.1
3		C7.2	-	C10.2
4		C10.3	-	C13.3
5		C13.4	-	C16.4
6		C16.5	-	C19.5

[1] Loading buffer which is not interfering with the PCR

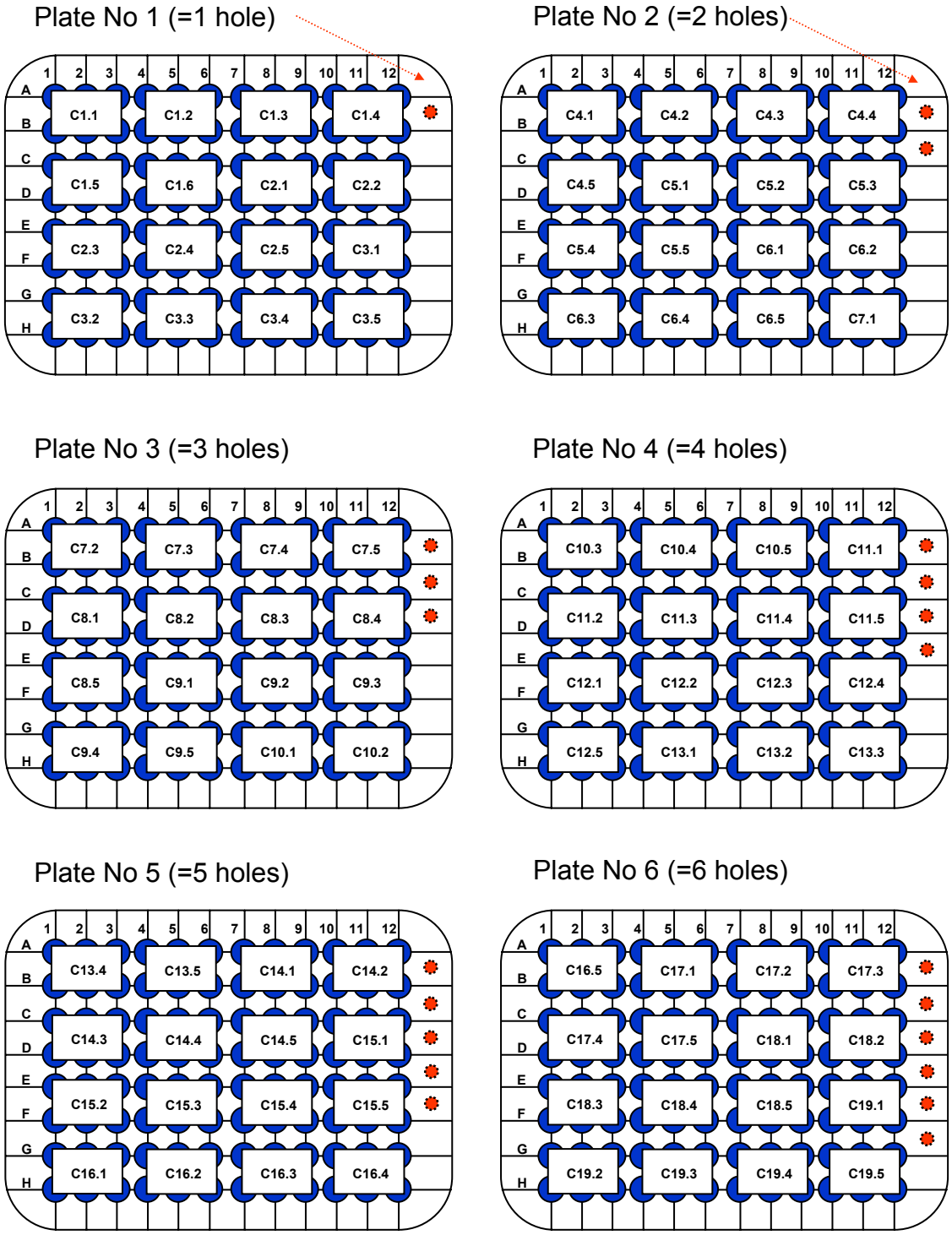
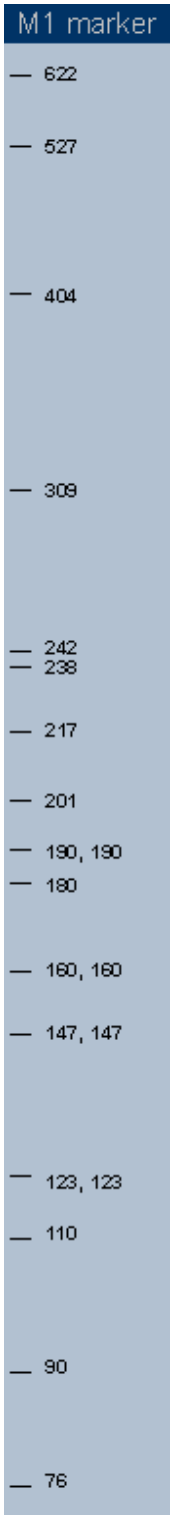


Figure 3. PCR plates Content. The 6 PCR plates are marked with holes at the right edge of the plates. In each row there are groups of 3 wells that contain the same primer pairs. The adjacent rows A/B, C/D, E/F, G/H contain the same primer pairs.

4.3 Map for the M1 DNA size marker



5 Quality control

All the primer pairs are tested with PCR on the DNA of both control mice.

6 Additional material required

PCR related:

Product	Recommended product	Company	Order Number for the recommended product
Hot Start DNA Polymerase with buffer	AmpliTaq Gold 5U/µl, with Gold Buffer and $MgCl_2$	ABI	4311806
$MgCl_2$	Included in the AmpliTaq Gold Kit	ABI	
dNTPs	Set of Deoxy-Nucleotides, PCR-Grade	Roche	1969064
Tween® 20		Roche	93773
Sealing for PCR plates	Adhesive PCR Film	Abgene	AB-0558
Electrophoretic system	Basic Electrophoretic System with SEA2000	Elchrom Scientific AG	2061E
Centrifuge for 96-well plates	Regular		
PCR machine with a 96 well plate adaptor	Regular		

Electrophoresis related:

Product	Recommended product	Company	Order Number for the recommended product
30mM TAE buffer	Protocol 4/or Elchrom	Elchrom Scientific AG	3031
Ethidium Bromide	Regular		
SYBR® Gold or SYBR® Green	SYBR® Gold SYBR® Green	Invitrogen	S11494 or S7563

If regular electrophoretic unit is used instead of SEA2000:

Product	Recommended product	Company	Order Number for the recommended product
Catamaran to secure 1/2 of a Wide Mini gel in a smal regular unit.	Catamaran S-13/50 (h 10.5cm x w 13.5cm)	Elchrom Scientific AG	2520
Catamaran to secure 1 Wide Mini gel in a large regular unit.	Catamaran S-26/400 (h 10.5cm x w 17.5cm)	Elchrom Scientific AG	2020
Special forceps for the gel	Special forceps	Elchrom Scientific AG	2366

7 Plate Preparation Protocol for PCR

7.1 Plate preparation

Before unsealing the plate:

- Spin down the plate

Resolving the primers and PCR start:

- Add 5µl Tween® 20 in H_2O (1:1000) to each well (Protocol 2)
- Spin down the plate
- Incubate the plate for 30 minutes at 35-45°C
- Add 5µl of the 2x PCR master mix (Table 3) including Hot Start DNA polymerase, polymerase buffer, dNTPs, DNA, Tween 20 in H_2O

7.2 Recommended preparation of 2x PCR master mix for one mouse (96 markers)

The setup of the GenoMouse-Speed Kit has been validated by using the protocol described in this section. For optimal results we strongly recommend to follow this protocol.

Table 3. 2x PCR master mix. Prepare the following 2x PCR master mix for one mouse (one 96 well plate, 96 markers):

Reagent	Amount
AmpliTaq Gold Puffer (or other hot start polymerase buffer)	100µl
AmpliTaq Gold Polymerase (5U/ml) (or other hot start polymerase)	2µl
dNTP (2mM each)	100µl
$MgCl_2$ (25mM)	60µl
Tween® 20 (1:10 diluted in H_2O)	5µl
ddH_2O	240µl
Genomic mouse DNA[2]	67ng-2.3µg

Prepare one 2x PCR master mix and aliquot the solution in different tubes before adding the different mouse DNA.

7.3 PCR program

Cycles	Procedure	Temperature	Time
1 Cycle	Initial Denaturation	94°C	20 min
35 Cycles	Denaturation	94°C	15 sec
	Annealing	64°C*	90 sec
	Elongation	72°C	30 sec
1 Cycle	Final elongation	64°C*	30 min
	storage	8°C	for ever

*Annealing temperature has to be adapted to the primer and can vary between the PCR-machines.

[2] Isolation of genomic DNA is usually performed from the tail. DNA isolation methods like phenol chlorophorm extraction and DNeasy Tissue Kit from QIAGEN have been tested to give appropriate quality of DNA to use in the GenoMouse-Speed

8 Protocol for Electrophoresis

Detailed protocol for electrophoresis in SEA2000:

- Prepare 2l of 30mM TAE running buffer (Protocol 4). The buffer level should be 2-3mm above the upper electrode. If not add or remove TAE buffer.
  Tipp: It takes about 45 minutes to heat up SEA 2000 to 55°C, but the power supply has to be running.
- Preparation of the hydrogel: Cut the aluminum bag with scissors on one side and take out the gel in its polyethylene (PE) bag. Cut PE bag on three sides and peel away the PE foil from the gel. Hold the gel with Elchrom forceps on the plastic backing overhang.
- If the hydrogels are taken directly from 4°C, place the gel **onto** the catamaran into the apparatus and preheat the hydrogel for 30 minutes. Before loading the samples place the hydrogel into the buffer and fix it with the catamaran.
- Check that the pump delay is switched off before loading the samples!
- For your convenience load the samples with a multichannel pipette according to the scheme in Figure 4: Load 4µl of each PCR sample on Spreadex® EL 500 S-4x25 hydrogel from Elchrom Scientific without adding any additional loading buffer.
- Set the power supply to 144V and 70 minutes.
- Close the lid, start the power supply and set pump delay to 1.5.
- After the run take out the hydrogel from the apparatus. Remove the plastic backing with nylon string that is provided. Mark each of the four gel parts individually by cutting the edges. Grip the plastic backing with Elchrom forceps and place the gel **upside down** into the Easy Staining tray, so that the plastic backing can easily be peeled away.

Detailed protocol for electrophoresis in regular unit:

- Prepare necessary volume of 30mM TAE buffer for the regular electrophoretic unit.
- Preparation of the hydrogel: Cut the aluminum bag with scissors on one side and take out the gel in its polyethylene (PE) bag. Cut PE bag on three sides and peel away the PE foil from the gel. Hold the gel with Elchrom forceps on the plastic backing overhang. If a small electrophoretic unit is used cut the hydrogels in the middle.
- Place the hydrogel into the buffer and fix it with the catamaran.
- For your convenience load the samples with a multichannel pipette according to the scheme in Figure 4: Load 4µl of each PCR sample on Spreadex® EL 500 S-4x25 hydrogel from Elchrom Scientific without adding any additional loading buffer.
- Set the voltage of power supply to 10V/cm (cm: distance between the electrodes) and run for 70 minutes.
- After the run take out the hydrogel from the apparatus. Remove the plastic backing with nylon string that is provided. Mark each of the four gel parts individually by cutting the edges. Grip the plastic backing with Elchrom forceps and place the gel **upside down** into the Easy Staining tray, so that the plastic backing can easily be peeled away.

9 Staining

- Stain the hydrogel with SYBR Gold (or SYBR Green II) or Ethidium Bromide:
  - With SYBR Gold (or SYBR Green II): Dilute 5µl of SYBR dye in 50ml DST solution (or 30mM TAE) in plastic beaker (not glass!). Stain on shaker for 30 minutes and cover the staining tray to protect the gel from light.
  - With Ethidium Bromide: Dilute 50 µl of stock solution (1mg/ml) in 100ml ddH_2O in glass beaker. Stain on shaker for 30 minutes.
- Destain the hydrogel with DST for 30 minutes if necessary (by this procedure a better contrast will be developed between the markers and the gel background).

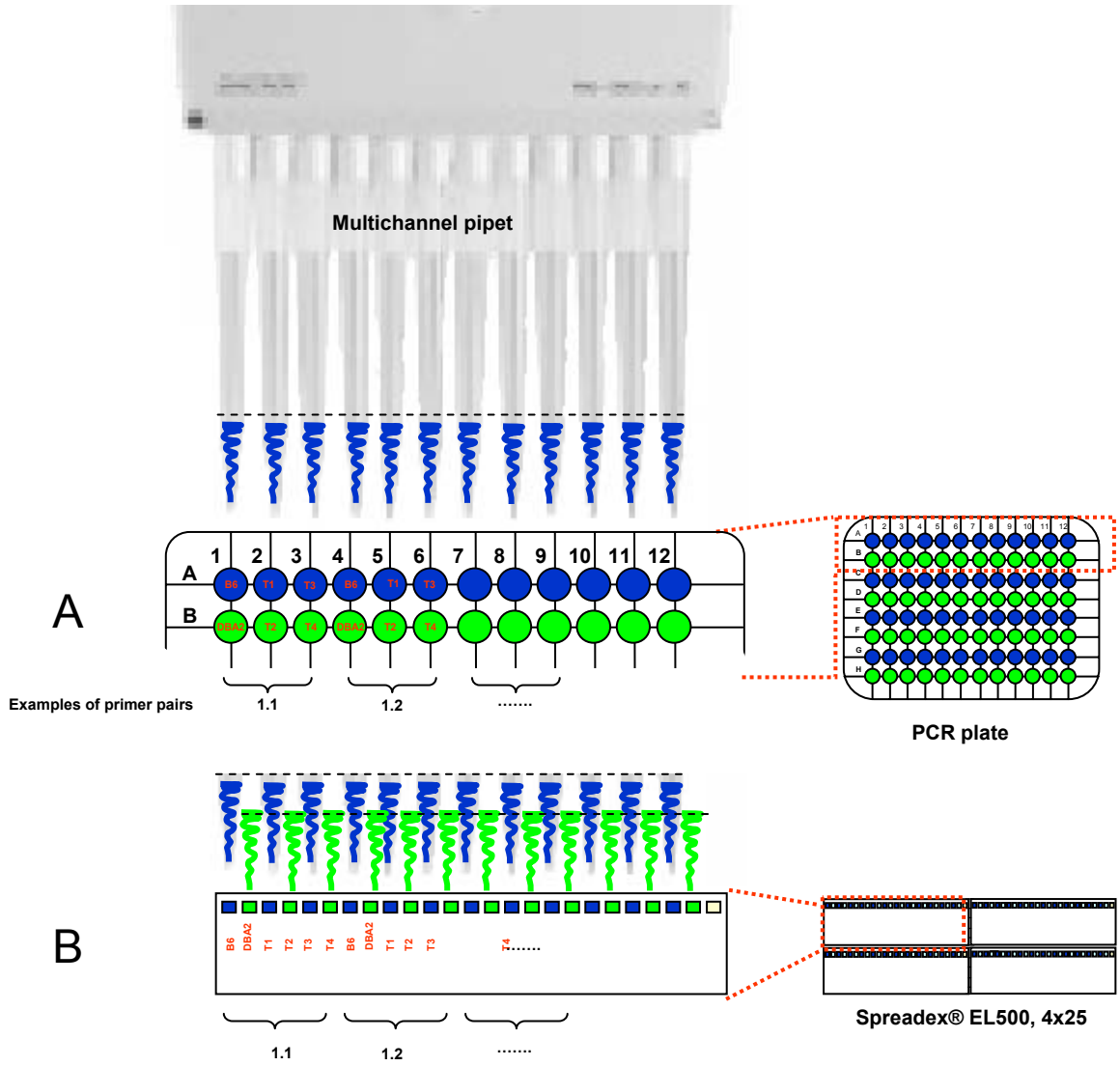


Figure 4. Gel loading scheme. For user's convenience it is recommended to load the PCR plate as shown in A. To have both control mice (for example B6/DBA2) next to each other on the hydrogel, place them in the PCR plates to the positions A1/B1, A4/B4 etc. The four mice to be tested (T1-T4) should be placed to positions A2,3, B2,3, A5,6, B5,6 etc. The gel loading should be performed by using a multichannel pipet. Every tip of a conventional multichannel pipet reaches every second well in Elchrom's S-4x25 format gels.

10 Protocols for Buffers

Protocol 1. Tween® 20 (1:10)

Components	Amount
Tween® 20	100µl
H_2O	900µl

Protocol 2. Tween® 20 (1:1000) for one 96 well plate

Components	Amount
Tween® 20 (1:10)	6µl
H_2O	600µl

Protocol 3. 40 x TAE (stock solution)*

Components	Amount for 1l
Tris (hydroxymethyl) aminomethane	145.37g
$Na_2EDTA \times 2H_2O$	11.16g
Acetic Aced (glacial)	34.4g

* can be ordered from Elchrom Scientific AG

Protocol 4. 30mM TAE **(**1x TAE)

Components	Amount for 2l
40x TAE	50ml
H_2O	1.950l

Protocol 5. DST solution*

Components	Amount for 1l
30mM TAE buffer	200ml
DST 100x conc	10ml
H_2O	up to 1l

* can be ordered from Elchrom Scientific AG

11 Ordering information

11.1 Speed congenics

P/N	GenoMouse-Speed Kits for Accelerated background Crossing (Speed Congenics)	Quantity
5001	GenoMouseSpeed B6/DBA2, 96 markers/animal, M1 size marker, Box of 6 Spreadex EL 500 Wide mini S-4x25 gels, technical manual.	6 mice
5002	GenoMouseSpeed B6/129, 96 markers/animal, M1 size marker, Box of 6 Spreadex EL 500 Wide mini S-4x25 gels, technical manual.	6 mice
5003	GenoMouseSpeed B6/FVB, 96 markers/animal, M1 size marker, Box of 6 Spreadex EL 500 Wide mini S-4x25 gels, technical manual.	6 mice
5004	GenoMouseSpeed B6/Balb/c, 96 markers/animal, M1 size marker, Box of 6 Spreadex EL 500 Wide mini S-4x25 gels, technical manual.	6 mice
5005	GenoMouseSpeed B6/NOD, 96 markers/animal, M1 size marker, Box of 6 Spreadex EL 500 Wide mini S-4x25 gels, technical manual.	6 mice

11.2 Strain identification

P/N	GenoMouse- Strain Detection Kits	Quantity
5101	GenoMouseStrain B6/12-48, 12 markers/animal, M1 size marker, Box of 6 Spreadex EL 500 Wide mini S-4x25 gels, technical manual.	48 mice
5102	GenoMouseStrain B6/24-24, 24 markers/animal, M1 size marker, Box of 6 Spreadex EL 500 Wide mini S-4x25 gels, technical manual.	24 mice
5103	GenoMouseStrain DBA/2-12-48, 12 markers/animal, M1 size marker, Box of 6 Spreadex EL 500 Wide mini S-4x25 gels, technical manual.	48 mice
5104	GenoMouseStrain DBA/2-24-24, 24 markers/animal, M1 size marker, Box of 6 Spreadex EL 500 Wide mini S-4x25 gels, technical manual.	24 mice
5105	GenoMouseStrain FVB-12-48, 12 markers/animal, M1 size marker, Box of 6 Spreadex EL 500 Wide mini S-4x25 gels, technical manual.	48 mice
5106	GenoMouseStrain FVB-24-24, 24 markers/animal, M1 size marker, Box of 6 Spreadex EL 500 Wide mini S-4x25 gels, technical manual.	24 mice
5107	GenoMouseStrain 129-12-48, 12 markers/animal, M1 size marker, Box of 6 Spreadex EL 500 Wide mini S-4x25 gels, technical manual.	48 mice
5108	GenoMouseStrain 129-24-24, 24 markers/animal , M1 size marker, Box of 6 Spreadex EL 500 Wide mini S-4x25 gels, technical manual.	24 mice
5109	GenoMouseStrain Balb/c-12-48, 12 markers/animal, M1 size marker, Box of 6 Spreadex EL 500 Wide mini S-4x25 gels, technical manual.	48 mice
5110	GenoMouseStrain Balb/c-24-24, 24 markers/animal, M1 size marker, Box of 6 Spreadex EL 500 Wide mini S-4x25 gels, technical manual.	24 mice
5111	GenoMouseStrain NOD-12-48, 12 markers/animal, M1 size marker, Box of 6 Spreadex EL 500 Wide mini S-4x25 gels, technical manual.	48 mice
5112	GenoMouseStrain NOD-24-24, 24 markers/animal, M1 size marker, Box of 6 Spreadex EL 500 Wide mini S-4x25 gels, technical manual.	24 mice

12 Service

GenoMouseSpeed Service: Please note that Elchrom Scientific offers the Service for speed congenics and the Service for mouse strain identification. Please contact us to get more information.

13 General Claims

Use of Elchrom's GenoMouse Kits requires performance of the polymerase chain reaction (PCR), which is the subject of European Pat. Nos. 201, 184 and 200, 362 and U.S. Pat. Nos 4, 683, 195, 188-4, 965, 188 and 4, 683, 202 owned by Hoffmann-La Roche. Purchase of Elchrom's GenoMouse Kits do not include of provide a license with respect to these patents or any other PCR-related patent owned by Hoffmann-La Roche or others.

14 Contact information

Elchrom Scientific AG
Gewerbestrasse 8
Postfach 5044
6330 Cham
Switzerland

Tel. +41 41 747 25 50
Fax +41 41 743 25 36

service@elchrom.com
www.elchrom.com