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Clinical

Checking the genetic integrity of laboratory mice with the help of conventional methods

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Abstract

Animal models are well documented in the literature and are used for experimentation due to their specific characters or response to the chemicals. Any deviation of these animals from the reported characters or response renders the animal unsuitable for the research. Therefore, use of genetically defined laboratory animal model is equally important to that of use of pure chemicals in any biomedical research. Procurement of pure lines and their sustained maintenance in the animal facility is of utmost importance for the existence and functioning of any animal facility. Keeping in view of the fact that despite careful and defined breeding program, genetic contamination and strain alterations may occur. The present study was undertaken to ascertain the genetic purity of strains of laboratory mice maintained at ACTREC. Skin grafting, coat colour testing and seven biochemical markers were studied. The results of all these tests showed that the rodent strains maintained at ACTREC have no genetic contaminations/ drift even after maintaining the strains for as many as 136 generations. The data indicates that merely conducting these simple tests, it is possible to establish in-house genetic quality control program and check the authenticity of the laboratory animals.

Key words: genetic monitoring, mice, ACTREC

Introduction

Animals with heritable and genetically defined characteristics are one of the most important requirements for the pharmaceutical and biomedical research. Animal models are selected based on their scientifically published qualities. These qualities may be phenotypic, genotypic, enzymatic, biochemical parameters or specific disease producing capacity. Availability of required strain qualities depends on the integrity of the strains (Groen, 1977; Randelia *et al.,* 1983). Inbred animals are the choice of animals because of their features like homozygosity, heritability, long term stability, identifiability, international distribution etc. (CRL, Fall 1991). Achieving homozygosity and its maintenance in laboratory rodents are important factors for precision, accuracy and reproducibility of the results of any experiment. Use of genetically contaminated animal results in loss of time, money and research manpower. Continuous supply of pure strains of laboratory rodents obtained from stock which is carefully maintained and genetically monitored that yields consistent results are always expected by the researchers (Cui *et al.,* 1993). Heterozygosity can be avoided by use of animals derived from brother x sister mating for at least 20 consecutive generations. This makes the animals homozygous for the given characters. Homozygocity does not carry the unexpressed recessive genes within the colony and expresses phenotypic and genotypic similarities. However, mutation or genetic mixing by strain contamination may result in phenotypic or genetic changes. To detect these changes or variations, genetic monitoring program is indispensable.

Several tests can be performed to identify the purity of rodent strains (Festing, 1979; Hedrich, 1981; Nitzki *et al.*, 2007). Some of the methods used for strain identification or checking purity of the rodent strains are -

- Morphological and physiological traits- coat colour, mandible size etc.
- Biochemical markers- proteins and enzyme variants.
- Immunogenetic- differentiation alloantigen loci, erythrocytes alloantigen loci, histocompatibility loci.
- Cytogenetics- karyotyping and banding patterns.
- Molecular (DNA markers)- Restriction fragment length polymorphisms, SNP.

Since the coat colours of many strains resembles and there is inaccuracy in measurement of mandible size, morphological markers are least adopted for genetic monitoring. However, coat colour crosses between different colour strains with DBA/2 strain gives defined coat colour at F1 generation. Any variation from the defined coat colour is an indication of strain alterations due to genetic contamination or mutation. Since the method does not involve use of any equipment, it can be used with ease for some of the strains. Out of large number of variants of the coat colour of mice, only few are used in genetic monitoring. Coat colour markers routinely used for genetic monitoring are shown in the table 1.

There is a strong preference for albino strains of mice for laboratory research. Therefore, majority of the strains in animal facility are of albino coat colour. Even if these strains are crossed amongst themselves, the resultant progeny masks the genetic contamination because of the similar coat colour. The coat colours can be distinguished by naked eyes as it requires no special equipments to test them. For this reason, this could be a very efficient and cost effective method of genetic monitoring provided the knowledge of coat colour genetics and interpretations of the results are proper.

Classification of immuno-genetic loci as cell membrane associated alloantigen loci is based on their localization and function. Methods used for detection of these loci are based on hemagglutination, cytotoxicity, hemolysis, complement fixation, radio-immunoassay, fluorescent antibody techniques and immuno-diffusion (Nomura *et al.*, 1984).

Based on the intensity of allograft reactions, the histocompatibility loci are designated as either ‘strong’ or ‘weak’ H locus. Strong H locus is the one which prevents the progressive growth of all transplants and causes rapid rejection of skin homograft in donor and recipient. Weak H locus is the one which permits the progressive growth of all transplants and fails to cause rapid rejection of skin homograft in donor and recipient. Experiments have shown that H-2 locus is the only strong H locus and all other H loci are weak loci. Weak loci are also called as non H-2 loci or minor histocompatibility complex loci, while H-2 is referred to as the major histocompatibility complex locus in mice (Klein, 1975). Skin grafting is a classical and crucial technique for characterizing inbred strains. It was developed by Billingham and Medawar in 1951 to study histocompatibility (CRL, Winter 1992). Acceptance of reciprocal skin graft is an indication that the animals are isogenic. In the present study, skin grafting was assessed to check the major and minor histocompatibility differences in the mice strains maintained at ACTREC.

Skin grafting is easiest way to check the purity of the strain and contamination in the inbred strains. For skin grafting, numbers of donor/ recipient pairs are selected depending on the size of the rodent colony. Animals should be randomly selected from each strain. Male recipient animals should be selected for male graft donor; similarly female recipient animals should be selected for female graft donors. In mice and rats, tail skin is generally used for the graft. However, shoulder skin has also been used for the skin graft. To test

the major histocompatibility, graft is observed up to 10-12 days whereas differences in minor histocompatibility need observation of skin graft up to at least 90 days.

Checking purity of the laboratory rodents using one of the above methods should be the routine procedure of any animal facility maintaining different strains of laboratory rodents. The animals may be infection-free, but genetic contamination may render them useless or non-reproducible as far as the results of the research are concerned.

Traditional methods of genetic screening makes use of genetic variation in antigenic determinants, such as those associated with blood groups or histocompatibility; or use of genetically determined variation in the electrophoretic mobility of protein molecules, such as in the enzymes of intermediary metabolism. Both of these methods of genetic markers are very useful, but they have limitations that the numbers of polymorphic genetic markers are relatively small. An additional practical limitation is that each of the polymorphic genes requires somewhat different serological or enzymatic assay and therefore implementation of large-scale screening can be complicated and time consuming. Considering all these factors, in the present study regularly used markers like coat colour testing, skin grafting and biochemical markers were used to check the authenticity of the mice strains maintained at ACTREC.

Material and Methods

Animal strains and their maintenance:

Eleven different strains of mice such as A/J (-), BALB/c (71), C3H/J (14), C57BL/6 (69), CD1 (49), CFW (-), DBA/2 (70), FVB/NJ (42), ICRC (136), SENCAR (43) and Swiss bare (119) mice were used in the present study. Figures in parenthesis indicate number of inbreeding generations of each strain. The quality control proposal for use of the animals under this study was approved by the Institutional Animal Ethics Committee of the ACTREC vide proposal no. 22/2010. These animals were maintained under controlled environmental conditions with a relative humidity around 55 ± 5% and temperature at 23 ± 2°C with 12 hrs each cycle of dark and light. All mice were provided with easy access to in-house pelleted feed and *ad libitum* UV treated water from the commercial water purifier (Alfa Water Purifiers, Bangalore). As far as possible, old and retired breeders were used for this study.

Mating of animals for coat colour crosses:

Test crosses uncover the exact coat colour genotype. In order to ascertain the hidden coat colours in nine different mice strains, they were mated with the DBA/2 strains of mice. For this purpose, two each randomly selected 6-8 weeks old females of A/J, BALB/c, C3H/J, C57BL/6, CD1, CFW (Swiss), FVB/NJ, ICRC and, SENCAR strains were mated with the males of DBA/2 strain. Standard coat colour genotypes of these strains of mice were taken from the published references (Nomura, 1984; CRL, Fall 1991). Based on the coat colour expression in the F1 progeny, coat colour genotype was calculated and ascertained.

Skin grafting:

Skin grafting procedure for A/J, BALB/c, C3H/J, C57BL/6, CD1, CFW, DBA/2, FVB/NJ, ICRC and SENCAR strains of mice was performed as described by Festing, 1979 and Simpson *et al.*, 1997. Two pairs each of donor and recipient mice of same sex were selected from each of the eleven strains. Donor as well as recipient animals were anesthetised using general anaesthetic, Avertin (Sigma Chemicals, USA), by injecting intraperitoneally at the dose of Avertin- 0.015 x body weight. The tail skin was cleaned with disinfectant solution and allowed it to dry. Thin layer of tail skin of donor animal was removed using 11 or 20 no. BP blade. In case of mice, the skin graft should be ~ 8 mm long and 3 mm wide. Since it is likely that excessive bleeding might lead to rejection of the graft, care was taken to ensure that the cut is not too deep. Similarly, thin layer of tail skin of recipient animal was removed on the same blade. Alternatively, the graft of the donor animals was held on sterile wet tissue paper towel soaked in normal saline till implanted. Blood oozing from the graft bed, if any, was wiped out using sterile tissue paper/ cotton. The graft from one animal was exchanged on to the other animal, and *vice versa*. The grafts were placed in a reverse direction to the hair growth. The graft was protected by placing a plastic tube of slightly larger diameter than the tail. The plastic tube was secured in place using adhesive cotton tape. The animals were kept under table-lamp for fast recovery from the anaesthetic. The adhesive tape and plastic tubes were removed after 48 hrs, by which time the graft becomes firmly attached. These animals were observed regularly up to a period of 3 months for acceptance or rejection of the grafts. Photographs of the accepted grafts were taken on 7 and 100 days for record purposes.

Biochemical markers:

Seven biochemical markers testing from five different chromosomes were performed in the present study. The biochemical markers were Alkaline phosphatase (Akp-1), Haemoglobin beta-chain (Hbb), Esterase-3 (Es-3), Glucose-6-phosphate dehydrogenase-1 (Gpd-1), Glucose phosphate isomerase-1 (Gpi-1), Isocitrate dehydrogenase-1 (Idh-1) and Malic enzyme supernatant (Mod-1). The loading samples for these seven markers were prepared either from blood or kidney.

Plasma and Red Blood Cells (RBC) hemolysate

For preparation of plasma, RBC or hemolysate, approximately 200 µl of blood was collected from the orbital plexus of the animals of each strain in 1.8 ml capacity

heparinised/ EDTA eppendorf tubes using heparinised capillaries (Top tech Lab Equipments Pvt. Ltd. Mumbai). The blood samples were held on ice till they were processed. These blood samples were spinned down in a cold centrifuge (Plastocraft, Mumbai) at the rate of 3000 rpm for 15 min at 4ºC. Top layer of plasma was carefully collected in a new eppendorf tube with the help of 100 µl capacity micropipette. Three-fold volume of Milli-Q water was added in the settled RBC's to lyse them (Groen, 1977; Nomura *et al.*, 1984). Both the plasma as well as RBC hemolysate was stored at -80 ºC till further use.

Kidney homogenate:

Two mice from each strain were sacrificed by cervical dislocation. One kidney was removed aseptically and homogenized in five-fold volume of Milli-Q water in glass tubes. The homogenates was centrifuged at 300 rpm at 4ºC for 30 min. using cold centrifuge. The supernatant was collected in 1.8 ml capacity eppendorf tubes. The samples were stored at -80 ºC till further use (Groen, 1977; Nomura *et al.*, 1984).

Cellulose Acetate plates:

For Hbb, Titan III-H Cellulose Acetate (CA) plates (cat. no. 3022) and for rest of the proteins/ enzyme markers, Titan III Cellulose Acetate (CA) plates (cat. no. 3033) supplied by Helena Laboratories, USA were used in the present study.

Sample loading comb, well plate and platform:

Readymade sample loading comb, super Z-12 applicator (cat. no. 4090), well plate (cat. no. 4096), and platform (cat. no. 4094) were purchased from Helena Laboratories (Fig. 1).

Electrophoresis tank and power pack:

In-house made horizontal electrophoresis apparatus with platform to hold the CA plates horizontally (Fig. 2) was used for running the reactions. Standard power packs (Bangalore Genie and Techno Source, Mumbai) were used as a electric source to run the samples on the CA plates.

Buffer:

Four types of buffers were used for running all biochemical markers as shown in table 2.

Sample application and run:

Appropriate amount of the respective buffer was added in both the chambers of the electrophoresis apparatus (Fig. 2). Two wicks made from Whatman filter paper no.1 were prehydrated in the electrophoresis tank (Fig. 2). Ten ml of the respective sample was added in each well of the sample holding chamber (Fig. 1B). Prior to the sample application on CA plate, excess buffer was removed from the pre-hydrated CA plate by soaking the plate on a tissue paper towel. The CA plate was kept on the sample-loading platform (Fig. 1C). Sample loading comb was dipped in the sample in the wells to load the samples onto the comb. Using the applicator comb, respective samples were applied onto the hydrated CA plates. After sample application, the CA plate was kept onto the platform provided on the electrophoresis tank such that the cellulose coated side is up (Fig. 2). Excess buffer was removed from the pre-hydrated wicks by touching them to the sides/ borders of the tank. The wicks were kept on the CA plate such that one end touches the cellulose coat of the plate and the other is sufficiently immersed in the buffer (Fig. 2). The electrophoresis power pack was switched 'ON' allowing appropriate voltage and current to pass through the CA plate (Nomura *et al.*, 1979). After the stipulated time of the run is complete, the electrophoresis power pack was switched 'OFF'. Using the blunt forceps, the plate was removed from the platform of the electrophoresis apparatus. Excess buffer was removed from the plate by gentle soaking onto the tissue paper towel. The CA plates were stained with appropriate stain. Readings were noted and photograph of the band pattern were taken for permanent record. Stained plates were preserved for record and future reference.

Results

Coat colour crosses of different strains yielded expected colour coats in the progeny as shown in table 3. Based on the coat colour expressions, genotypes for progeny of all strains were calculated. Results of all the mating suggested that there are no hidden recessive genes present in the strains in question.

All mice subjected for skin graft had accepted the grafts from the respective donor mice. Representative accepted grafts are shown in Fig. 3.

Biochemical markers

All eleven strains tested in this study showed standard biochemical marker as per the literature (The Jackson Laboratory, 2001- 2002). Results of these eleven strains tested for seven biochemical markers are summarised in table 4 and fig. 4-11. Description of the alleles for all seven biochemical markers and their migration of on CA plate is given in table 4 A.

Discussion

Several reasons are reported for variations in the genetic make up of the animals. Most frequent cause of the variations in the genetic make up is by deliberate or accidental breeding between strains which have similar coat colours (Groen, 1977; Groen and Lagerwerf, 1979; Lovell *et al.*, 1984; Simpson *et al.*, May 1997; Nitzki *et al.*, 2007). An observation of specific strain characteristics in the animal strains is the first step in genetic monitoring program. Variance in inbred strain characteristics such as body size, weight, skeletal structure, behaviour, reproductive performance, tumor susceptibility, lifespan, etc. may be an indicator of spontaneous mutation, breeding error or genetic contamination (Festing, 1979).

Moreover, even inbred strains do not remain genetically stable over long period (Groen, 1977).

In Indian scenario, quite a few animal facilities are opting for national and international accreditation/ certification. In light of this, validation and verification of the available strains is not only a requirement from the regulatory authority point but is also required to ascertain reproducibility of the results. Validation and verification refers to periodic genetic screening of representative samples of animals selected from the available populations (Lovell *et al.*, 1984).

Genetic contamination (i.e. failure to maintain inbred strain integrity) in most cases is difficult to determine from examination of the strain characteristics unless it is accompanied by a coat colour change. Correct coat colour is the first observation made when evaluating a strain. The most common genes responsible for the colour are 3 alleles at the agouti locus, agouti (A), non-agouti (black) (a), and white bellied agouti (Aw); two alleles at the tyrosinase locus, albino (Tyrc) and chinchilla (Tyrc-ch); the brown locus (Tyrp1b); the pink-eyed dilution locus (p); and the dilute locus, Myo5ad (Festing, 1979; CRL, Fall 1991).

Albino strains carry hidden coat colours. Therefore, in order to express them phenotypically, the albino strains were crossed with DBA/2 strain. If there are contaminations, the coat colours segregate in crosses and express phenotypically in the progeny (Lovell *et al.*, 1984). F1 progeny of all strains crossed in this study showed no deviation from the expected coat colour. This indicates that the strains in question maintained at ACTREC have no hidden segregating coat colour alleles. In short, coat colour crosses study revealed that the strains are pure lines and have no mutations/ genetic drift of coat colours in these strains.

If an unexpected change in coat colour appears in the breeding colony, it is advisable to test the animal/s by coat colour crossings to determine if mutational event or genetic contamination has occurred in the animals. The genetic integrity of strains may be further determined from their isoenzyme profile, immunological assays and/ or molecular markers.

Skin grafting is an age old but useful technique of genetic monitoring. Tail-skin grafting is used primarily to determine minor histocompatibility differences between strains. The minor histocompatibility loci cause tissue rejection, as does H2, but usually at a much slower rate. Skin grafting can be used to determine sub-strain differentiation and to check for residual heterozygosity. It has the advantage of simultaneously screening for hundreds or thousands of incompatible loci. Results of the present study indicate that the strains tested for skin grafting have no minor as well as major histocompitability differences as evidenced from the acceptance of grafts even after 100 days.

Isoenzymes (or isozymes) are proteins that perform similar functions but exhibit different physical characteristics, such as electrophoretic mobility. Large numbers of enzymes and protein variants are available in laboratory rodents but most of them have rare variation among different strains. Therefore, limited variants are practiced for detection of strain differences - contaminations. They are useful biochemical markers for determining genetic purity when this difference is strain specific. Isoenzyme determination is quick, technically simple, readily reproducible, and inexpensive. Many isoenzymes are expressed in several tissues and determinations can often be made from plasma or RBC lysates.

Biochemical markers are polymorphic and, like the H-2 markers, are located on chromosomes throughout the genome. Most inbred strains can be identified by five or fewer isoenzymes plus coat colour crosses. Since cellulose acetate electrophoresis system, developed by Helena Laboratories, USA, is accurate, quick, easy and sensitive, it is most preferred over any other method.

Variety of proteins/ enzymes can be checked in laboratory rodents but in our case only four biochemical markers differentiated seven of our strains. C3H/J, CFW (Swiss), FVB/NJ and SENCAR possesses similar variants for the seven biochemical markers tested in this study. Using these seven biochemical markers, it is difficult to differentiate these four strains from each other. However, their coat colour genetics differs distinctly from each others and that makes the differentiation of these strains easy. However, checking more biochemical markers makes the genetic quality control program more stringent. For this reason, it is advisable to select atleast one marker from each chromosome of the animal. These markers also need to be checked for several generations to ascertain the stability and consistency in the homogeneity. The standard allele patterns of all the biochemical markers available from the literature formed the basis for this study (Lovell *et al.*, 1984; The Jackson Laboratory, 2001). All biochemical markers studied in the present study helped the genetic quality control program of ACTREC to establish the fact that the mice strains maintained in ACTREC Animal Facility have no genetic contaminations. Maintaining the integrity of all these strains could have been possible because of strict maintenance of the breeding program, proper record keeping of the strains as well as availability of proper skilled technical staff to maintain these strains over the years.

Efforts are required to acquire techniques, develop the manpower and establish the reliable practical solutions in India for providing the commercial services of genetic quality testing of the laboratory animals. Efforts are also required to sensitize the scientists about the importance of checking purity of their experimental animals. More importantly, the editors should also insist for the evidence of the purity of the laboratory rodent strains used for the experiments. A cheap, sensitive, consistent and practical approach of genetic monitoring tests is need of the hour.

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Table 1. Coat colours alleles and chromosomal positions

Sl. No.	Name of coat colour	Alleles	Chromosomes
1.	Agouti- Non-agouti	A a	2
2.	Black- brown	B b	4
3.	Pigmented- albino	C c	9
4.	Dilution- non dilution	D d	7

Table 2. Preparation of buffers used for separation of alleles on CA plates

1) Tris citrate, pH 8.2 for Akp-1 • 10.5 g Tris • 3.0 g Citric acid • Make up to 1 litre with MilliQ water	**2) Tris EDTA borate, pH 8.4 for Es-3, G6pd-1 and Hbb** • 10.91 g Tris • 0.60 g EDTA disodium salt • 3.10 g Boric acid • Make up to 1 litre with MilliQ water
3) Tris glycine, (pH 8.5) for Gpi-1 • 3.00 g Tris • 14.4 g Glycine (NH_3 free) • Make up to 1 litre with MilliQ water	**4) Tris citrate (pH 7.6) for Idh-1 and Mod-1** • 12.10 g Tris • D/W 600 ml • Adjust pH with 10% citric acid. • Make up to 1 litre with MilliQ water

Table 3. Resultant phenotypes of the coat colour crosses

Coat colour and genotype of the standard strain	Test mice strains, coat colour and their original genotypes	F1 phenotypes and their acquired genotypes
DBA/2 (Dilute brown) C/C a/a b/b d/d	A/J (Albino) c/c a/a b/b D/D	Dilute brown C/c a/a b/b D/d
	BALB/c (Albino) c/c A/A b/b D/D	Brown-agouti C/c A/a b/b D/d
	C3H/J (Agouti) C/C A/A B/B D/D	Black-agouti C/C A/a B/b D/d
	C57BL/6 (Black) C/C a/a B/B D/D	Black C/C a/a/ B/a D/a
	CD1 (Albino) c/c A/A B/B d/d	Black –agouti C/c A/a B/b d/d
	CFW (Albino) c/c A/A B/B d/d	Black- agouti C/c A/a B/b d/d
	FVB/NJ (Albino) c/c A/A B/B D/D	Black-agouti C/c A/a B/b D/d
	ICRC (Albino) c/c a/a b/b D/D	Dilute brown C/c a/a b/b D/d
	SENCAR (Albino) c/c a/a B/B d/d	Black C/c a/a B/b d/d

Table 4. Results of the seven biochemical markers of A/J, BALB/c, C3H/J, C57BL/6, CD1, CFW (Swiss), DBA/2, FVB/NJ, ICRC, SENCAR and Swiss bare mice

Sl. No.	Strain	Coat colour	Results of the tests						
			Akp-1	Hbb	Es-3	G6pd-1	Gpi-1	Idh-1	Mod-1
1.	A/J	Albino	b/b	d/d	c/c	b/b	a/a	a/a	a/a
2.	BALB/c	Albino	b/b	d/d	a/a	b/b	a/a	a/a	a/a
3.	C3H/J	Agouti	b/b	d/d	c/c	b/b	b/b	a/a	a/a
4.	C57BL/6	Black	a/a	s/s	a/a	a/a	b/b	a/a	b/b
5.	CD-1	Albino	b/b	s/s	c/c	a/a	b/b	a/a	b/b
6.	CFW (Swiss)	Albino	b/b	d/d	c/c	b/b	b/b	a/a	a/a
7.	DBA/2	Dilute brown	a/a	d/d	c/c	b/b	a/a	b/b	a/a
8.	FVB/NJ	Albino	b/b	d/d	c/c	b/b	b/b	a/a	a/a
9.	ICRC	Albino	b/b	s/s	c/c	b/b	b/b	a/a	a/a
10.	SENCAR	Albino	b/b	d/d	c/c	b/b	b/b	a/a	a/a
11.	Swiss/ba	Hairless	b/b	d/d	c/c	b/b	a/a	b/b	a/a

Table 4 A. Description of alleles for each of the seven biochemical markers and their migration

Sl. No.	Marker	Alleles			Migration
1.	Akp	a = fast	b = slow		Anodal
2.	Hbb	s = single	d = diffuse		Anodal
3.	ES-3	a = absent	b = fast	c = slow	Anodal
4.	Gpd-1	a = slow	b = fast		Anodal
5.	Gpi-1	a = slow	b = fast		Cathodal
6.	Idh-1	a = slow	b = fast		Anodal
7.	Mod-1	a = fast	b = slow		Anodal

Fig. 1. Helena kit for Cellulose Acetate Electrophoresis

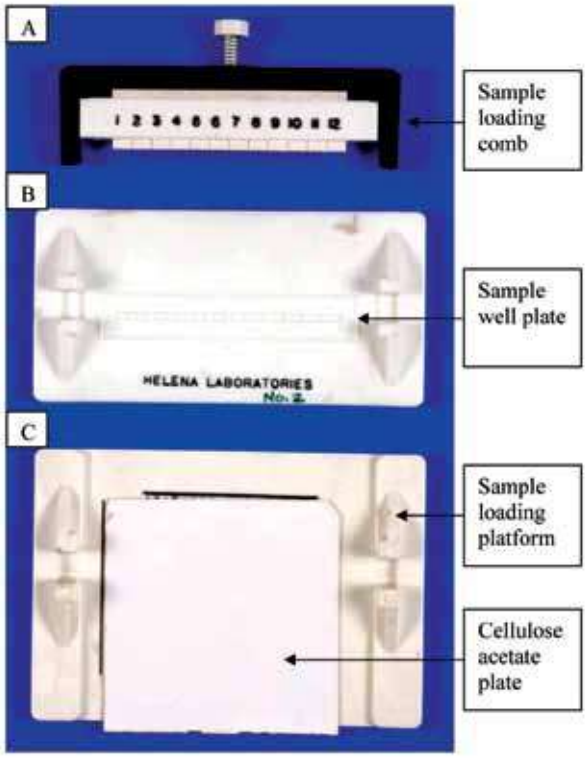


Fig. 2. Positioning of the loaded CA plates and wicks

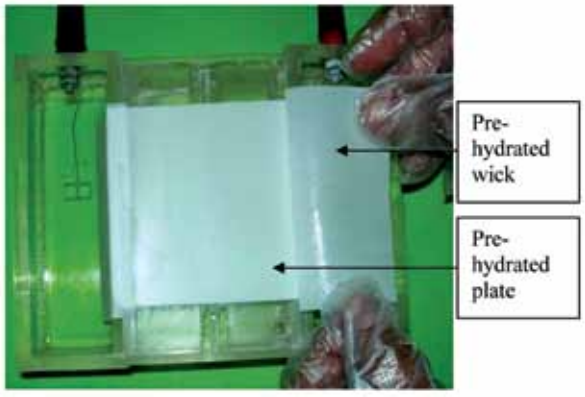


Fig. 3. Accepted Skin Grafts from representative strains after 100 days

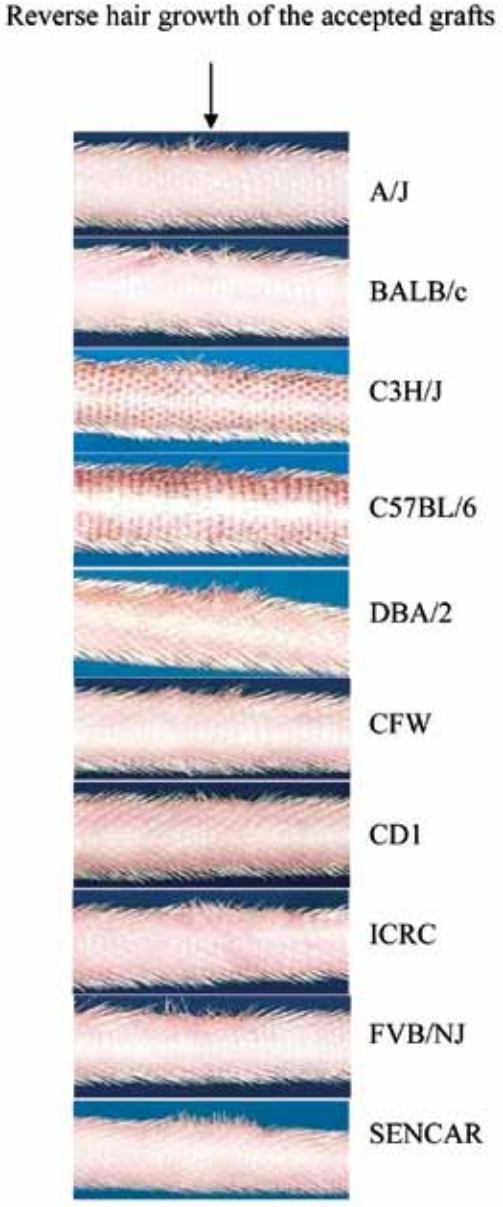
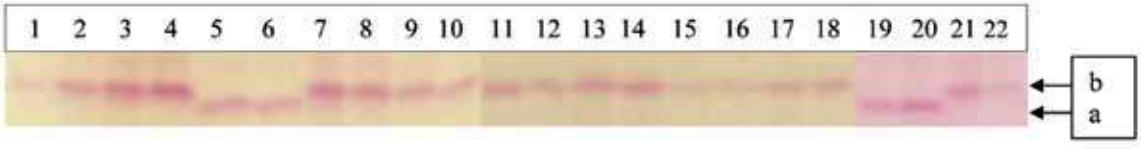


Fig. 4. Allelic band pattern of Akp-1 marker after staining.

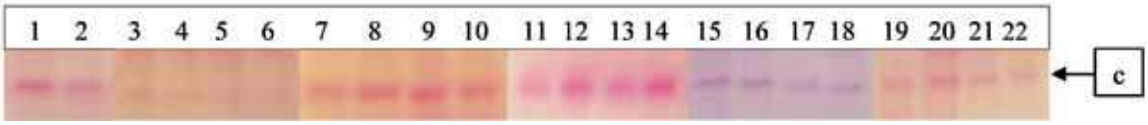


Lane 1 and 2- A/J; 3 and 4- BALB/c; 5 and 6- C57BL/6; 7 and 8- C3H/J; 9 and 10- CD1; 11 and 12- FVB/NJ; 13 and 14- ICRC; 15 and 16- CFW; 17 and 18- Swiss bare; 19 and 20- DBA/2; 21 and 22- SENCAR.

Fig. 5. Allelic band pattern of Hbb marker after staining

Lane 1 and 2- A/J; 3 and 4- DBA/2; 5 and 6- CFW; 7 and 8- SENCAR; 9 and 10- FVB/NJ; 11 and 12- CD1; 13 and 14- C3H/J; 15 and 16- Swiss bare; 17 and 18- ICRC; 19 and 20- BALB/c; 21 and 22- C57BL/6.

Fig. 6. Allelic band pattern of Es-3 marker after staining

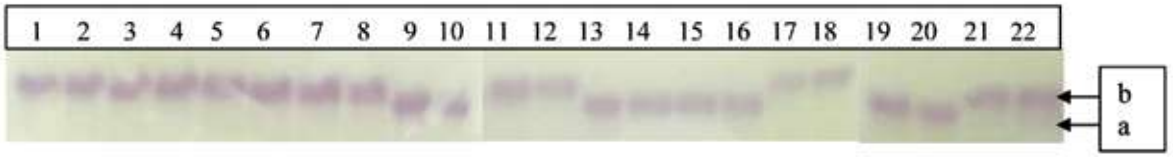


Lane 1 and 2- A/J; 3 and 4- BALB/c; 5 and 6- C57BL/6; 7 and 8- C3H/J; 9 and 10- DBA/2; 11 and 12- CD1; 13 and 14- FVB/NJ; 15 and 16- SENCAR; 17 and 18- Swiss bare; 19 and 20- ICRC; 21 and 22- CFW.

Fig. 7. Allelic band pattern of Gpd-1 marker after staining

Lane 1 and 2- ICRC; 3 and 4- FVB/NJ; 5 and 6- SENCAR; 7 and 8- Swiss bare; 9 and 10- C3H/J; 11 and 12- C57BL/6; 13 and 14- BALB/c; 15 and 16- A/J; 17 and 18- CD1; 19 and 20- DBA/2; 21 and 22- CFW.

Fig. 8. Allelic band pattern of Gpi-1 marker after staining



Lane 1 and 2- ICRC; 3 and 4- FVB/NJ; 5 and 6- SENCAR; 7 and 8- CD1; 9 and 10- Swiss bare; 11 and 12- CFW; 13 and 14- DBA/2; 15 and 16- A/J; 17 and 18- C3H/J; 19 and 20- BALB/c; 21 and 22- C57BL/6.

Fig. 9 and 10. Allelic band pattern of Idh-1 and Mod-1 markers after staining

Lane 1 and 2- C57BL/6; 3 and 4- A/J; 5 and 6- BALB/c; 7 and 8- DBA/2; 9 and 10- C3H/J; 11 and 12- SENCAR; 13 and 14- CD1; 15 and 16- FVB/NJ; 17 and 18- ICRC; 19 and 20- CFW; 21 and 22- Swiss bare.