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Genetics

FVB/N: An inbred mouse strain preferable for transgenic analyses

(ES cells/blastocyst chimera/germ-line transmission)

MAKOTO TAKETO*†, ALLEN C. SCHROEDER*‡, LARRY E. MOBRAATEN*, KERRY B. GUNNING§, GERRI HANTEN§, RICHARD R. FOX*, THOMAS H. RODERICK*, COLIN L. STEWART¶, FRANK LILLY‖, CARL T. HANSEN**, AND PAUL A. OVERBEEK§

*The Jackson Laboratory, Bar Harbor, ME 04609; §Howard Hughes Medical Institute, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030; ¶Department of Cell and Developmental Biology, Roche Institute of Molecular Biology, Nutley, NJ 07710; ‖Department of Molecular Genetics, Albert Einstein College of Medicine, Bronx, NY 10461; and **National Institutes of Health Animal Genetic Resource, Bethesda, MD 20892

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ABSTRACT **FVB/N mice offer a system suitable for most transgenic experiments and subsequent genetic analyses. The inbred FVB/N strain is characterized by vigorous reproductive performance and consistently large litters. Moreover, fertilized FVB/N eggs contain large and prominent pronuclei, which facilitate microinjection of DNA. The phenotype of large pronuclei in the zygote is a dominant trait associated with the FVB/N oocyte but not the FVB/N sperm. In experiments to generate transgenic mice, the same DNA constructs were injected into three different types of zygotes: FVB/N, C57BL/6J, and (C57BL/6J × SJL/J)F_1. FVB/N zygotes survived well after injection, and transgenic animals were obtained with efficiencies similar to the F_1 zygotes and much better than the C57BL/6J zygotes. Genetic markers of the FVB/N strain have been analyzed for 44 loci that cover 15 chromosomes and were compared with those of commonly used inbred strains. In addition to the albino FVB/N strain, pigmented congenic strains of FVB/N are being constructed. These features make the FVB/N strain advantageous to use for research with transgenic mice.**

The generation of transgenic mice by pronuclear microinjection and their subsequent breeding has been more efficient with F_1 or F_2 zygotes than with zygotes of inbred strains because inbred mice generally have a relatively poor reproductive performance (1). However, an inbred genetic background is preferable for genetic analyses such as the transfer of one allele of a mouse gene into a strain carrying a different allele (2). Likewise, for insertional mutagenesis experiments, inbred strains eliminate ambiguity caused by different genetic backgrounds and segregating markers in the progeny. As reported here, the inbred strain FVB/N is a good breeder with large litters, and the fertilized eggs of this strain have large prominent pronuclei, which facilitate microinjection of DNA.

The ancestor of FVB/N is an outbred colony of Swiss mice N:GP (NIH general purpose mouse) established at the National Institutes of Health in 1935. From the N:GP colony, a second colony N:NIH (NIH mouse) was established in the early 1940s. In 1966, a project was begun to develop two populations of N:NIH mice. Mice were inoculated with pertussis vaccines, followed by a challenge with histamine diphosphate. Two strains were selected for sensitivity and resistance and were designated as histamine sensitivity factor sensitive (HSFS/N) and histamine sensitivity factor resistant (HSFR/N), respectively. In the early 1970s, a group of mice at the eighth inbred generation from HSFS/N line were determined to carry the *Fv-1*^{b} allele for sensitivity to the B strain of Friend leukemia virus, in contrast to N:NIH mice, which were sensitive to the N strain of this virus (*Fv-1*^{n}) (F.L., unpublished results). These mice were then inbred and offspring were selected for *Fv-1*^{b} homozygosity. To avoid confusion with the HSFS/N strain that is *Fv-1*^{n}, the *Fv-1*^{b} strain was designated as FVB for Friend virus B-type susceptibility. This strain has been maintained since the late 1970s as an inbred strain without selection for either pertussis vaccine sensitivity or virus type. In this report, we provide a detailed characterization of the genetic background of the FVB/N strain and the advantages of using the strain to generate and study transgenic mice.

MATERIALS AND METHODS

Mice. FVB/N mice (F38) were obtained from the National Institutes of Health Animal Genetic Resource.

Pronuclear Measurement. Embryos were obtained by *in vitro* fertilization of superovulated oocytes as described (3). Embryos developing pronuclei between 6 and 7 hr postinsemination were cultured an additional 5–6 hr and photographs were taken with Nomarski optics. Pronuclear volumes were calculated from their diameters measured along the equatorial planes perpendicular to the location of the polar bodies and excluding the zonae pellucidae. Only embryos exhibiting both pronuclei were used for analysis.

Generation of Transgenic Mice. Pronuclear microinjections were performed by standard techniques (1). Mice were maintained on a cycle of light from 6:00 a.m. to 8:00 p.m. Superovulation was induced by administration of 5 international units of pregnant mare serum gonadotropin (Calbiochem) between 1:00 and 2:00 p.m., followed by 5 international units of human chorionic gonadotropin (Sigma) 48 hr later. DNA solutions were injected at a concentration of 2.5 μg/ml in 10 mM Tris·HCl/0.1 mM EDTA, pH 7.4. Embryos that survived microinjection were reimplanted the same day into pseudopregnant ICR/Hsd females that had been random-mated to vasectomized BDF_1 males.

Recombinant Plasmid DNA Constructs. Standard procedures (4, 5) were used for recombinant DNA experiments. Construct C2TAg contains the mouse αA-crystallin promoter driving simian virus 40 early region [modified from Mahon *et al.* (6)]. Construct VISC is based on a genomic clone (OVE1B; ref. 7) and contains the αA-crystallin promoter linked to a truncated simian virus 40 early region, flanked by mouse genomic sequences. Both C2TAg and VISC constructs cause cataracts in transgenic mice (P.A.O., unpublished results).

Screening of Potential Transgenic Mice. Tail DNAs were isolated by the procedure of Thomas *et al.* (8). Transgenic mice were identified by polymerase chain reaction (9) using primers GTCCTTGGGGTCTTCTACCTTTCTC and GTGAAGGAACCTTACTTCTGTGGTG (nucleotides 4407–

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†To whom reprint requests should be addressed at: Department of Microbiology and Immunology, Box 3020, Duke University Medical Center, Durham, NC 27710.
‡Deceased September 16, 1990.

4432 and 4707–4682, respectively, in the simian virus 40 genome; ref. 10). Both C2TAg and VISC can be amplified to give a 300-base-pair fragment. Mice were also screened by visual inspection for lens cataracts.

Analysis of Genetic Markers. Biochemical markers have been determined according to established methods (R. R. Fox, unpublished). Coat color markers have been determined by crossing FVB/N strain with linkage testing strains P/J, ABP/Le, and V/Le (11).

Computer Analyses. The MATRIX program that allows statistical screening of nearly 500 genetic markers over 1000 strains was written by Roderick and Guidi (12) and loaded in a SUN workstation at The Jackson Laboratory. It is accessible outside The Jackson Laboratory.

Formation and Analysis of Blastocyst Chimeras. The methods described by Bradley (13) were used.

Analysis of Friend Virus Susceptibility (*Fv-1*) Locus. Methods are described in ref. 14 and references therein.

RESULTS

Reproductive Performance of FVB/N Strain. The fecundity of the FVB/N strain was assessed by data from nine breeding pairs, which produced 43 litters. Litter size ranged from 7 to 13, with a mean value of 9.5. (First litters were generally smaller.) This is superior to other commonly used inbred strains; for example 6.7 for C57BL/6J, 6.6 for SJL/J, 5.4 for 129/J, or 5.0 for DBA/2J (15). A typical breeding pair mated at every postpartum estrous cycle and continued breeding for at least half a year, usually longer. The sex ratio of weaned animals did not significantly deviate from a 1:1 ratio (53% females and 47% males). FVB/N mice are easy to handle, with mild behavior patterns.

Pronuclear Volume of FVB/N Zygotes. About 6–7 hr after fertilization, male and female pronuclei appear in the periphery of the zygote. They then move toward the center while the first round of DNA replication takes place, and the sizes of the pronuclei increase accordingly (1). Because spontaneous ovulation is asynchronous, sperm penetration *in vivo* occurs over a period of 2–3 hr (1). To determine the size of the pronuclei, *in vitro* fertilization was used to synchronize the process. Groups of eggs from FVB/N and control (C57BL/6J × SJL/J)F_1 (abbreviated as (B6SJLF$_1$) were fertilized with FVB/N or control B6SJLF$_1$ sperm in a 2 × 2 factorial design. FVB/N sperm had a lower efficiency of fertilizing either FVB/N or B6SJLF$_1$ eggs compared with B6SJLF$_1$ sperm (50% of FVB/N embryos were fertilized with FVB/N sperm compared with 88% with the F_1 sperm). Nevertheless, fertilized eggs from all groups that were cultured further developed to the morula and blastocyst stages at similar frequencies. As shown in Table 1, there was no difference in the mean volume of FVB/N and control B6SJLF$_1$ zygotes ($\approx 2.7 \times 10^5$ μm^3 or 270 pl). However, the volumes of both male and female pronuclei in the zygotes from FVB/N eggs were significantly larger than those in fertilized B6SJLF$_1$ eggs, independent of the source of the sperm. Moreover, the pronuclei in the zygotes derived from FVB/N eggs were visually prominent under the microscope (Fig. 1). The phenotype of large and prominent pronuclei is unique to FVB/N when compared with other inbred strains such as C57BL/6J, SJL/J, LT/SvJ, etc. (data not shown). This phenotype appears to be a dominant trait because FVB6F$_1$ females produced from FVB/N female and C57BL/6J male pairs generated eggs that showed large prominent pronuclei (Table 1). The same results were found with B6FVF$_1$ embryos obtained from the reciprocal cross (data not shown). When FVB6F$_1$ females were backcrossed with C57BL/6J males and eggs from the progeny females were fertilized with B6SJLF$_1$ sperm, 9 such females produced zygotes with large and prominent pronuclei, whereas 19 animals produced zygotes with small pronuclei. These results suggest that the large and prominent pronuclei in FVB/N strain is controlled by more than one gene (see *Discussion*).

Table 1. Pronuclear volumes in embryos derived from FVB/N and (C57BL6/J × SJL/J)F_1 gametes

Ovum strain	Sperm strain	Male pronucleus vol	Female pronucleus vol	Embryo vol × 10^{-5}
FVB/N	FVB/N	5430 ± 302[a]	2430 ± 113[a]	2.75 ± 0.064[a]
FVB/N	B6SJLF$_1$	5650 ± 184[a]	2700 ± 119[b]	2.69 ± 0.059[a]
B6SJLF$_1$	FVB/N	3960 ± 122[b]	1910 ± 77[c]	2.74 ± 0.062[a]
B6SJLF$_1$	B6SJLF$_1$	3820 ± 168[b]	1780 ± 71[d]	2.72 ± 0.060[a]
FVB6F$_1$	B6SJLF$_1$	5530 ± 196	2640 ± 110	2.73 ± 0.059[a]

Mean vol is expressed in μm^3 ± SEM for 54 embryos measured. Within a column, mean values with different superscripts (a, b, c, and d) are significantly different as determined by Student's *t* test ($P > 0.001$). The sample number in FVB6F$_1$ was also 54; however, statistical analysis was not performed.

Survival of Embryos after Injection of DNA. FVB/N, C57BL6/J, and B6SJLF$_1$ zygotes were injected with two test DNA constructs (Table 2). Most (94%) of the FVB/N embryos from mated females were fertilized, had a healthy morphology upon examination under the microscope, and were subjected to injection. In contrast, nearly 25% of the zygotes obtained from the C57BL/6J and B6SJLF$_1$ donors were abnormal and were not injected. Overall, 74% of injected FVB/N embryos, 72% of C57BL/6J, and 70% of B6SJLF$_1$ zygotes survived injection. The FVB/N embryos showed significantly better survival rates after reimplantation into pseudopregnant foster mothers. As a consequence, the percent of injected embryos that yielded newborns was nearly twice as high for the FVB/N embryos (23%) as it was for the B6SJLF$_1$ embryos (13%), and three times higher than it was for C57BL/6J embryos (7%). In FVB/N embryos, the pronuclei become prominent ≈3 hr earlier than the C57BL/6J and B6SJLF$_1$ embryos, and pronuclear development is relatively well synchronized even after *in vivo* fertilization.

Efficiency of Generating Transgenic Mice. As shown in Table 2, 18% (15/85) of the FVB/N newborns were transgenic, somewhat lower but not significantly different than the 27% (8/30) for the B6SJLF$_1$ newborns ($P > 0.3$ by χ^2 test). The FVB/N percentage was higher than that for the C57BL6/J newborns (8%; 1/13), but it was also nonsignificant by χ^2 test because of the small number of C57BL/6J newborns ($P > 0.4$). The fraction of injected FVB/N embryos that produced transgenic newborns was not significantly different from that of B6SJLF$_1$ embryos ($P > 0.8$); an average of 3.7% (15/407) vs. 3.2% (8/248). The FVB/N matings also matched the B6SJLF$_1$ matings in terms of the number of transgenic mice per mated donor (58% vs. 42%) and the percentage of transgenic mice that express the transgenic inserts (85% vs. 83%). In nearly every aspect of generating transgenic mice, the inbred strain FVB/N was comparable to the more traditional B6SJLF$_1$ mice. Our overall efficiencies with the B6SJLF$_1$ embryos (3.2%) were the same as those previously reported by Brinster *et al.* in comparing the efficiency of B6SJLF$_1$ with C57BL/6J mice (16).

Genetic Markers in FVB/N. To help the genetic analyses of FVB/N transgenic mice, 44 common biochemical and coat color markers located on 15 chromosomes are shown in Table 3. Chromosomes not included are 10, 13, 16, 18, X, and Y. Also listed for these markers are alleles of seven commonly used inbred strains. With the use of the MATRIX computer program (12), we screened most inbred strains and analyzed their relative relatedness to FVB/N. Strains such as BDP/J, P/J, I/St, and *Mus molossinus* are most distant from FVB/N (<50% of the markers matching), whereas strains such as HSFS/N, NFS/N, and SWR/J are most closely related (>80% matching), reinforcing the fact that these strains were derived from Swiss mice (data not shown). Genetically

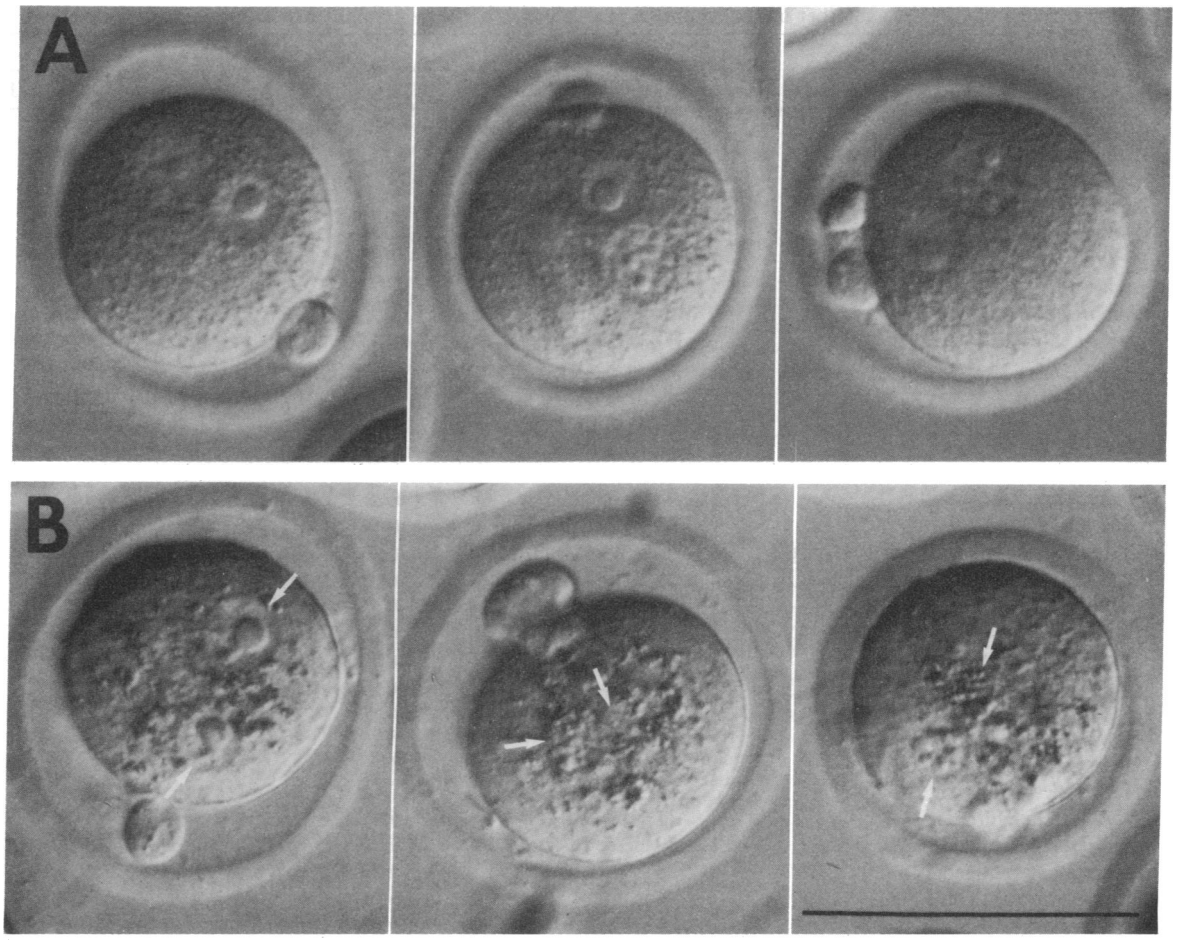

FIG. 1. Photomicrographs of zygotes. (*A*) FVB/N. (*B*) C57BL/6J. Arrows in *B* indicate pronuclei, which are not as visible as those in *A*. (Bar = 100 μm.)

distant strains such as P/J will be useful in analyzing and mapping mutations occurring in the FVB/N strain.

Congenic FVB/N Strains with Pigmented Coats. Because of the albino mutation in the *c* locus, FVB/N mice are not suitable for analyses of some genes that affect coat color. For such experiments, it is desirable to use zygotes of inbred strains with pigmented coats. To this end, we are constructing a congenic strain of FVB/N that carries a black coat. F_1 animals between FVB/N and C57BL/6N (agouti coats) were backcrossed with FVB/N, and the agouti N_1 animals were bred to each other, and mice with black coats were selected (F_2 generation homozygous at the *a* locus). This alternate inbreeding and backcrossing has been repeated for 18 cycles so far (N_{18}). When the mice reach the 20th backcross generation, they can be considered congenic with FVB/N and will be designated as FVBB/N (FVB black). Pigmented transgenic FVB/N mice have also been generated by microinjection of a tyrosinase minigene. The coloration of the coat varies between independent transgenic strains.

Formation of Chimeras Using FVB/N Blastocysts. The fecundity of the FVB/N strain and its difference in glucose phosphate isomerase 1 isotype (Table 3) make FVB/N a possible host for ES cells derived from the 129/Sv strain. ES cell lines D3 (20) and CS1 (21) have been described, and both give germ-line chimeras at a frequency of 25–30% of chimeric offspring when injected into C57BL/6J blastocysts. LG-1 is a parthenogenetic ES cell line, which so far has not produced any germ-line chimeras (C.L.S., unpublished data). As

Table 2. Efficiency of transgenic mouse construction using FVB/N, C57BL/6J, and B6SJLF$_1$ zygotes

Zygote strain	DNA construct	Number injected	Number survived (% of injected)	Number of newborns (% of injected)	Number screened	Number transgenic (% of injected)
FVB/N	C2TAg	189	136 (72)	45 (24)	40	6 (3.2)
	VISC	218	165 (76)	48 (22)	45	9 (4.1)
	Total	407	301 (74)	93 (23)	85	15 (3.7)
C57BL/6J	C2TAg	110	72 (65)	10 (9)	10	1 (0.9)
	VISC	85	68 (80)	3 (4)	3	0 (0.0)
	Total	195	140 (72)	13 (7)	13	1 (0.5)
B6SJLF$_1$	C2TAg	99	54 (55)	10 (10)	9	3 (3.3)
	VISC	149	120 (81)	21 (14)	21	5 (3.4)
	Total	248	174 (70)	31 (13)	30	8 (3.2)

See *Materials and Methods* for DNA constructs C2TAg and VISC. B6SJLF$_1$ females were fertilized by C57BL/6J males. Some of the newborns were cannibalized shortly after birth and were not screened.

Table 3. Genetic markers of FVB/N and other inbred strains

Symbol	Locus name	Chromosome no.	Allele in strain							
			FVB	129	C3H	C57BL/6	DBA/2	P	SJL/J	SWR
a	Agouti	2	+*	A^w	+	*a*	*a*	*a*	+	+
Ah	Aromatic hydrocarbon responsiveness	12	*b*	*d*	*b*	*b*	*d*	*b*	*d*	*d*
Akp-1	Alkaline phosphatase 1	1	*b**	*b*	*b*	*a*	*a*	*b*	*b*	*b*
Amy-1	Amylase 1	3	*a**	*a*	*a*	*a*	*a*	*a*	*a*	*a*
Apoa-1	Apolipoprotein A1	9	*b**	*b*	*b*	*a*	*b*	*a*	*a*	*b*
b	Brown	4	+*	+	+	+	*b*	*b*	+	+
c	Albino	7	*c**	c^{ch}	+	+	+	+	*c*	*c*
Car-2	Carbonic anhydrase 2	3	*b**	*a*	*b*	*a*	*b*	*a*	*b*	*b*
Ce-2	Kidney catalase	17	*a*	(*b*)	*b*	*a*	*a*	*a*	*a*	*a*
d	Dilute	9	+	+	+	+	*d*	*d*	+	+
Es-1	Esterase 1	8	*b**	*b*	*b*	*a*	*b*	*b*	*b*	*b*
Es-3	Esterase 3	11	*c**	*c*	*c*	*a*	*c*	*a*	*c*	*c*
Es-10	Esterase 10	14	*a**	*b*	*b*	*a*	*b*	*a*	*b*	*a*
Es-11	Esterase 11	8	*a**	*a*	*a*	*a*	*a*	*b*	*a*	*a*
Fv-1	Friend virus susceptibility 1	4	*b**	*n*	*n*	*b*	*n*	*n*	.	*n*
Glo-1	Glyoxylase 1	17	*a**	*a*	*a*	*a*	*a*	*a*	*a*	*b*
Got-2	Glutamate oxaloacetate transaminase 2	8	*a**	*b*	*b*	*b*	*b*	*b*	*b*	*a*
Gpd-1	Glucose-6-phosphate dehydrogenase 1	4	*b**	*a*	*b*	*a*	*b*	*a*	*b*	*b*
Gpi-1	Glucose phosphate isomerase 1	7	*b**	*a*	*b*	*b*	*a*	*a*	*a*	*b*
Gpt-1	Glutamic-pyruvic transaminase 1	15	*a**	*a*	*a*	*a*	*a*	*a*	*a*	*a*
Gr-1	Glutathione reductase 1	8	*a**	*a*	*a*	*a*	*a*	*a*	*b*	*b*
Gus-s	β-Glucuronidase structural	5	*b*	(*b*)	(*h*)	*b*	*b*	*b*	*b*	*b*
H-2	Histocompatibility 2	17	*q**	(*b*)	*k*	*b*	*d*	*p*	*s*	*q*
Hba	Hemoglobin α-chain complex	11	*c*	(*a*)	*c*	*a*	*g*	*h*	*c*	*c*
Hbb	Hemoglobin β-chain complex	7	*d**	*d*	*d*	*s*	*d*	*d*	*s*	*s*
Hc	Hemolytic component (C5)	2	(0*)	(1)	1	1	0	1	1	0
Idh-1	Isocitrate dehydrogenase 1	1	*a**	*a*	(*a*)	*a*	*b*	*b*	*b*	*a*
Ly-1	Lymphocyte antigen 1	19	*b*	*b*	*a*	*b*	*a*	*b*	*b*	*b*
Ly-2	Lymphocyte antigen 2	6	*b*	*b*	*a*	*b*	*a*	.	*b*	*b*
Ly-3	Lymphocyte antigen 3	6	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*
Mod-1	Malic enzyme	9	*a**	*a*	*a*	*b*	*a*	*b*	*a*	*a*
Mpi-1	Mannose phosphate isomerase 1	9	*b**	*b*	*b*	*b*	*b*	*b*	*b*	*b*
Mup-1	Major urinary protein 1	4	*c*	*a*	(*a*)	*b*	*a*	*b*	*a*	*a*
Neu-1	Neuraminidase 1	17	*b**	*b*	*b*	*b*	*b*	*b*	*b*	*b*
p	Pink-eyed dilution	7	+*	(*p*)	+	+	+	*p*	*p*	.
Pep-3	Peptidase 3	1	*b**	*b*	*b*	*a*	*b*	*c*	*b*	*b*
Pgm-1	Phosphoglucomutase 1	5	*a**	*a*	(*b*)	*a*	*b*	*b*	*b*	*b*
Pgm-2	Phosphoglucomutase 2	4	*a**	*a*	*a*	*a*	*a*	*a*	*a*	*a*
rd	Retinal degeneration	5	*rd**	+	*rd*	+	+	*rd*	*rd*	*rd*
Thy-1	Thymus cell antigen 1 (θ)	9	*a*	*b*	*b*	*b*	*b*	.	*b*	*b*
Trf	Transferrin	9	*b**	*b*	(*b*)	*b*	*b*	*b*	(*b*)	*b*

Three additional coat color markers were tested but are not listed above because all strains showed the wild-type alleles. They are belted (*bt*) on chromosome 15, leaden (*ln*) on chromosome 1, and piebald (*s*) on chromosome 14. Many of these markers have also been reported in refs. 17 and 18. Data for other strains were obtained from the MATRIX program (12). For the *rd* mutation, see also ref. 19. Inbred strains are shown as composites of their various substrains. When some of their substrains carry different alleles from others or their alleles have not yet been determined, data are placed in parentheses. A complete listing of all substrains is available from the authors. Periods indicate markers not determined.

*Data obtained or confirmed by screening by the authors.

shown in Table 4, ≈40% of mice derived from FVB/N blastocysts injected with CS1 and LG-1 were chimeric. However, the tissue contribution of the injected ES cells in these chimeras was low, with no individuals exhibiting >50% chimerism in the coat. Only one chimera derived from the CS1 line was chimeric in the germ line. ES cell line D3, which has produced chimeras up to 95% tissue contribution when injected into C57BL/6J blastocysts, produced only four chimeric animals. In all four FVB/N-derived chimeras, coat color contribution was very weak and none of them were germ-line chimeras (see *Discussion*).

Table 4. Efficiency of chimera formation and germ-line transmission of 129/Sv-derived ES cells injected into FVB/N blastocysts

ES cell line	Blastocysts injected and transferred	Newborn mice	Chimeric mice (% of newborn)	Germ-line chimeras
CS1	62	31	13 (42)	1
D3	101	61	4 (6.5)	0
LG-1	60	29	12 (41)	0

DISCUSSION

We have studied the inbred mouse strain FVB/N for reproductive performance, pronuclear morphology, and efficiencies in the generation of transgenic mice and blastocyst chimeras. One important feature of this strain is its superior fecundity compared with most inbred strains of mice. Although FVB/N females do not typically superovulate to give >25 embryos per female, this is not a serious drawback because of the consistently high mating frequency and high percentage of fertilized, healthy embryos. Furthermore, the number of superovulated eggs can be improved by a high fat

diet and/or by adjusting the superovulation protocol to 3.5 units of pregnant mare serum gonadotropin and 5.0 units of human chorionic gonadotropin (unpublished observation). The large and prominent pronuclei of FVB/N zygotes facilitate microinjection of DNA. The overall efficiency of generating transgenic mice is as high with FVB/N embryos as with B6SJLF$_1$ embryos (Table 2). Use of FVB/N zygotes in transgenic experiments is becoming more common, and successful construction of transgenic mice has been reported (e.g., see refs. 22–25).

In transgenic experiments, new mutations are often generated by insertional mutagenesis (e.g., ref. 26). If F$_1$ or F$_2$ zygotes are used, analysis will be complicated by the hybrid nature of the mutant animals, and extensive backcrossing will be needed to establish mutants with an inbred background. The vigorous reproductive activity of FVB/N and its well-defined inbred background should simplify genetic analyses. We propose using FVB/N as one of the standard strains not only for transgenic studies but also for other experiments in mouse genetics.

The large and prominent pronuclear morphology is caused by the FVB/N oocyte and is independent of the genotype of the fertilizing sperm. Although this phenotype is dominant, it is likely to be controlled by more than one gene because females of an FVB6F$_1$ × C57BL6/J backcross showed unequal distribution in producing zygotes containing large and small pronuclei. These results are consistent with a two-gene model (1:3 ratio), although the sample numbers are insufficient to establish an inheritance pattern convincingly. Comparison of genetic markers between FVB/N and its direct ancestor HSFS/N revealed different alleles in three genes in addition to the *Fv-1* locus on chromosome 4; they are *Ly-3* (chromosome 6), *Mup-1* (chromosome 4), and *Thy-1* (chromosome 9). It remains to be determined whether any of these four genes is responsible for the large pronuclei. Because N:NIH mice have been maintained as an outbred colony, it is conceivable that genes were still segregating when the *Fv-1^b* allele was found at the eighth inbred generation in the normally *Fv-1^n* HSFS/N strain. It is also possible that mutations occurred in the HSFS/N colony to cause the FVB/N phenotype.

The fertilization efficiency by FVB/N sperm *in vitro* was somewhat lower than B6SJLF$_1$ sperm; the efficiency may be improved by adjustments in the conditions for *in vitro* fertilization—e.g., improvement of capacitation. Attempts to form germ-line chimeras using 129/Sv-derived ES cell lines were not very successful with FVB/N blastocysts. The contribution of injected cells to the internal tissues and coat color of the chimeric animal was much less than that observed when C57BL/6J zygotes were used. In this context, it would be interesting to reverse the combination; to establish ES cell lines from the FVB/N strain and test their efficiency of forming germ-line chimeras. FVB/N mice have been observed at National Institutes of Health Animal Genetic Resource, The Jackson Laboratory, and Baylor College for spontaneous tumors (carcinomas, sarcomas, leukemias, lymphomas, etc.). No significant incidence of tumors has been detected, although we have not made a systematic study for any particular type of neoplasm. Construction of mouse strains that have pigmented coats and are congenic with FVB/N, used in conjunction with FVB/N, can provide useful options for a variety of experiments, including experiments in which ES cells derived from one strain are transferred into blastocysts of the other.

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