Characterisation of genetically modified animal lines:

Standard form

C. Mertens/T. Rülicke: a project of the 3R Research Foundation, Switzerland (Grant 59-97)

Update 2003

I. General information

A. Basic form

Species:

Name of the line(s) [In accordance with: rules and guidelines for gene, allele, and mutation nomenclature, International Committee on Standardized Genetic Nomenclature for Mice (2001) http://www.informatics.jax.org/mgihome/nomen/]

Name/ address of the person(s) responsible for the present characterization:

The present descriptive characterisation is:

- within the scope of the creation of new genetically modified lines? ☐
- in view of modification/further development of an existing line? ☐
- in view of an experimental application? ☐
- in view of breeding of an existing line? ☐

Short description of the induced geno- and phenotypes; important application areas (please see detailed information p. 7 et sqq.):

Name/ address/ first publication of the creator of the line:

Source(s) for the supply of and information on the line [name(s), address(es)]:

Further publications (own and others'- please enclose a publication list):

- Final date of the present characterisation:
- Date of the last modification of the basic information (A – D):
- Date of the last modification of the detailed information (E – G):

B. Genotype: mode of origin of the line; construct or mutant locus

B 1. Gene addition

	Pronucleus injection/ ES-cell transfection	**Viral vector**
Construct		
Origin (species)		
Coding sequence		
Qualitative property (e.g. oncogene)		
Regulatory elements (promotor, enhancer, etc.)		
Genetic background of zygote or embryo		
Vector	n/a	
Helper cell line	n/a	
Biological risk potential *	1 ☐ 2 ☐ 3 ☐ 4 ☐	1 ☐ 2 ☐ 3 ☐ 4 ☐

* according to the announcement form of the Swiss Federal Commission for Biological Safety (EFBS form).

B 2. Targeted mutation in ES Cells

- Mutation: loss-of-function ☐ or gain-of-function ☐
- Mutated locus:
- ES cell line:
- Genetic background of the mutant mouse line:

B 3. Breeding of multiple mutants

Designation of the parental lines:

Females	
Males	

Additional remarks:

C. Phenotype and clinically manifest burden: survey

In general, the wild-type animals (the genetically unmodified animals with the same genetic background as the transgenic line) are considered to be the references.

	Yes	No	Maybe	Details p.
1. Does any breeding data exist which can be evaluated?	☐	☐		9
2. Do any differences exist between hemi-/heterozygous and homozygous animals?	☐	☐	☐	10
3. Do sex-specific differences exist?	☐	☐		11
4. Does an increased lethality exist (prenatal, perinatal, postnatal)?	☐	☐		11
5. Are there any disturbances in the ontogenetic development?	☐	☐		12
6. Are there any external visible disturbances or malformations?	☐	☐		13
7. Are there any malformations of the inner organs?	☐	☐	☐	13
8. Are there any disturbances in the behaviour of the individual?	☐	☐		14
9. Are there any disturbances in the social behaviour (non-reproduction)?	☐	☐		14
10. Are there any disturbances in the reproductive behaviour?	☐	☐		15
11. Are there any disturbances in the reproductive performance?	☐	☐		15
12. Do strain-related diseases occur?	☐	☐		16
If yes, which one(s): _____				
13. Are there any disturbances in the immune reaction?	☐	☐		17
14. Did/do the animals breed on a different genetic background?	☐	☐		17
15. Is/will continued crossing (be) performed to create double and multiple mutants?	☐	☐		17
16. Has any detailed information been collected?	☐	☐		18

Please fill in the respective detail sheet for each positive answer.

The data was collected on the following level of hygiene: gnotobiotic, SPF, OHB, conventional

Suitability of the line for the research project:

First, estimate the suitability of the line for your research project or area, because or despite of the phenotypical characteristics. Second, estimate its usefulness:

- The animal line is suitable ☐ / unsuitable ☐ to gain the desired results
- The usefulness is high ☐ / moderate ☐ / low ☐ / not yet obvious ☐ / too early for an estimation ☐

D. Evaluation of the line with respect to ethical and animal welfare aspects

D 1. Age at occurrence of clinical symptoms

If you observed any clinical symptoms in the animals, specify the age in which they were seen (chronologically):

Symptom:	**Age:** Day, Week, or Month	**Genotype:** Hemi-/Hetero or Homozygous	**Load:** Slight (1), Moderate (2), Serious (3)

D 2. Overall load considering all symptoms and stages of life

In general, the 'wild-type animals' are considered to be the references, i.e. the genetically unmodified animals with the same genetic background as the transgenic line.

	Hemizygous/ Heterozygous	**Homozygous**
Mortality peri- and postnatal: unchanged (-), increased (+), high (++)		
Morbidity: unchanged (-), increased (+), high (++)		
Vitality: unchanged (-), reduced (--), low (---)		
Life expectancy: unchanged (-), reduced (--), low (---)		
Mortality prenatal (estimated in % of the fetuses)		
Mortality perinatal and during the first 3 weeks of life; in % of n = _____ offspring		
Mortality during the first 3 months of life; in % of n = _____ offspring		
Mortality during the first year of life; in % of n = _____ (sample)		

E. Information on breeding, husbandry and transportation

- line breeds normally ☐
- can only be bred under SPF conditions or gnotobiotic conditions ☐
- only hemi-/ heterozygous breeding ☐
- do not breed with transgenic females/males ☐
- husbandry/breeding only under special conditions
  (climate, nutrition, cage facility, and/ or social structure) ☐
- special conditions for transportation
  (none, sedation, temperature, drinking water, filter cages, etc.) ☐
- cryoconservation (if possible) ☐
- cryoconservation by all means (i.e. breeding only with permission) ☐
- euthanasia latest at the age of/ at occurrence of _____ ☐

	Female	**Male**
Hemi-/ Heterozygous		
Homozygous		

Additional remarks (e.g. ICSI, IVF, fostering, ovary transplantation, cryoconservation of embryos and/or sperm cells):

Therapeutic steps to safe viability and to increase vitality and health

Steps to safe viability

- are/ were: necessary ☐ possible ☐
- are/ were: taken ☐ not taken ☐

description of steps:

Note: steps were taken before the age of_____ or steps were not taken, until the age of_____

Steps to increase vitality and health:

- are/ were: necessary ☐ possible ☐
- are/ were: taken ☐ not taken ☐

description of steps:

II. Detailed information on geno- and phenotype

F. Gene-expression on molecular and cellular level; inducibility

F 1. Prospective
Projected expression, incl. timing and spatial patterns of expression and inducibility:

F 2. Retrospective

F 2.1. Integration site and number of integrated copies per locus

- Multiple integration Yes ☐ No ☐
- Chromosome/linkage group ☐ Not examined ☐
- Number of copies: ☐ Not examined ☐

F 2.2. Results of the expression analysis

	Expression on RNA level (y/n)		Expression on protein level (y/n)		Temporal occurrence (y/n)	Inducibility (y/n)
Location	**studied**	**found**	**studied**	**found**		
Blood cells (specify)						
Brain (parts; specify)						
Tongue						
Thymus gland						
Salivary glands						
Thyroid gland						
Heart						
Lung						
Stomach						
Colon						
Small intestine						
Liver						
Spleen						
Bladder						
Mammary glands						
Testicles						
Uterus						
Skin						
Muscles						
Pancreas						
Kidneys						
others (specify)						

Additional remarks on the spatial and temporal inspection and the inducibility:

F 2.3. Physiological functionality of the transgenic protein

- Tested? Yes ☐ No ☐
- Method employed?

- Functionality given Yes ☐ No ☐

F 2.4. Sex-specific differences in the expression

- Tested? Yes ☐ No ☐
- Differences? Yes ☐ No ☐
- If the answer is yes to the above question (s), please describe:

F 2.5. Influence of the genetic background on the expression

- Tested? Yes ☐ No ☐
- Applied background for comparison? _____
- Any influence determined? Yes ☐ No ☐
- If the answer is yes to the above question (s), please describe:

G. Detailed information on the phenotype

All lines (which are bred further) have to be characterised separately.

- How many different founders have been created? I do not know: ☐ _____ founders
- How many lines are being bred further? I do not know: ☐ _____lines
- Are there any differences between the lines, in the total load, according to point C?
  I do not know: ☐ Yes ☐ No ☐

G 1. Differences between hemi-/heterozygous and homozygous animals

Differences exist in the following areas (multiple selections possible):

G 2. Sex-specific differences ☐
G 3. Statistical breeding data ☐
G 4. Lethality, vitality, morbidity, mortality ☐
G 5. Disturbances in individual development ☐
G 6. Externally visible disturbances and malformations ☐
G 7. Functional disorders and malformations of the inner organs ☐
G 8. Disturbances in individual behaviour ☐
G 9. Disturbances in social behaviour (non-reproduction) ☐
G 10. Disturbances in reproductive behaviour ☐
G 11. Disturbances of reproductive performance ☐
G 12. Clinical signs/ line-specific diseases ☐
G 13. Immunological characteristics ☐
G 14. Effects of the genetic background ☐
G 15. Continued crossing; double and multiple mutants ☐

Please fill in the corresponding complementary sheet for every aspect checked above and for each genotype separately. This will result in several complementary sheets [e.g. G1 + G5 (heterozygous) + G5 (homozygous) + G11 (heterozygous) + G11 (homozygous)]

G 2. Sex-specific differences

Differences exist in the following areas (multiple selections possible):

G 1. Between hemi-/heterozygous and homozygous animals ☐
G 4. Lethality, vitality, morbidity, mortality ☐
G 5. Disturbances in individual development ☐
G 6. Externally visible disturbances and malformations ☐
G 7. Functional disorders and malformations of the inner organs ☐
G 8. Disturbances in individual behaviour ☐
G 9. Disturbances in social behaviour (non-reproduction) ☐
G 10. Disturbances in reproductive behaviour ☐
G 11. Disturbances of reproductive performance ☐
G 12. Clinical signs/ line-specific diseases ☐
G 13. Immunological characteristics ☐
G 14. Effects of the genetic background ☐
G 15. Continued crossing; double and multiple mutants ☐

Please fill in the corresponding complementary sheet for every aspect checked above and for each genotype separately. This will result in several complementary sheets [e.g. G2 + G5 (heterozygous) + G5 (homozygous) + G11 (heterozygous) + G11 (homozygous)]

G 3. Statistical breeding data

The aim of this section is to obtain reliable breeding data. The sample considered on this page does not have to be identical with the population supplying the findings for the basic information (B – D) or the detailed information (G 1 & 2 , 4 - 15). In case such data exist(s) from more than one generation, please fill in separately (one generation per sheet).

Characterisation data have been collected in the following parental generations: F0, F1, F2, F3, etc.
and/or with the parental genotypes: female (wild-type, hemi-/ hetero-/ homozygous), male (wild-type, hemi-/ hetero-/ homozygous)

Breeding Information	**Numbers/ Ratios Recorded**	
No. of litters considered		
Total no. of offspring born		Of which were found dead:_____
Average litter size at birth		
Average time between two litters		
Total no. of offspring weaned		
Average litter size at weaning		
No. of transgenic offspring weaned		% of total offspring:_____
No. of homozygous offspring weaned		% of total offspring:_____
Sex ratio (male to female) of all offspring weaned		
Sex ratio (male to female) of mutants		
Sex ratio (male to female) of homozygous mutants		

G 4. Lethality

Characterise the life phases (prenatal, perinatal, postnatal until weaning, 1st – 3rd month of life, 4th – 6th month, rest of life) in which lethality occurs and state the applied unit(s) of measurement indicators, respectively, with regard to:

- The extent found:
- Probable causes for each case:
- Possibilities to prevent lethality:
- Conclusions considering the best strategy to breed the line:

G 5. Disturbances in individual development (Period of investigation: 1st-7th week)

Only fill in where you have performed enough tests to gain a valid result

Birth and the first 24 hours (circle those that apply):

- Conspicuous symptoms in the individuals: none, weight, stomach contents, skin colour (cyanosis)
- Conspicuous symptoms in the litter: none, weight differences in the litter

Neuronal state (period of investigation: day 1-17):

	Normal	Abnormal
Righting	☐	☐
Grasping	☐	☐
Walking (pivoting, crawling, walking)	☐	☐
Vibrissa placing	☐	☐
Auditory startle	☐	☐
Bar holding or grip strength	☐	☐
Blancier beam	☐	☐
Crossed extensor (spinally mediated reflex)	☐	☐
Corneal reflex	☐	☐
Finding the way back to the nest	☐	☐
_____	☐	☐
_____	☐	☐

Physical development:	Normal	Abnormal
Opening the eyes	☐	☐
Hair growth	☐	☐
Average weight at the age of 3 weeks	____	____
Average weight at the age of 6 weeks	____	____

Behaviour (juveniles between 3 and 7 weeks and between weaning and breeding age, respectively):

	Normal	Abnormal
Locomotion	☐	☐
Posture	☐	☐
Care of the body	☐	☐
Social behavior (social isolation/ aggression)	☐	☐
Attention/ novel stimulus	☐	☐
Prone to stress/ reaction to disturbances	☐	☐

Please describe the striking conspicuous symptoms and any 'abnormal' variables on a separate sheet

G 6. Externally visible disturbances and malformations (circle those that apply):

- Malformations concerning: skeleton, head, extremities, paws, musculature, skin, hair coat, eyes, ears, nose, mouth, tail, external genitals
- Body proportions, posture and position of the extremities: normal/abnormal
- Hair coat: clean, smooth, bright, dirty, rough, loss of hair, bald spots

Additional Remarks:

G 7. Functional disorders and malformations of the inner organs

Morphological (circle those that apply):

- Malformation of brain (parts), heart, lung, stomach, small intestine, colon, liver/gall, kidneys/bladder, lymphatic system/spleen, primary genitals, glands (thymus, thyroid, salivary, pancreas), skeleton, musculature
- Organ weight: brain, heart, lung, stomach, small intestine, colon, liver/gall, kidneys/bladder, spleen, testicles, uterus, thymus gland, thyroid gland, salivary glands, pancreas
- Tumors on/in brain, heart, lung, stomach, small intestine, colon, liver/gall, kidneys, bladder, lymph vessels, spleen, testicles, uterus, thymus gland, thyroid gland, salivary glands, pancreas

Histological results:

Molecular methods:

Additional Remarks:

Line Name: Genetic background:
Genetic state: hemi-/heterozygous; homozygous
Sex: male/ female

G 8. Disturbances in individual adult behavior (animals older than 7 weeks)

	Normal	Abnormal
• Locomotion, motor activity (walking, raising, digging, climbing)	☐	☐
• Posture when awake and during sleep	☐	☐
• Feeding and drinking behaviour	☐	☐
• Care of the body	☐	☐
• Exploration behaviour, attention	☐	☐
• Susceptibility to disturbances and stress during manipulations/ surgery	☐	☐
• Pain sensitivity	☐	☐
	Yes	No
• Stereotypes	☐	☐
• Automutilation	☐	☐

Additional Remarks:

G 9. Disturbances in social behaviour (non-reproduction)

• Aggressiveness	☐	☐
• Isolation	☐	☐
• Allogrooming	☐	☐
• Cannibalism	☐	☐
• Behavior when in contact with persons (passive, defensive, aggressive)	☐	☐

Additional Remarks:

G 10. Disturbances in reproductive behaviour

- Disturbances in the copulation due to the female Yes ☐ No ☐
- Disturbances in the copulation due to the male Yes ☐ No ☐
- Disturbances in the mother's care Yes ☐ No ☐
- Cannibalism Yes ☐ No ☐

Additional Remarks:

G 11. Disturbances in reproductive performance

Breeding conditions: monogamous/ polygamous *; mated permanent / intermittent *

Hetero-/ hemizygous parents (N = _____)

- Sterility of females yes ☐ no ☐
- Sterility of males yes ☐ no ☐
- Disturbances in the lactation yes ☐ no ☐
- Fostering necessary yes ☐ no ☐

Additional Remarks:

Homozygous parents (N = _____)

- Sterility of females yes ☐ no ☐
- Sterility of males yes ☐ no ☐
- Disturbances of lactation yes ☐ no ☐
- Fostering necessary yes ☐ no ☐

Additional Remarks:

G 12. Clinical signs/ line-specific diseases

- Weight: marked emaciation/ marked increase
- Dehydration (liquid lost): skin turgidity normal/ weak
- Musculature: normal/ atrophic; muscular tone: normal/ diminished
- Signs of pain/load: crooked back, hunched position, body extension, shifting of the body centre of gravity, licking, scraping, biting, trembling, convulsions, spasms
- Faeces (quantity, colour, form): normal, diarrhea, etc. _____
- Locomotion: normal, incomplete sequence of motion, ataxia (circle/ reel), taking care of particular extremities, careful walking, paralysis
- Prone to disease: _____
- Incidence (%) and initial time of manifestation: _____
- Lack of (physical, physiological, and behavioral): _____
- Incidence (%) and initial time of manifestation: _____
- Spontaneous death at the age of: _____

Additional Remarks:

G 13. Immunological characteristics

- Haplotype: _____
- Complete immunodeficiency yes ☐ no ☐
- Partial immunodeficiency yes ☐ no ☐

Description:

G 14. Alteration of the genetic background; effects of the genetic background

- Previous background:
- New genetic background:
- Current number of back-crossed generations:
- Detectable phenotypic differences compared with the original line:

G 15. Continued crossing; double and multiple mutants

Indicate data obtained:

H. Line-specific detailed information

For already established methods, give their name (e.g. „Morris water maze"), if possible, with a literature reference.

1. Have specific parameters or tests, based on line-specific characteristics, been developed which enable the refinement of phenotypic characterisation?

Yes ☐ No ☐

If yes, which?

2. Can further differences between hemi-/heterozygous and homozygous animals be found by line-specific investigations?

Yes ☐ No ☐

If yes, which?

Line Name: Genetic background:
Genetic state: hemi-/heterozygous; homozygous
Sex: male/ female

3. Can further sex differences be found based on these investigations?

Yes ☐ No ☐

If yes, which?

4. Please summarize the found characteristics with regard to the global description of the phenotype (part C), the ethical and animal protection relevant evaluation of the line (part D), and the recommendations for breeding, husbandry and transportation (part E).