



# Transgenic Outlook

Charles River Laboratories

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## PHENOTYPING SERVICES

This edition of Transgenic Outlook focuses on our comprehensive program of Phenotyping Services. Our services are designed to characterize genetic mutant models by assessing quantitative and qualitative changes in anatomy, physiology, and behavior. All services are carried out under the direction of our board-certified veterinarians and PhDs, providing skilled evaluation and interpretation in comparing genetic mutant models to their wild type counterparts. Based on phenotypic findings, further investigation may be warranted. Transgenic Services' phenotyping program is offered at many levels (i.e. targeted and comprehensive), allowing investigators to custom design their phenotyping panel based on the unique attributes of their model.

Also highlighted in this edition is our alliance with Rules-Based Medicine, Inc., through which we continue to deliver the most up-to-date technology to our customers. For further information on either feature, please call 1-877-CRIVER1, or email [askcrl@criver.com](mailto:askcrl@criver.com).

## Comprehensive Phenotypic Assessment

Comprehensive phenotypic assessment is commonly used when the researcher does not know the function of a gene. To help balance the value of comprehensive characterization with the cost of extensive testing, the screen may be divided into two tiers when developing a phenotype assessment program. In a tiered or phased approach, the first tier

consists of a general primary screen, with which all mutant models are evaluated.

The second tier consists of multiple secondary screens that are more focused. The results of the general primary screen determine which secondary screens are needed to evaluate each model. Mutant models are only tested with secondary screens that further define and characterize the data collected in the primary screen.

## Targeted Phenotypic Assessment

Targeted phenotypic assessment is performed when the function of the gene is known or there is interest in a specific phenotype. It is a focused approach consisting of screens that help define and characterize the model within the specific scope or area of research interest. Targeted phenotypic assessment is used when a researcher has prior knowledge of gene function, predictive data that suggests the function, or in the creation of mutant models with tissue-specific transgene expression. This type of assessment may also be used subsequent to random mutagenesis to study a specific disease.

Many institutions rely on targeted phenotypic assessment because of the technique and design used to create the mutant model, the focused scope of research, the investigator's interest in a very specific scientific area, researcher knowledge of gene function or predilected function, limitations on available resources, and the cost of assessment.



*Radiograph of a normal mouse*



*Radiograph of a mouse with Hydrocephalus*

  
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## Experimental Design

It is important to be aware of factors that may influence phenotype expression when developing a phenotype assessment program and interpreting collected data. Such factors include genetic background, age, health status, and environmental conditions such as husbandry practices, light cycle, and diet. Use of appropriate controls and sample sizes should minimize the effect of these variables on collected data. Appropriate sample size depends on the test or assay performed. For example, most behavioral tests require 10-20 animals per genotype to obtain statistically significant results. If it is not clear what the appropriate sample size is to achieve statistical significance, Charles River recommends to initially screen a minimum of 3-5 gene-altered and 2-3 wild type control mice matched for genetic background, zygosity, age, sex, parity, health status, and environment. Based on these results, in future screens, the appropriateness of the sample size can be reassessed and adjusted, as needed.

The age of the animals selected for testing will depend on the test or assay to be performed. If there is no scientific reason to examine animals of a specific age, a common age to start with is 8-12 weeks old, although early developmental characteristics may be missed, if they are present. In addition, some models may warrant characterization at multiple age points in order to fully assess disease progression over the life of the animal. Finally, it is generally recommended to screen both males and females.

We work closely with investigators and their research teams to develop customized phenotyping assays including challenge protocols. Facilitated by discussions with our technical staff, a customized phenotyping panel allows

## Phenotyping Screens

IN-LIFE ANALYSIS	
<i>Clinical Observation</i>	Assessment of physical characteristics and abnormalities, body weight, posture, and gait including digital photographs to document clinical observations.
<i>Primary Behavioral Observation</i>	Assessment of gross behavioral changes in sensory abilities, motor coordination, and temperament.
<i>Growth Curve</i>	Body weight obtained at different time points to develop a growth curve.
<i>Reproductive Performance</i>	Analysis of breeding records and genotype results to assess fertility, average litter size born and weaned, male to female ratio, and expected Mendelian ratio.
<i>DEXA Scan</i>	Measurement of body composition (i.e. percent body fat and bone density).
<i>Radiography</i>	Assessment of organ size, bone density, skeletal malformations, and potential tumor burden.
<i>Grip Strength Measurement</i>	Quantitative analysis of hind limb and forelimb muscle grip strength according to the Meyer procedure.
<i>Pain Sensitivity: Hot Plate Analgesia Meter and Tail Flick Analgesia Meter</i>	Measurement of thermal pain reflex: Tail Flick Test - spinal reflex Hot Plate Test - centrally mediated acute pain for narcotic type analgesic agents
<i>Activity Level - Open Field System</i>	Measurement of activity and movement including distance traveled, total number of movements, time spent in center of open field, and time spent in perimeter of open field.
<i>Cognitive Analysis: Holeboard System and Barnes Maze</i>	Holeboard System: Spatial learning/memory system used for testing animal's reactions to food rewards based on varying conditions. Barnes Maze: Spatial learning/memory task system using reinforcement and escape from a brightly lit platform to a recessed chamber.
<i>Motor Coordination and Balance - Rotarod</i>	Rotarod: Rotating cylinder measures motor coordination and balance
<i>Acoustic Startle Reflex</i>	Measurement of gross hearing ability and of auditory threshold.

PATHOLOGY	
<i>Necropsy</i>	Gross evaluation, organ collection, and gross photographs.
<i>Clinical Pathology</i>	Clinical chemistries, urinalysis, hematology, CBC, white blood cell differential and platelet count.
<i>Histopathology</i>	Routine H+E staining and photomicrographs. Special stains and techniques such as analysis of embryos to characterize embryonic lethal mutations are available for customized projects.
<i>Specialty Pathology</i>	Histomorphometry, immunohistochemistry, and in situ hybridization can be used to assess functional changes governed by the transgene.
<i>Proteomics</i>	Detection of specific proteins or characterization and comparison of protein patterns present in tissues of gene-altered vs. wild type controls both pre- and post-treatment or drug challenge.  Multi-Analyte Profiles (MAP) for a comprehensive evaluation of the responses to disease, drugs, and the environment in laboratory animals. Assays to measure markers of cancer, infectious disease, autoimmunity, cardiovascular risk, as well as hormones, cytokines/chemokines, and acute phase reactants are run on small volumes of blood.

investigators to explore and define the unique attributes of their model.

### Rules-Based Medicine's Multi-Analyte Profiles (MAPs)

Through an exclusive worldwide marketing and services agreement signed last year, Charles River offers Rules-Based Medicine's Multi-Analyte Profiles (MAPs) for phenotypic characterization of certain laboratory research models.

Rules-Based Medicine (RBM) began MAP development in 1998 to detect and understand the early signs of disease revealed by blood markers associated with cancer, infectious disease, autoimmunity and cardiovascular risk, as well as cytokines/chemokines, acute-phase reactants and other blood components.

Plasma Antigens		Autoimmune/Infectious Diseases
1. Apolipoprotein A1	31. IL-18	<b>Autoimmune Antibodies</b>
2. $\beta$ -2 Microglobulin	32. Insulin	60. $\beta$ -2 Glycoprotein
3. C-Reactive Protein	33. IP-10	61. Insulin
4. D-Dimer	34. KC/GRO $\alpha$	62. JO-1
5. EGF	35. Leptin	63. Mitochondrial
6. Endothelin-1	36. LIF	64. MPO
7. Eotaxin	37. Lymphotactin	65. PCNA
8. Factor VII	38. MCP-1	66. PR3 (cANCA)
9. FGF-Basic	39. MCP-3	67. Ribosomal P
10. FGF-9	40. MCP-5	68. RNP
11. Fibrinogen	41. M-CSF	69. Scl-70
12. GCP-2	42. MDC	70. Smith
13. GM-CSF	43. MIP-1 $\alpha$	71. SSA
14. Growth Hormone	44. MIP-1 $\beta$	72. SSB
15. GST	45. MIP-1 $\gamma$	<b>Infectious Disease Antibodies</b>
16. Haptoglobin	46. MIP-2	73. Adenovirus
17. IFN- $\gamma$	47. MIP-3 $\beta$	74. <i>Clostridium piliforme</i> (Tyzzer's)
18. IgA	48. Myoglobin	75. Cytomegalovirus
19. IL-1 $\alpha$	49. OSM	76. Ectromelia virus
20. IL-1 $\beta$	50. RANTES	77. EDIM (Epidemic diarrhea of infant mice)
21. IL-2	51. SCF	78. <i>Encephalitozoon cuniculi</i>
22. IL-3	52. SGOT	79. Hepatitis virus
23. IL-4	53. TIMP-1	80. Lymphocytic choriomeningitis virus
24. IL-5	54. Tissue Factor	81. Minute virus
25. IL-6	55. TNF- $\alpha$	82. <i>Mycoplasma pulmonis</i>
26. IL-7	56. TPO	83. Parvovirus
27. IL-10	57. VCAM-1	84. Pneumonia virus of mice
28. IL-11	58. VEGF	85. Polyoma virus
29. IL-12p70	59. von Willebrand Factor	86. Reovirus-3
30. IL-17		87. Sendai virus
		88. Theiler's mouse encephalomyelitis virus

Disease alters certain blood analytes in characteristic fashion. MAPs provide the researcher an unprecedented ability to discover these relationships. By comparing the blood of experimental animals versus control animals, relevant differences can be observed. Today, RBM's MAPs are used for retrospective studies of banked samples, prospective studies on ongoing clinical trials, post-drug approval surveillance monitoring, timed studies of a single mouse, correlation of a subject's genotype to phenotype, basic research on tissue culture supernatants, studies comparing gene expression to MAPs, qualifying clinical trial participants, as well as animal model phenotyping.

MAPs provide significant benefits to the animal researcher:

- Perform up to 90 assays from only 20 microliters of blood
- Repeated sampling from individual animals

- Low impact sampling from valuable founder animals
- Rapid assay development by an experienced team of scientists

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