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The application of cellular chemistry and proteomic approaches to the detection of gene doping

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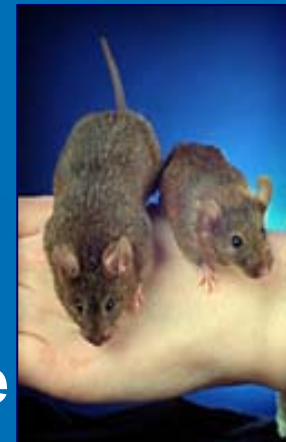
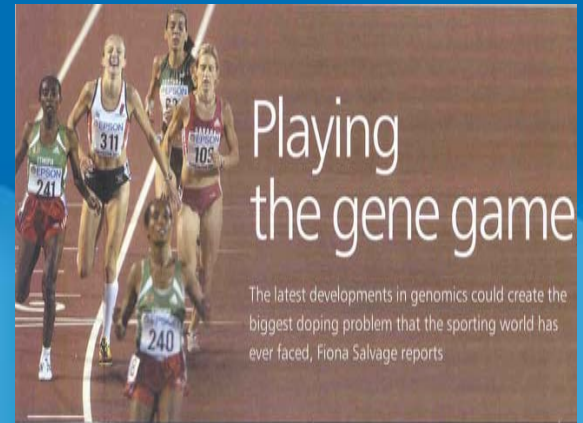


Objectives

- Identify and validate protein expression patterns (fingerprints)
 - Normal physiological conditions
 - GH/IGF-1 (protein / gene construct)
 - Mouse model
 - Application to humans (Y2-3)

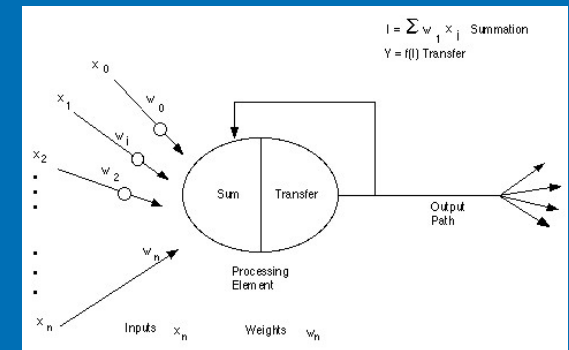
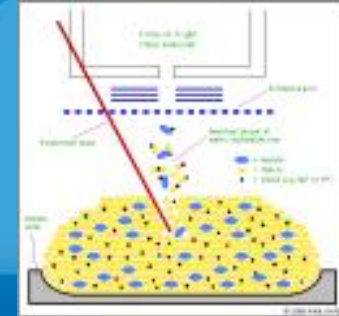
Year 1:

- Genetic manipulation results in change in biomarker fingerprint
- Detection by Mass Spectrometric pattern recognition



Experimental approach

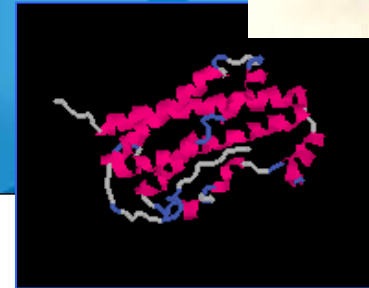
- Treated and Untreated mice
 - +/- GH
 - +/- GH gene vector (+ vector only)
 - Measure body weight
 - Collect serum, blood cells, muscle
- Serum sample analysis
 - Protein/peptide extraction
 - MALDI / nano LC-MS
 - IGF-1, GH levels (immunoassay)
- Data analysis
 - Apply pattern recognition e.g. ANNs
 - Characterise biomarkers by LC-MS/MS
- Application to GH treated humans
- Proof of principle of technique
 - Detect gene doping in humans



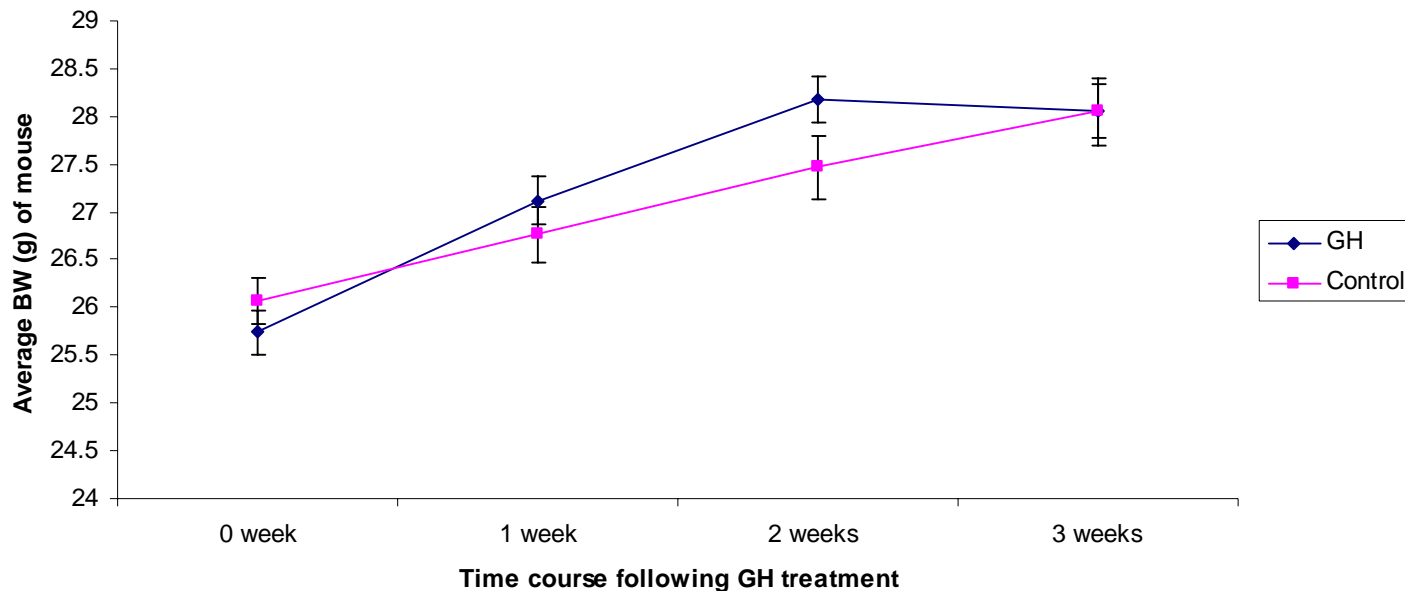
GH administration to mice

- **Porcine GH**

- s.c. implanted mini-osmotic pump
- 15 treated, 15 placebo
- 0.19 units/kg/day, 3 weeks
- blood collection (after 3 weeks)

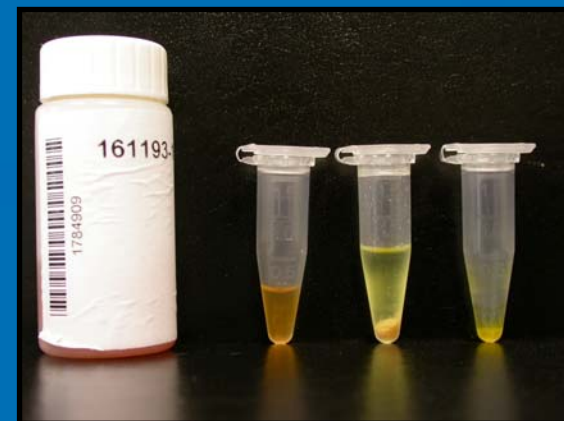


Body weight changes after GH treatment



Sample processing approaches for Mass Spectrometric analysis

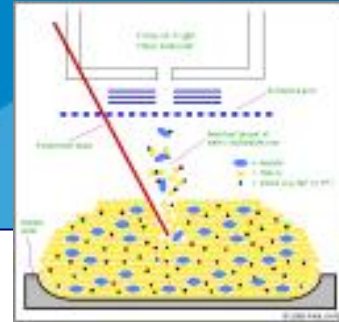
- Immuno-depletion e.g. albumin, IgG
- Dye-based protein removal
- Protein precipitation
 - Acids (TCA)
 - Salts (Ammonium sulphate)
 - Organic solvent (ACN)
- Ultrafiltration
- Dilution



Mass spectrometric analysis

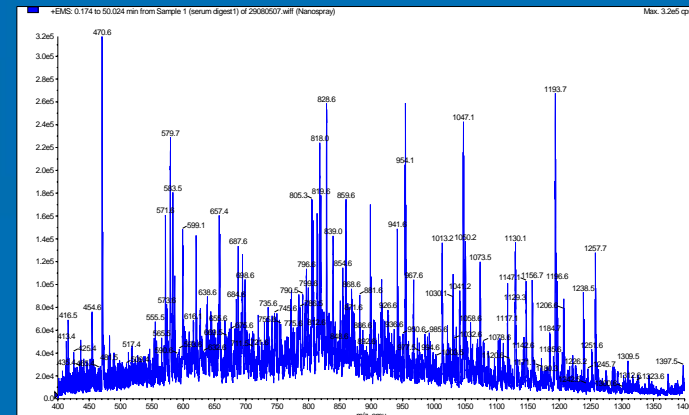
- **MALDI**

- Intact protein
- Axima *CFR*^{Plus} (Shimadzu)
- Matrix (Sinapinic acid)
- Mass range (1k-25k Da)



- **LC-MS-MS**

- Digested protein (peptides)
- HPLC Ultimate 3000 nanoflow
 - (LC Packings)
- MDS Sciex 4000 Q trap
 - (Applied Biosystems)
- Electrospray Ionisation
- Mass range (400 – 1600)
- Characterisation possible

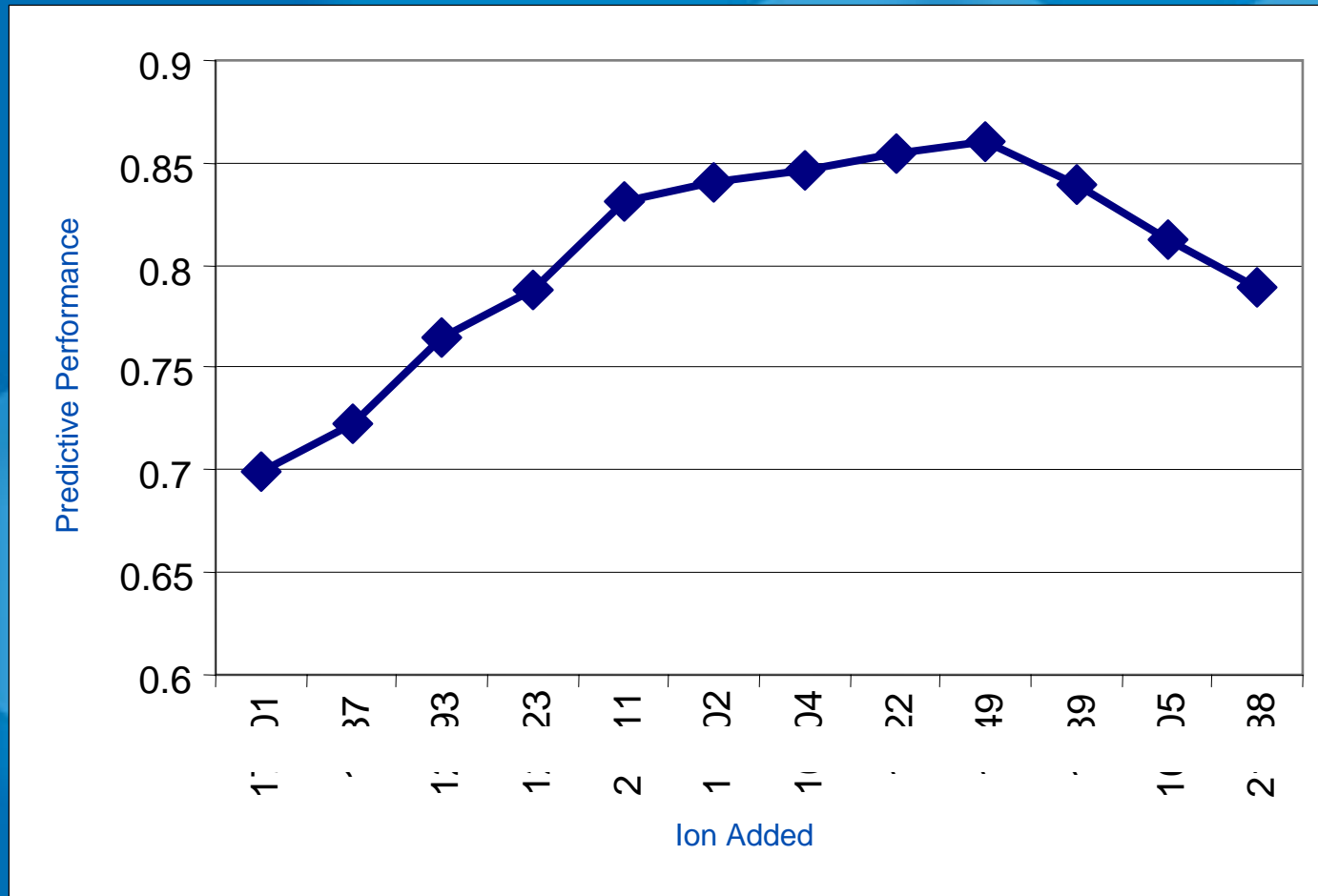


Data analysis - bioinformatics

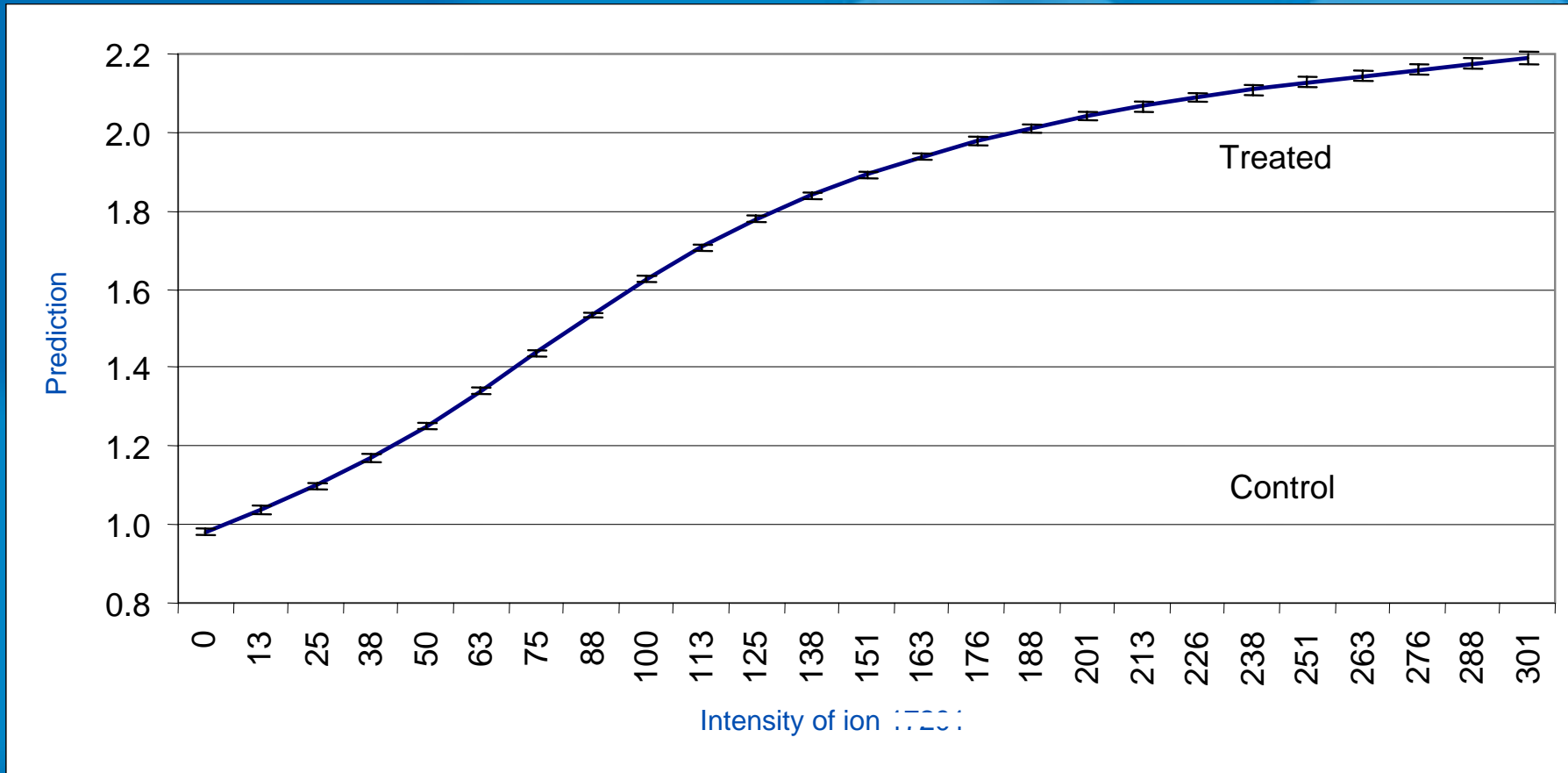
Artificial Neural Networks (ANNs)

- Data mine complex biological data
- Reduce complexity/dimensionality
 - determine key features (biomarkers) in a given biological class
 - e.g. GH treated vs Control
- Develop predictive models
 - determine best subset of parameters (biomarkers) to predict class
 - make prediction/ decision with a level of certainty
 - e.g. Treated vs Untreated

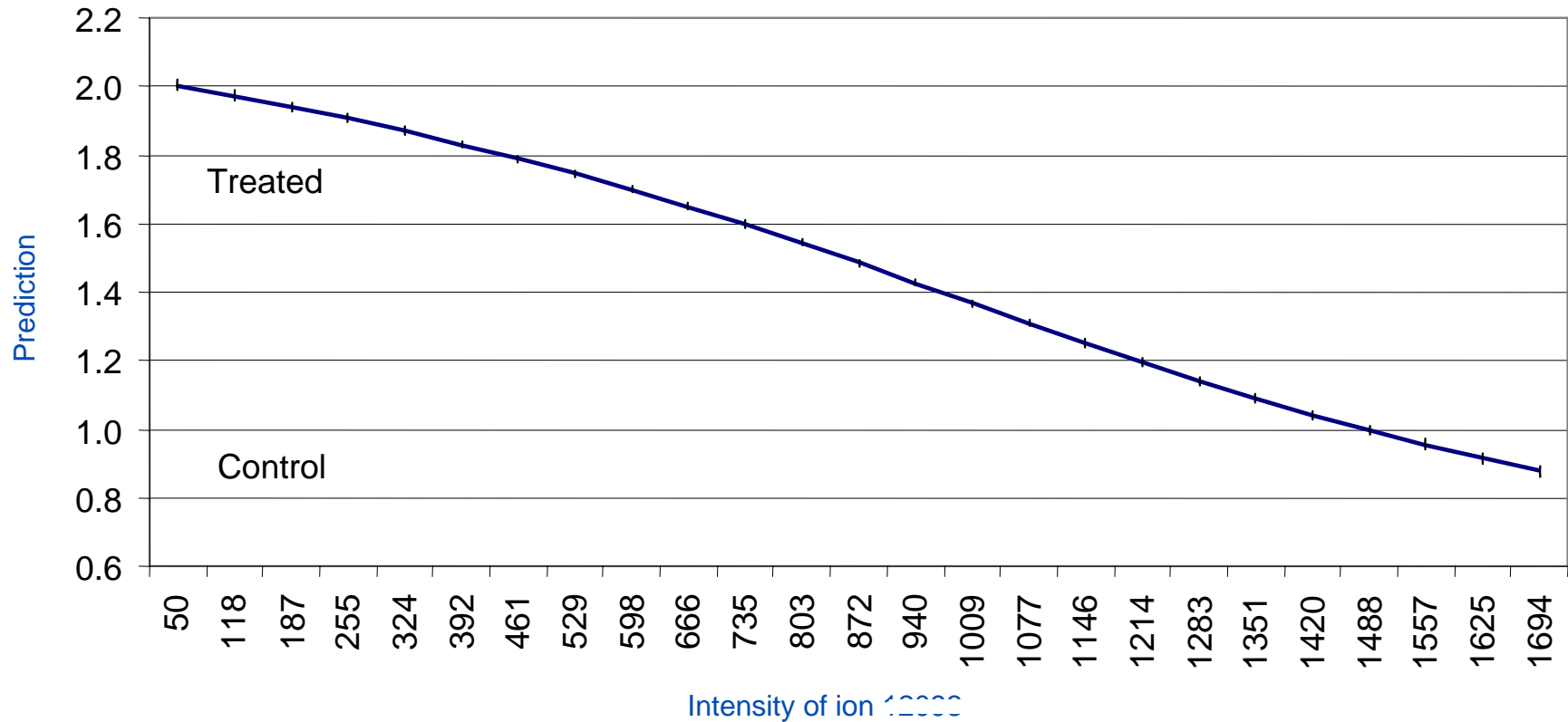
Artificial Neuronal Network (ANNs) Stepwise Learning



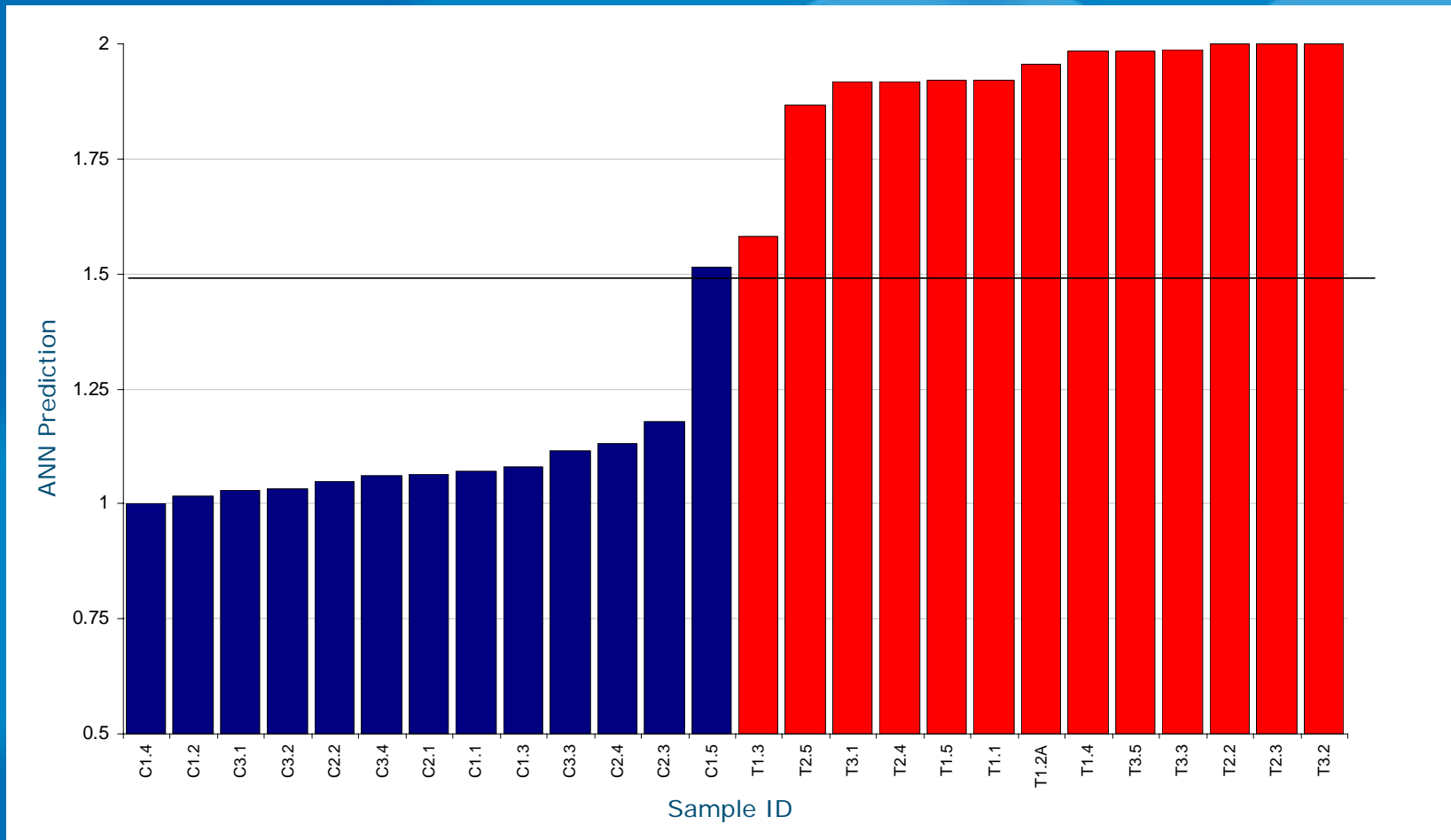
Response profile ion x



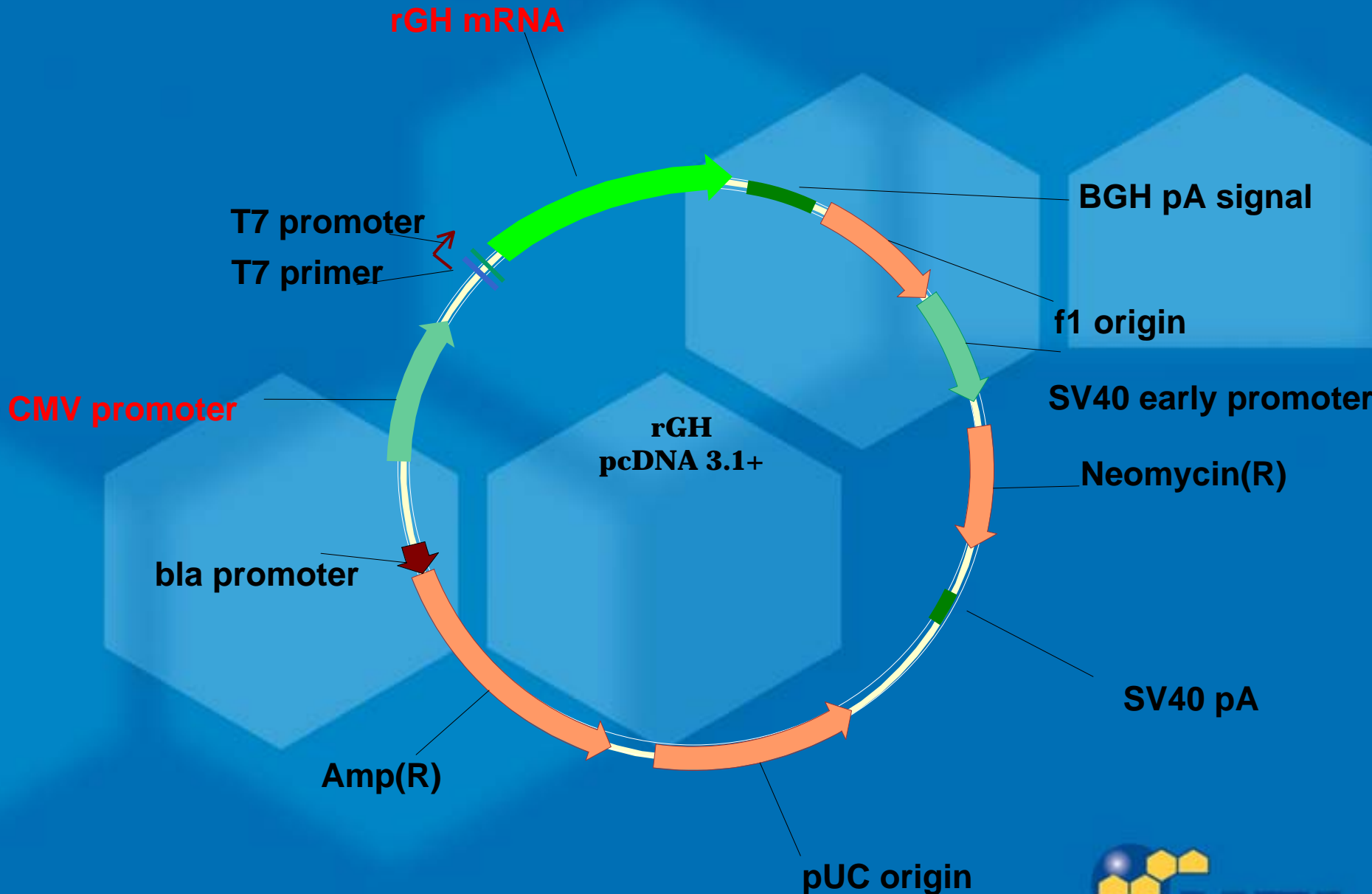
Response profile ion y



Prediction training data (population profile)



GH construct details



Mammalian Expression Vector rGH Construct

GH construct administration

- **3 Cohorts of 50 adult male mice**
 - PBS control
 - + GH construct
 - - GH construct (start codon disrupted)
- **Hind leg injections**
 - 240 ug plasmid
 - 1 in Tibialis Anterior
 - 3 in Gastrocnemius
- **Serum sample analysis**
 - Blood collection after 19 days
 - MALDI and LC-MS-MS
 - ANNs (more statistically valid)



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