

Contract Concept: Bioinformatics Support for DNTP and DIR

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NTP Board of Scientific Counselors Meeting
December 1 - 2, 2015



What is bioinformatics?

- **What?** Computational analysis, biostatistical treatment and visualization of large, multidimensional datasets in multidisciplinary research fields
- **Includes:** Omics technologies, chemistry, HTS, and literature data extraction
- **Innovation:** Datasets from various disciplines require support for adaptation of software tools or innovative procedures for new informatics approaches



Need for bioinformatic support

Large, complex datasets: NIEHS researchers conduct studies producing large amounts of data, varying in size and complexity.

Multidisciplinary data - Fields of scientific study are diverse, require innovative bioinformatics analysis, software tools and data visualization.

Emerging Bioinformatics Needs: NTP leadership decided a new contract was needed to address the growing bioinformatics needs of NTP and DIR and to support the magnitude and range of data and bioinformatics analysis for the studies conducted at NIEHS.

Data Volume and Existing Expertise - More studies and expertise than can be handled by existing bioinformatics support from Biostatistics and Computational Biology Branch to support NIEHS researchers.

Limits of Statistical Support Contract - NTP leadership recognized the informatics component was becoming distinctive from statistical support contract; integration with existing contract support.



General Requirements

- **Bioinformatic services** – independent of the government
- **Report data analysis** – report data analyses to NIEHS groups
- **New bioinformatic** – adapt or create new, bioinformatic procedures and analysis
- **Frequent interaction** – contract officer representative (COR), DNTP/DIR researchers; onsite and electronic



Bioinformatic support for:

- **Omics** – genomics, transcriptomics, proteomics, metabolomics
- **Genomic sequencing**– NextGen data
- **Epigenetics**– DNA methylation; histone modifications
- **Toxicogenomics** – microarray, RNA-seq
- **High Throughput Transcriptomics** – S1500+
- **Cheminformatics** – Tox21 HTS data, SAR, integration
- **Information Mining** – data or text
- **Impact Research** – NTP research products, impact
- **Informatics data extraction** – Tox21 chemical extraction
- **Study Design** – informatics support for study design teams
- **Innovative Bioinformatics** – adaptation of existing and new data pipelines for new bioinformatic approaches for NTP/DIR



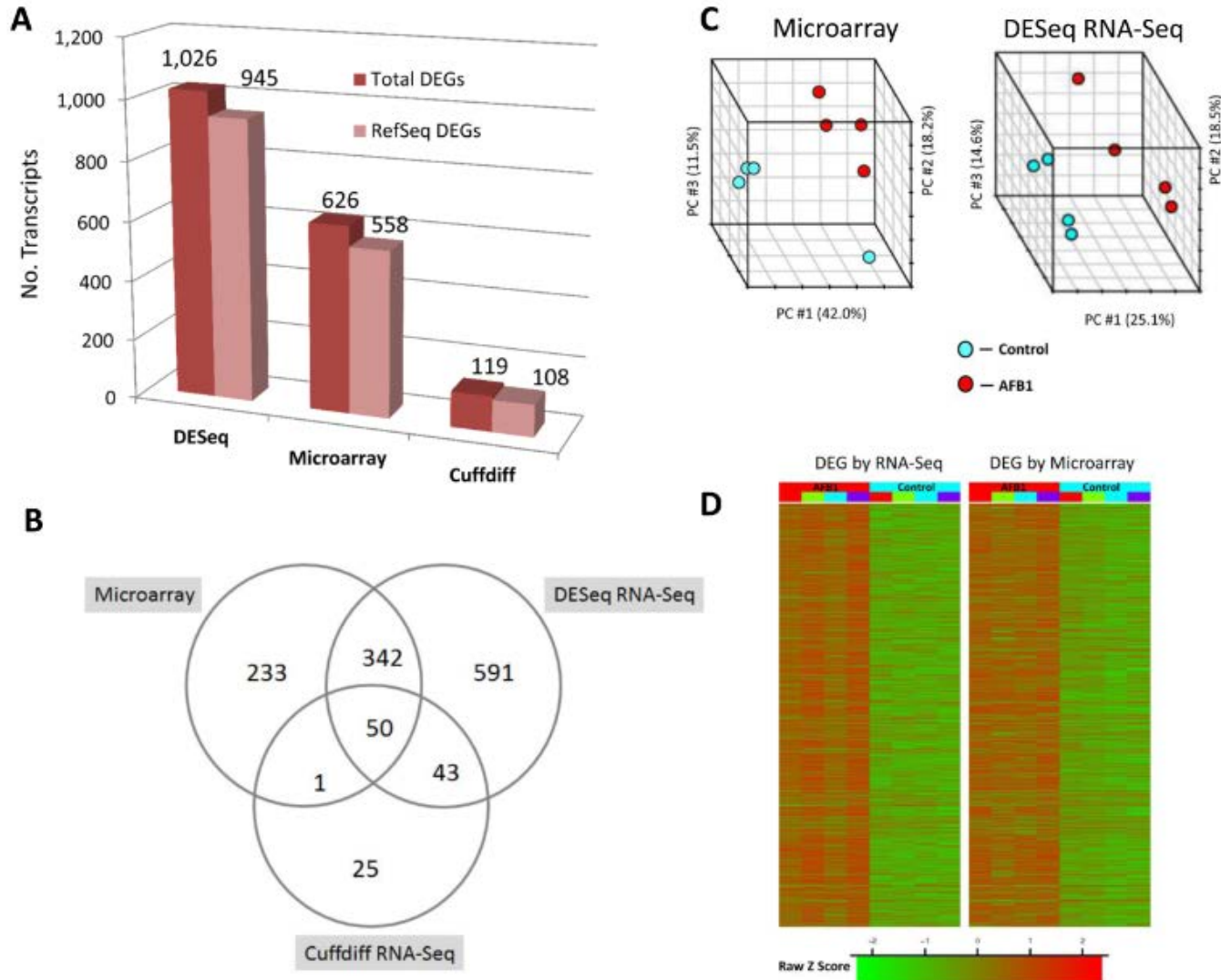
Bioinformatics Needs of Investigators

- **NextGen Sequencing – RNASeq**
- **Tox21 and Chemoinformatics**
- **HTT – High Throughput Transcriptomics** for Tox21 and toxicity screening projects
- Informatics for **systematic literature** reviews
- Informatics for **impact research**



Differentially expressed (DEGs) identified from RNA-Seq

RNA-Seq compared to microarray data





High Throughput Transcriptomics

Gene expression signatures as surrogate markers for chemical toxicity screens – ‘S1500+’

- **Selection of genes** to be used with HTT platform
- **Represent known pathways** - Select highly diverse, correlated and complementary set of genes that fully represent all known pathways
- **Data driven** - use of data to build transcriptome
- **HTT Data** – to recreate transcriptome
- **HTT Chemical Screening** - identify perturbed genes and pathways
- **Informatics** – identify gene modules and pathways impacted by chemical treatment
- **Prioritize** chemicals for further testing
- **Integrate** and analyze data from diverse datastreams

S1500+ details:

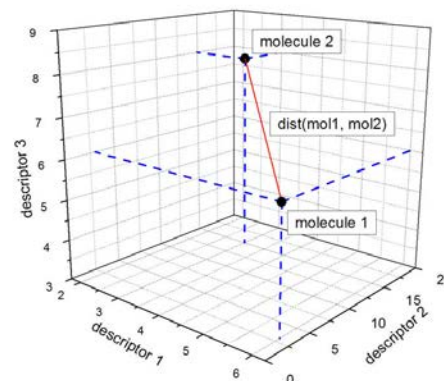
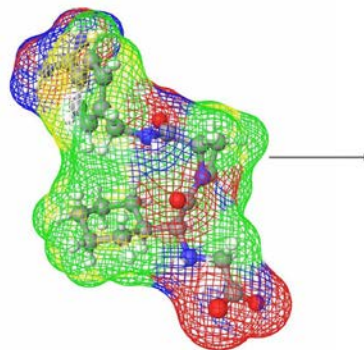
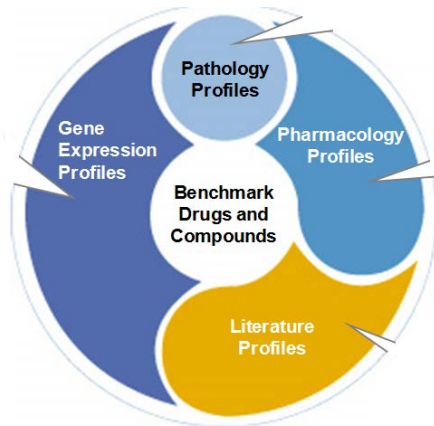
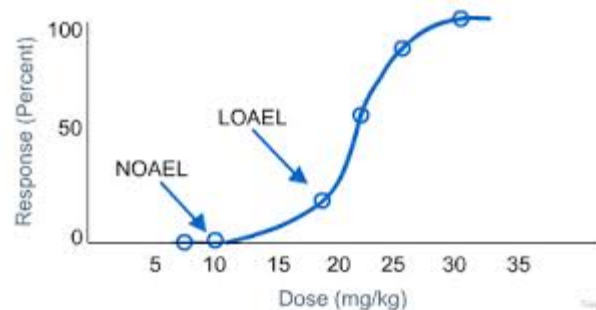
<https://ntp.niehs.nih.gov/results/hts/s1500-gene-set/index.html>





Tox21 and Cheminformatics

- Integration, Curation and Analysis of large volumes of assay, toxicology and animal study data
- Develop visualization tools to view HTS data
- Research and implement curve fit models
- Maintain Drug Matrix database and provide user support
- User of chemical descriptor, QSAR and cheminformatics approaches for chemical toxicity predictions, integrative analysis and chemical prioritization



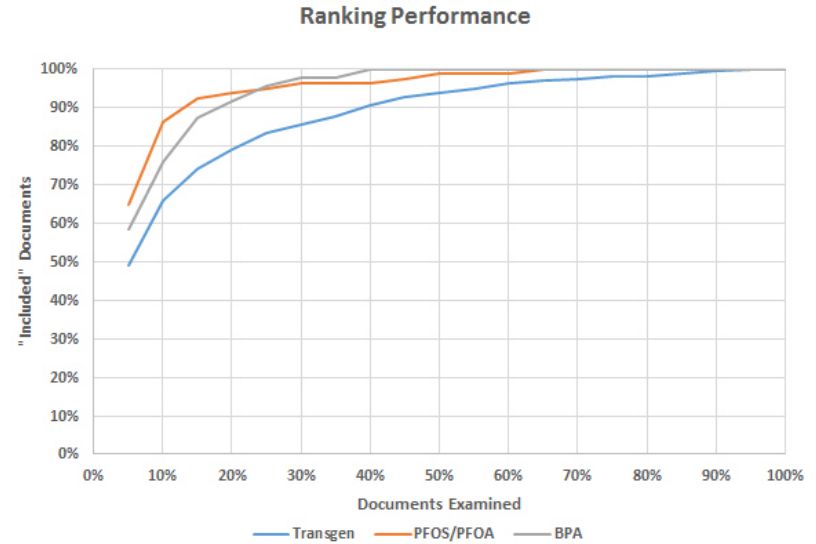
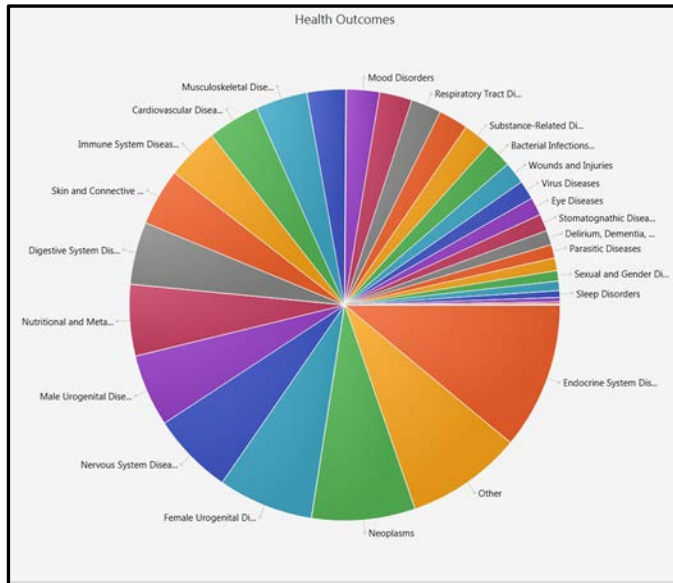
DNTP's *Drug Matrix Database*



Informatics for Systematic Reviews

Informatics capabilities and tools for:

- Scoping Reports
- Problem Formulation
- Topic Refinement
- Document Prioritization





Informatics for Impact Research

- Informatics expertise and tools to support impact research
- Identify and assess impact of NTP research

Methodologies under consideration:

- Bibliometrics: easily search, visualize, tag, organize, screen and research articles
- Web mining: perform extensive web mining using high performance computing and sift through results in rapid manner
- News story: organize and summarize news articles

(Costa, 1995), whereas Cr(III) is not a substrate for transport (Proctor et al., 2002) but is thought to enter cells via diffusion or phagocytosis. Consistent with this mechanism of transport, higher chromium tissue concentrations were observed with Cr(VI) compared with Cr(III) following equivalent exposures in drinking water (Costa, 1997; Costa and Klein, 2000; Mackenzie et al., 1998).

Both extracellular and intracellular reduction of Cr(VI) to Cr(III) occur. As a result of the lower bioavailability of Cr(III), extracellular reduction, primarily in the stomach, has been suggested to be protective against the toxic and carcinogenic effects of Cr(VI) following oral exposure (De Flora, 2000; De Flora et al., 1997, 1999; Pauterbach et al., 2003; Proctor et al., 2002). In contrast, intracellular reduction is thought to be a mechanism of carcinogenesis because of DNA damage that occurs when Cr(VI) is reduced through Cr(VI) and Cr(IV) to Cr(III) (reviewed in O'Brien et al., 2003). Because of its large redox potential, Cr(III) is not expected to oxidize to Cr(VI) in vivo. In summary, absorption and retention of chromium depend on a number of factors: the rate of reduction of Cr(VI) to Cr(III) outside the cells, the pH of the milieu, the rate of transport of Cr(VI) into the cells, the rate of reduction of Cr(VI) to Cr(III) inside the cells, and the rate of diffusion of Cr(III) from the cells (NTP, 2008a).

As part of the NTP chronic oral studies of Cr(VI) (NTP, 2008a; Skala et al., 2008a) and Cr(III) (NTP, 2008b; Skala et al., 2008b), total Cr was measured in selected tissues and excreta of additional groups of male and female mice at selected time points. The objective of these studies was to determine the effect of administered species [Cr(VI) or Cr(III)] on Cr uptake and tissue distribution. Because Cr(VI) is reduced to Cr(III) in vivo, and current analytical procedures do not allow for the speciation of Cr extracted from biological samples, speciation of absorbed Cr(VI) was inferred by comparing tissue uptake of Cr following exposure to Cr(VI) or Cr(III). These are the first studies that provide a comprehensive comparison of chronic toxicity and carcinogenicity with tissue distribution in rats and mice following exposure to Cr(VI) and Cr(III) and may ultimately aid in the extrapolation of the Cr(VI) carcinogenicity data to humans.

MATERIALS AND METHODS

Chemical and Dose Formulations

SOD (CAS 7782-429) was obtained from Aldrich Chemical Company (Milwaukee, WI). The purity was determined using differential scanning calorimetry (DSC) and elemental analysis, gravimetric analysis, and emission spectroscopy. Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used to determine the purity of the SOD. The overall purity of the chemical was 99%. The dose formulations were prepared monthly by mixing Cr(VI) with feed. Homogeneity and stability of the dose formulations were assessed by HPLC with UV detection. Periodic analysis confirmed that all dose formulations were within 20% of the target concentrations. Concentrations of total chromium in vehicles were 0.044 to 0.233 ppm in feed [Cr(III) acetate was added because Cr(III) is thought to be an essential element] and below 0.005 mg/l in tap water.

Animals and Animal Maintenance

The studies were conducted at Southern Research Institute (Birmingham, AL). Male F344/N rats and female B6C3F1 mice were obtained from Taconic Farms (Copenhagen, NY). Rats and mice were quarantined for 21 days (Cr(VI)) or 34 days (SOD) and were 5–6 (Cr(VI)) or 6–7 (SOD) weeks old at the beginning of the studies. Animals were distributed randomly into groups of similar mean body weights and identified by tail tattoos. Rats were housed three to a cage. Mice were housed five to a cage. Feed and tap water were available ad libitum. For the SOD study, feed was irradiated NTP-2000 water. For the Cr(VI) study, feed was irradiated NTP-2000 open formula meal diet. Both feeds were obtained from Ziegler Brothers, Inc. (Gardens, PA). Animals were killed by asphyxiation with CO₂.

Animal care was in accordance with the United States Public Health Service policy on humane care and use of laboratory animals and the Guide for the Care and Use of Laboratory Animals. These studies were conducted in compliance with the Food and Drug Administration Good Laboratory Practice Regulations (21CFR, Part 58).

Study Design

All part of the NTP chronic oral studies of SOD (NTP, 2008a; Skala et al., 2008a) and Cr(VI) (NTP, 2008b; Skala et al., 2008b), additional animals were randomly assigned for measurement of total Cr in selected tissues and excreta. These animals were treated the same as the core study animals used for evaluation of toxicity and carcinogenicity with respect to measure, housing, and handling. SOD was administered in drinking water to groups of 40 male rats and female mice at concentrations of 0, 0.45, 2.25, 11.25, and 56.25 mg/l. Cr(VI) was administered in feed to groups of 30 male rats and female mice at concentrations of 0, 2000, 10,000, and 50,000 ppm. On days 0, 11 and 140 (Cr(VI) and SOD), and on day 140 (SOD) only, up to 10 rats and mice per measure group were removed from treatment and placed in individual metabolism cages for separate collection of urine and feces. Animals were provided undisturbed drinking water or feed ad libitum during this period to allow undisturbed chromium to be excreted. Two collections of urine and feces were made to include the intervals from days 24 and 24 to 48 h; measured values were combined to yield the reported 48-h values. The 48-h without period was based on an elimination half-life of 8–21 h (Ege and van Duyn, 1981; Vandenbrouck et al., 2003). On days 6, 13, and 142 (SOD) and Cr(VI), and on day 171 (SOD) only, at the end of the 48-h period, the animals were anesthetized with CO₂, and blood was taken from the retro-orbital sinus into heparinized tubes. Urine and feces were collected separately. Although the animals were still under anesthesia, the abdominal wall was opened and the area was swabbed. The liver, kidneys, and the stomach (separated into glandular stomach



Contractor Request for Information

Problem and Scope of Work

- Work plan – informatics approach to areas of need
- Informatics resources – pipelines, biocomputational skills

Relevant Organizational Experience/Management

- Bioinformatic project experience
- Management plan
- Facilities and equipment
- Conflict of interest

Quality Assurance

RFI issued on 8-23-2015 – NIHES2015048-RFI



Questions?

