

HUPO PLASMA PROTEOME PROJECT (PPP)

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OVERALL SCIENTIFIC GOALS OF PPP

Comprehensive analysis of plasma protein constituents in normal humans, with large cohorts

Determination of the extent of variation in plasma proteins

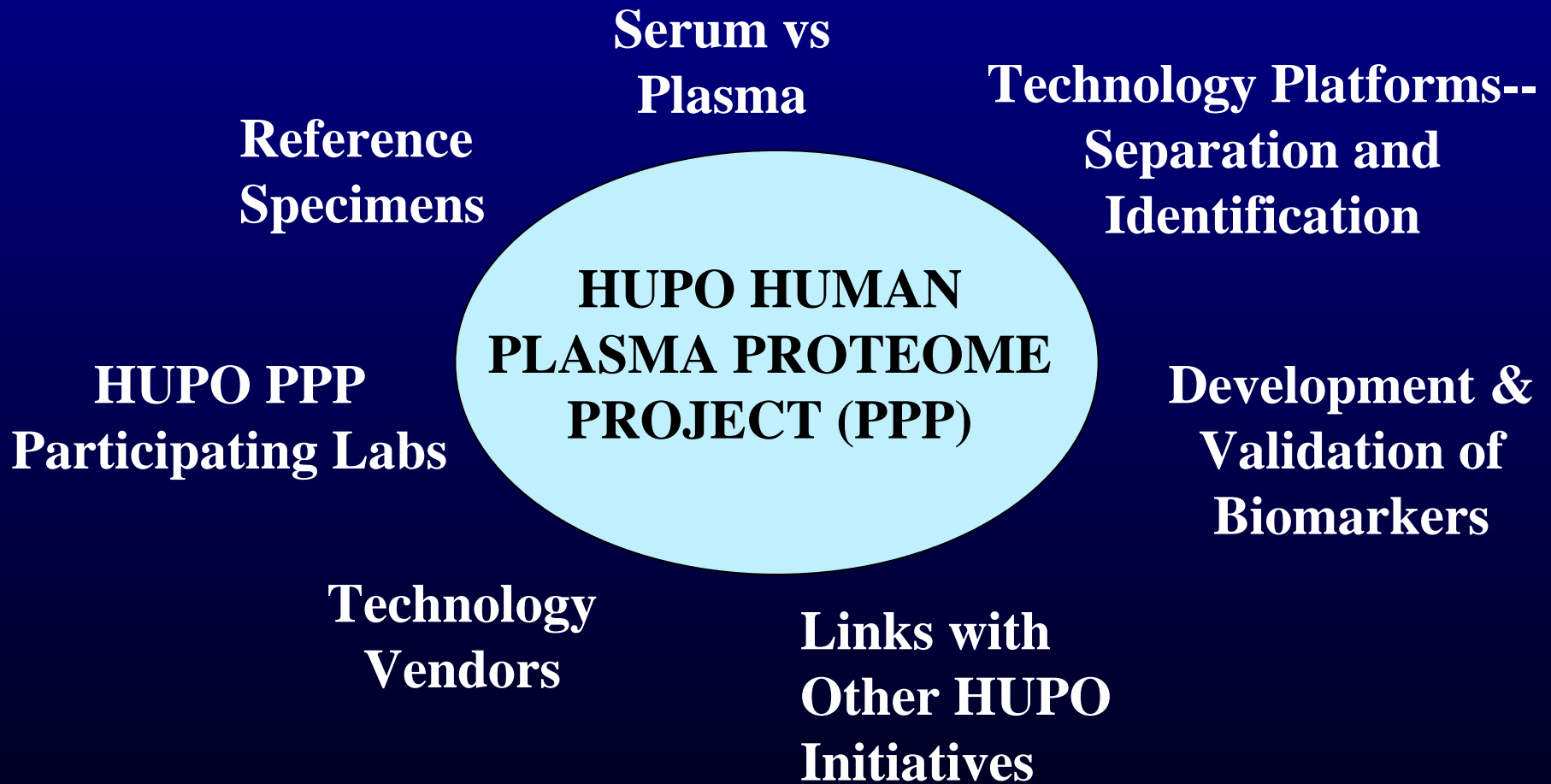
Across populations (around the world)

Within populations (in various countries)

Identification of biological sources of variation within individuals over time, with validation of biomarkers

Age, sex/menstrual cycle, exercise

Common medications, selected diseases



LINKS WITH THE OTHER HUPO WORKING GROUPS

Bioinformatics

Cell & Tissue Models (Liver)

Antibodies

Technology & Resources

MAJOR ACTIVITIES IN VERSAILLES

PPP Progress (Nov, 2002)

ELICITED INTEREST/PROVIDED INFO ABOUT ROLES

**HEARD PROPOSALS FOR REFERENCE SPECIMENS
AND DECIDED WHICH TO DEVELOP**

**INVITED QUESTIONS ABOUT TECHNOLOGY PLATFORMS
AND SENSITIVITY OF DETECTION OF DYNAMIC RANGE**

ELICITED INTEREST FOR COMMITTEES:

Specimens Committee

Technology and Protocol Review/Feedback Committee

Data Management and Bioinformatics Committee

Executive Committee (including corporate & govt partnerships)

CHALLENGES/ISSUES FOR PILOT PHASE

- 1. Sensitivity of various techniques for dynamic range of proteins and of peptides**
- 2. Technical aspects of specimen collection, handling, storage: need to standardize; antiprotease needed?**
- 3. Methods of depleting or fractionating high abundance proteins**
- 4. Comparisons of serum and plasma**
- 5. Enumeration and categorization of proteins: post-translational modifications, tissue of origin**

CHALLENGES/ISSUES (cont'd)

- 6. Separation of protein digests vs proteins**
- 7. Liquid phase multi-dimensional methods vs gel-based methods**
- 8. Parameters for high-throughput link to MS**
- 9. MALDI and SELDI comparisons**
- 10. Various labeling methods for comparison of paired samples**

AIMS FOR PILOT PHASE OF PPP

1. Compare a broad range of technology platforms for the characterization of proteins in human plasma and serum. Assess resolution, sensitivity, time, cost, volumes of sample required, practicality.
2. Clarify influence of various technical variables in specimen collection, handling, and storage; especially anti-coagulation and plasma/serum.
3. Determine whether (and, if so, how) the most abundant plasma proteins should be depleted, and whether anti-protease cocktails are necessary or desirable.

AIMS FOR PILOT PHASE (cont'd)

4. Develop a database structure and repository for HUPO PPP results
5. Establish international collaborations for later-phase characterization of the normal human plasma proteome in the major ethnic groups.
6. Lay groundwork through evaluation of technology platforms and specimen handling for future studies of circulating proteins (biomarkers) in health and disease.

TECHNOLOGY PLATFORMS (HUPO PPP

Sept 2002 Ann Arbor Presentations as Examples)

- Liquid phase separations--Sam Hanash (Michigan)
- Free Flow Electrophoresis--Askar Kuchumov (Tecan Inc)
- Microsol IEF Pre-Fractionation--David Speicher (Penn)
- 2-D LCMS/Automated - Steve Cohen (Waters)
- Systems Approach with Peptide Digest--Karin Rodland (Pacific Northwest National Laboratory, Dept of Energy)
- Swellgels - Walid Qoronfleh (PerBio, Pierce Chemical)
- AntiBio Mix - Moncef Jendoubi (Milagen Inc)
- Chicken IgY Anti-Human Antibodies—Zhang (GenWay)

GENERAL REFERENCE SPECIMEN FREEZE-DRIED PLASMA

**National Inst Biological Standards & Control U.K.
(Drs. Trevor Barrowcliffe & David Wood)**

Reference Specimen for Hemostasis and Thrombosis
(for Intl Soc Thrombosis & Hemostasis/Standards Committee).

Prepared from 25 donors as whole blood anticoagulated with citrate-phosphate-dextrose; double-spun, HEPES added to 0.05M; aliquot tested for HIV, HBV, HCV; 1 ml aliquots in 5000 ampoules frozen at -70°C . Thawed at room temperature to avoid cryoprecipitation, then freeze-dried at -35°C for 4 days and desiccated over P_2O_5 for 6 days, filled with dry N_2 . Tested for longterm stability. Will be assayed for clinical analytes by HUPO.

GENERAL REFERENCE SPECIMEN

with American Red Cross

(Dr. Kenneth Ingham)

Purpose: broadly available reference specimen for comparisons of many different technologies for separation and identification of proteins.

Utilize Red Cross general pool of plasma (citrated) from several thousand donors of blood (or plasma) at various centers, frozen and thawed at plasma processing centers, tested for pathogens, aliquoted to 0.25-0.5-1.0 ml volumes in appropriate tubes, frozen at -80°C , and stored for distribution to HUPO labs. Conduct extensive clinical lab analyses for future use.

[Defer until after Pilot Phase]

SERUM AND PLASMA REFERENCE SPECIMENS

in cooperation with BD Biosciences
(Bruce Haywood & Dr. David Warunek)

1. Recruit and consent equal numbers of healthy, fasting, ethnically diverse male and female donors (volume required to be determined, according to number of participating labs) under an IRB-approved donor Program. [Separate ethnic pools will be prepared.]
2. Draw blood into bags or tubes with appropriate concentrations of K-EDTA, lithium heparin, or sodium citrate for plasma and without clot activator for serum. [Leave anti-protease cocktail to side experiments in individual laboratories.]

3. Centrifuge donor specimens at 2-6C, pool, filter, aliquot, freeze and store at -70C within 60 min of processing.
4. Distribute to each participating HUPO PPP laboratory a set of specimens with 1 ml (in four aliquots) each from Serum Pool and from K₃EDTA, sodium citrate and lithium heparin-anti-coagulated Plasma Pools for use with PPP-approved protocols.
5. Distribute internationally through regional lead sites.

PROPOSAL FROM CHINESE ACADEMY OF MEDICAL SCIENCES (Dr. Xiaohang Zhao)

The Chinese Academy of Medical Sciences will collect a large pool from plasma donors at its hospitals, under procedures agreed upon with the HUPO PPP. Specimens can be prepared also from individuals.

Specimens will be characterized carefully for pathogens and for clinical analytes. Aliquots of the large pool will be made available for distribution to HUPO PPP participating laboratories especially in Asia-Oceania HUPO.

[Plan to prepare identically to BD specimens.]

MOST ABUNDANT PLASMA PROTEINS (mg/ml)

From Chemical Rubber Handbook of Biochemistry 1970, pp C-36-39,
and current data from GenWay, Inc, at (Sept 2002 PPP Workshop)

albumin 35-45

haptoglobin 0.3-2

IgG, IgA, IgM 12-18

alpha-1 acid

fibrinogen 2-6

glycoprotein 1

alpha-1 antitrypsin 2-5

hemopexin 1

alpha-2 macroglobulin 2-4

pre-albumin 0.3-0.4

transferrin 2-3

ceruloplasmin 0.3

alpha-2 + beta-lipoproteins (LDL) 4-7

alpha-lipoproteins (HDL) 0.6-1.5

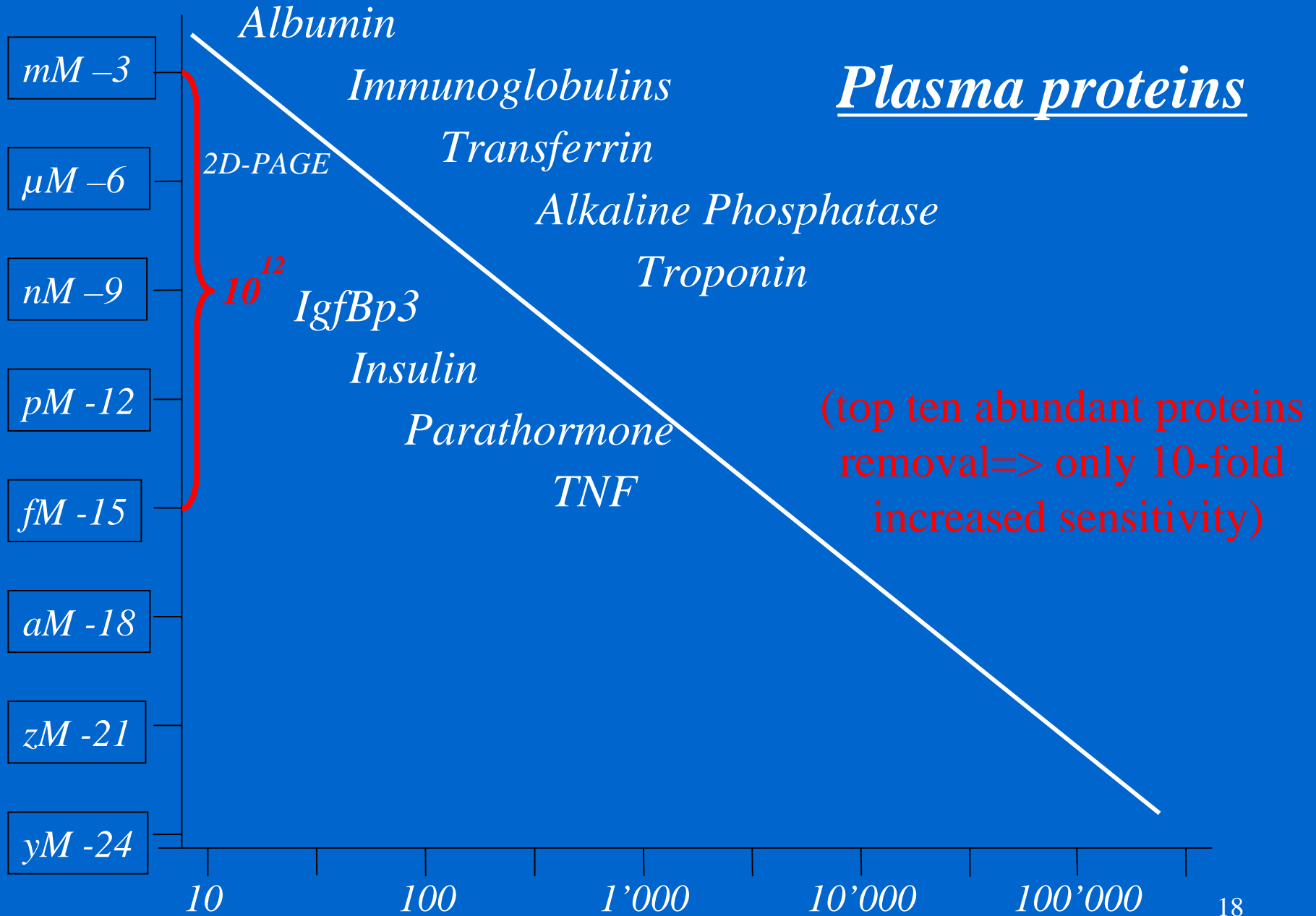
Comments at Versailles:

We face tremendous protein chemical diversity, great protein dynamic changes, and large protein concentration range.

Strategy of GeneProt cardiovascular project: begin with 6L plasma to fractionate and detect low-abundance proteins

Strategy of Pacific NW Lab: begin with peptide digest, identify about 500 proteins.

Beware interferences: plastic filters, even centrifugation, and cold (cryo-release) may activate some proteins; anti-protease cocktails and heparin often interfere with antibody and chip methods.



PPP PILOT PHASE

1. Prepare HUPO PPP reference specimens
2. Define initial roles for All PPP Participating Labs
 - a) Run reference specimen set, as well as any others chosen by the Lab, using each lab's preferred technology platform(s), after review.
 - b) Evaluate depletion of up to 5-10 most abundant proteins versus fractionation of abundant proteins; analyze depletion for additional proteins removed.

PILOT PHASE (cont'd)

3. In subset of labs (with assistance from vendors):
 - a) Run the three alternative plasma preps (EDTA, heparin, citrate) & serum from other ethnic groups
 - b) Compare with vs without anti-protease cocktail and with vs without depletion of albumin or gamma globulins (or both).
 - c) Test technical parameters of sample handling, e.g. duration at various temps before frozen, temp of frozen storage, duration of storage, freeze/thaw (see Chan/Moore, www.hupo.org).

4. Database development

- a) Develop methods for identification, enumeration, and comparison of proteins, accommodating a broad array of technology platforms for separation and then identification, including mass spectrometry and antibody arrays
- b) Assure specimen tracking and methods comparisons
- c) Link with databases of HUPO/PSI, Swissprot, EBI; DOE/PNNL; PIR; others
- d) Build inventory of plasma/serum proteins from HUPO PPP studies

Data Development (cont'd)

- e) Consider system for identifying and classifying related proteins (in cooperation with EBI); an example:
 - 1.0 Primary amino acid backbone sequence of gene-coded protein
 - 1.1 Modification of backbone: cleavage (activation, inactivation)
 - 1.2 Modification of side-chains
 - 1.2.1 Phosphorylation (sites) and dephosphorylation
 - 1.2.2 Glycosylation (sites)
 - 1.2.3 Other classes of post-translation modifications
 - 1.3 Aggregation (dimers, etc)
 - 1.4 Interactions: protein-protein,-nucleic acid,-others

PILOT PHASE (cont'd)

5. Administration and Dissemination

- a) Develop administrative hub (responsibility assigned by HUPO to U. Michigan)
- b) Raise funds for budget: private & public
- c) Clarify intellectual property matters: committed to public domain, with ample opportunity for proprietary development of biomarkers and targets/agents
- d) Link with related HUPO initiatives: liver, PSI, brain

Administration & Dissemination (cont'd)

- e) Organize PP Workshop in 2003 for presentation of results of pilot studies and to determine further work plans (presumably at 2nd World Congress, Montreal, 8-12 October).
- f) Publish presentations with comparisons of samples, of technologies, from different populations around the world, from participating laboratories (already arranged for Special Issue of Proteomics).

PRESENT LIST OF HUPO PPP PARTICIPATING LABS

- Michigan Proteomics Alliance for Cancer Research (Hanash, Omenn, Andrews, Guan at U.Michigan; Pisano at Proteomic Research Services; Haab at Van Andel)
- Johns Hopkins, Baltimore (Chan)
- Wistar/Penn, Philadelphia (Speicher)
- Institute for Systems Biology, Seattle (Aebersold)
- Eastern Virginia Medical School (Semmes)
- Mt. Sinai, New York (Wang)
- Roswell Park, Buffalo (Park)
- Thomas Jefferson, Philadelphia (Steel)
- UCLA (Ping/Loo)
- Stanford (Utz/Robinson)
- Ohio State (Wu)
- Keck Graduate School, Claremont, CA (Chakravarti)
- Wayne State, Detroit (Novak)
- Barnett Institute, Northeastern Univ (Hancock)
- Univ of Texas/Southwestern (Zhao)

Participating Labs, US Govt Agencies

Department of Energy (DOE)

Pacific NW Lab (Rodland, Pounds, Adkins)

Lawrence Livermore Natl Lab (McCutchen-Maloney)

Lawrence Berkeley Lab (Benner)

National Institutes of Health & FDA

NCI/FDA (Petricoin, Liotta, Veenstra)

NIEHS (Merrick, Tomer, Tennant)

NIMH (Merril)

HUPO PPP PARTICIPATING LABS (non-US)

McGill, Montreal, Canada (Bergeron)

Genome British Columbia/Univ of Victoria (Olafson)

German Proteomics Network (Meyer, 5 laboratories)

Institute of Biomedical Chemistry, Russia (Archakov)

University of Southern Denmark, Odensee (Mann)

Geneva Proteomics Centre (Hochstrasser)

Technion-Israel Institute of Technology, Haifa (Admon)

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HUPO Laboratories (non-US) cont'd

Asia-Pacific

Joint Proteomics Center, Melbourne, Australia (Simpson)

Australian Natl Liver Transplant Unit, Sydney (Sun)

Macquarie Univ, Sydney (Plata)

Yonsei Proteome Research Center, Korea (Paik)

Beijing Institute of Radiation Medicine, China (Qian, He)

Chinese Academy of Medical Sciences/PUMC (Zhao)

Genome Institute of Singapore (Leung)

Academia Sinica Taiwan (Wu)

Tokyo Univ of Science (Murakami)

Yamaguchi Univ (Nakamura)

**LIST OF COMPANIES (so far) expressing interest as
participating labs, providers of existing data,
providers of samples and technologies, sponsors**

Amersham	BD Biosciences	Tecan
Johnson&Johnson	Ciphergen	Millipore
Bristol Myers Squibb	Invitrogen	GenWay
Lilly Res Labs	MDS Sciex	Milagen,Chemicon
GlaxoSmithKline	Millenium	Agilent
Pfizer	Pierce Chemical	IBM
Roche	Waters	Confirmant Solutions
Celera	Beckman Coulter	BioVision
Oxford GlycoSciences	Aventis	SynX Pharma

LIST OF U.S. AGENCIES EXPRESSING INTEREST

American Red Cross

**National Institute for Standards &
Technology (NIST)**

**National Institutes of Health (NCI, NINDS,
NHLBI, NIDDK, NIEHS, NIMH, NICHD)**

Dept. of Energy (PNNL, LBL)

Dept. of the Army

Food and Drug Administration

PPP COMMITTEE STRUCTURE

- **Reference Specimens and Specimen Handling Issues**
(Dan Chan, chair)
- **Review of Proposals & Protocols for Evaluating**
Technology Platforms with HUPO PPP Reference
Specimens (Richard Simpson, chair)
- **Database Development and Links with EBI (HUPO/PSI)**
(Henning Hermjakob, chair)
- **Education & Training Committee (Peipei Ping, chair)**
- **Executive Committee (including Partnerships) (Omenn)**

