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SITC/iSBTc Cancer Immunotherapy Biomarkers Resource Document

Online Resources and Useful Tools - A Compass in the Land of Biomarker Discovery

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SITC/iSBTc Cancer Immunotherapy Biomarkers Resource Document: Online resources and useful tools - a compass in the land of biomarker discovery

Bedognetti *et al.*

REVIEW

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SITC/iSBTc Cancer Immunotherapy Biomarkers Resource Document: Online resources and useful tools - a compass in the land of biomarker discovery

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Abstract

Recent positive clinical results in cancer immunotherapy point to the potential of immune-based strategies to provide effective treatment of a variety of cancers. In some patients, the responses to cancer immunotherapy are durable, dramatically extending survival. Extensive research efforts are being made to identify and validate biomarkers that can help identify subsets of cancer patients that will benefit most from these novel immunotherapies. In addition to the clear advantage of such predictive biomarkers, immune biomarkers are playing an important role in the development, clinical evaluation and monitoring of cancer immunotherapies. This Cancer Immunotherapy Resource Document, prepared by the Society for Immunotherapy of Cancer (SITC, formerly the International Society for Biological Therapy of Cancer, iSBTc), provides key references and online resources relevant to the discovery, evaluation and clinical application of immune biomarkers. These key resources were identified by experts in the field who are actively pursuing research in biomarker identification and validation. This organized collection of the most useful references, online resources and tools serves as a compass to guide discovery of biomarkers essential to advancing novel cancer immunotherapies.

Introduction

Immunotherapy has emerged as an important treatment strategy for patients with cancer. With several recent approvals by the U.S. Food and Drug Administration (FDA), cancer immunotherapy has become the latest addition to the toolbox of effective cancer treatments that includes chemotherapy, signal transduction inhibitors, anti-angiogenic agents, radiotherapy, and surgery.

Successful development and testing, regulatory approval and clinical application of cancer immunotherapies require the identification and validation of biomarkers of efficacy. The importance of reliable biomarkers to guide immune-based and personalized cancer therapies is clear. Biomarkers can aid in early disease diagnosis, help clinicians identify patients most likely to benefit from these expensive treatments, and

facilitate drug discovery, development and biological/clinical evaluation of cancer immunotherapies.

For over twenty-five years the Society for Immunotherapy of Cancer (SITC; formerly the International Society for Biological Therapy of Cancer, iSBTc) has advanced the science, development and application of biological therapy/immunotherapy of cancer. The society has long recognized the importance of biomarkers for cancer immunotherapy, which has been the focus of a number of SITC/iSBTc symposia and workshops [1-5], and has published recommendations [6] and summaries [7-10].

To support the efforts of investigators involved in research to identify and validate biomarkers for cancer immunotherapy, the authors and members of the SITC Biomarkers Taskforce have identified key biomarker references and online resources and organized these into this SITC/iSBTc Cancer Immunotherapy Biomarkers Resource Document. This document provides an overview of suggested publications and resources for studies on

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biomarkers for cancer immunotherapy. This resource document is divided into two sections: Part I: Immunotherapy Biomarker References; and Part II: High Throughput and New Technologies for Biomarker Discovery: Arrays, Platforms, Tools for The Bench and Online Resources. While many important references and resources in the field are included in this document, it does not intend to represent an exhaustive list of all relevant publications, products or resources in the growing, and important field of immune biomarkers. A comprehensive list of online tools for bioinformatics and molecular biology research is available from the Bioinformatics Links Directory [11,12].

A draft of the present document was originally provided to attendees of the SITC/iSBTC Symposium on Immuno-Oncology Biomarkers, 2010 and Beyond: Perspectives from the iSBTC Biomarker Task Force [1], which was held September 30, 2010 at the National Institutes of Health in conjunction with the society's 25th Annual Meeting. Following the symposium, the draft document was posted on the society's website for open comment. The comments were reviewed by the authors and incorporated into this manuscript. The references and online resources are organized as outlined in Table 1.

Part I. Immunotherapy Biomarker References

1. Guideline and Meeting Reports

Recommendations from the iSBTC/FDA/NCI Workshop on Immunotherapy Biomarkers

Butterfield LH, Disis ML, Fox BA, et al.: A systematic approach to biomarker discovery; Preamble to "The iSBTC-FDA Taskforce on Immunotherapy Biomarkers". *J Transl Med* 6:81, 2008

Butterfield LH, Palucka AK, Britten CM et al.: Recommendations from the iSBTC-SITC/FDA/NCI Workshop on Immunotherapy Biomarkers, *Clin Cancer Res* 17:3064-3076, 2010

2009 Report of the US - Japan Workshop on Immunological Biomarkers in Oncology

Tahara H, Sato M, Thurin M, et al.: Emerging concepts in biomarker discovery; The US-Japan workshop on immunological molecular markers in oncology. *J Transl Med* 7:45, 2009

2010 Guidelines of the NCI-Investigational Drug Steering Committee (IDSC) Biomarker Task Force

Dancey JE, Dobbin KK, Groshen S, et al.: Guidelines for the development and incorporation of biomarker studies in early clinical trials of novel agents. *Clin Cancer Res* 16:1745-1755, 2010

2010 Report of the Translational Research Cancer Centers Consortium (TrC3) Immunotherapy Network Annual Meeting

Lesinski GB, Carson WE, Repasky EA, et al.: Meeting Report: The 13th Annual Meeting of the Translational

Research Cancer Centers Consortium (TrC3); Immune Suppression and the Tumor Microenvironment, Columbus, Ohio; March 1-2, 2010. *J Immunother*, 2010
2008 Report on EU-USA Workshop on System Biology in Cancer Research

Aebersold R, Auffray C, Baney E, et al.: Report on EU-USA workshop: how systems biology can advance cancer research (27 October 2008). *Mol Oncol* 3:9-17, 2009
2007 AACR-FDA-NCI Cancer Biomarkers Collaborative Consensus Report

Khleif SN, Doroshow JH, Hait WN: AACR-FDA-NCI Cancer Biomarkers Collaborative Consensus Report: Advancing the Use of Biomarkers in Cancer Drug Development. *Clin Cancer Res* 16:3299-3318, 2010

2005 Report on Workshop on Cancer Biometrics Held at NIH Masur Auditorium

(This manuscript also includes a brief description of high throughput technologies)

Lotze MT, Wang E, Marincola FM, et al.: Workshop on cancer biometrics: identifying biomarkers and surrogates of cancer in patients: a meeting held at the Masur Auditorium, National Institutes of Health. *J Immunother* 28:79-119, 2005

2002 Report on Workshop on Immunologic Monitoring of Cancer Vaccine Trials

Keilholz U, Weber J, Finke JH, et al.: Immunologic monitoring of cancer vaccine therapy: results of a workshop sponsored by the Society for Biological Therapy. *J Immunother* 25:97-138, 2002

2. Clinical Trials

A. Recent Immunotherapy Trials in Cancer Patients - Positive Randomized Phase III Studies

Examples

gp100:209-217 (210M) Peptide Followed by High-Dose IL-2 - Metastatic Melanoma Schwartzentruber DJ, Lawson D, Richards J, et al: gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma. *N Engl J Med* 364:2119-27, 2011

Anti-GD2 Antibody with GM-CSF, Interleukin-2, and Isotretinoin - High-Risk Neuroblastoma Yu AL, Gilman AL, Ozkaynak MF, et al.: Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma. *N Engl J Med*. 363:1324-34, 2010

Ipilimumab and Dacarbazine in Metastatic Melanoma Patients - Untreated Metastatic Melanoma Robert C, Thomas L, Bondarenko I, et al.: Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N Engl J Med* 364:2517-26, 2011

Ipilimumab in Metastatic Melanoma Patients - Previously Treated Metastatic Melanoma Hodi FS, O'Day SJ, McDermott DF, et al.: Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 363: 711-23, 2010

Table 1 Structure of the Biomarker References and Online Resources Provided

Part I. Immunotherapy Biomarker References
1. Guideline and Meeting Reports
2. Clinical Trials
A. Recent Immunotherapy Trials in Cancer Patients - Positive Randomized Phase III Studies
B. Emerging Biomarkers for Immunotherapy of Cancer - Clinical Trials
C. Correlation between Immune Response and Clinical Outcome in Cancer Patients; Positive Studies
3. Gene Expression Profiling in Cancer Immunotherapy
4. Useful Reviews
5. Bioinformatics for Biomarkers Discovery
6. New Technologies for Biomarkers Discovery
7. Standardization and Harmonization of Sample Collection and Use of Immunological Assays
A. Processing, Storage and Shipping of Blood Samples and Serum for Immunological Studies - Technical Considerations
B. Cellular Immunotherapy: Characterization of Cellular Products
C. Assay Standardization and Harmonization
D. Assays for Determination of Antitumor Immune-Response in Clinical Trials
8. Reporting Biomarkers Data in Publications
Part II. High Throughput and New Technologies for Biomarker Discovery: Arrays, Platforms, Tools for The Bench and Online Resources
1. Genomic Biomarkers Discovery
A. Single Nucleotide Polymorphisms (SNPs) Arrays
B. Comparative Genomic Hybridization (CGH) Arrays
C. Mitochondrial Genome Arrays
2. Epigenomic Biomarkers Discovery
A. Methylation Arrays
B. microRNA (miRNA) Arrays
C. Chromatin Immunoprecipitation (ChIP) Arrays (ChIP on chip)
3. Transcriptomic Biomarkers Discovery
A. Expression Arrays
B. Quantitative Assays
4. Proteomic Biomarkers Discovery
A. Protein and Phosphoprotein Assays
B. Multicolor Cytometric Systems
5. Next Generation Sequencing: Whole Genomic and Transcriptomic Sequencing Systems
6. Software and Tools for Data Analysis
A. Microarrays/Sequencing Data Analysis
B. Next Generation Sequencing Data Analysis
C. Multicolor Cytometric Data Analysis
7. Software and Tools for Function and Pathway Analysis
8. siRNA Libraries
9. Public Databases
10. Tools for the Bench and Other Useful Websites
A. Primer Design Software
B. Transcription Factors Binding Sites Prediction Software
C. Design of Antisense Oligonucleotides, Nucleic Acid Probes, siRNA Software
D. miRNA Prediction
E. Alternative Splicing Analysis
F. Linkage Disequilibrium Analysis
G. Analysis Support, Laboratory Optimization and Other Useful Websites
H. Nanotechnology
I. Clinical Trials Registries

Sipuleucel-T Immunotherapy - Metastatic Prostate Cancer Kantoff PW, Higoano CS, Shore ND, et al.: Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med* 363:411-422, 2010

Interferon α Adjuvant Treatment - High Risk Melanoma Eggermont AMM, Suciu S, Santinami M, et al.: Adjuvant therapy with pegylated interferon alfa-2b versus observation alone in resected stage III melanoma:

final results of EORTC 18991, a randomised phase III trial. *Lancet* 372:117-126, 2008

Idiotype Vaccine Therapy - Complete Remission Follicular Lymphoma Schuster SJ, Neelapu SS, Gause BL, et al: Vaccination with patient-specific tumor-derived antigen in first remission improves disease-free survival in follicular lymphoma. *J Clin Oncol* 20: 2787-2794, 2011

B. Emerging Biomarkers for Immunotherapy of Cancer - Clinical Trials

Examples

Parameter: HLA Cw*06 Allele

Setting: Interferon α Adjuvant Treatment - High-Risk Melanoma Gogas H, Kirkwood JM, Falk CS, et al.: Correlation of molecular human leukocyte antigen typing and outcome in high-risk melanoma patients receiving adjuvant interferon. *Cancer* 116:4326-33, 2010

Parameter: CCR5 and CXCR3 Polymorphisms and Gene Expression

Setting: Adoptive Therapy - Metastatic Melanoma Bedognetti D, Uccellini L, Wang, et al.: Evaluation of CXCR3 and CCR5 polymorphisms and gene-expression as predictive biomarkers of clinical response to adoptive therapy in melanoma patients. *J Immunother* 33:860, 2010 (Meeting Abstract)

Parameter: Differentiated Effector Phenotype, "B and T Lymphocyte Attenuator" (BTLA) Expression in CD8 + TIL

Setting: Adoptive Therapy - Metastatic Melanoma Laszlo G, Radvanyi, Chantale Bernatchez, Minying Zhang, et al. Adoptive T cell therapy for metastatic melanoma: The MD Anderson experience. *J Immunother* 33:863, 2010 (Meeting Abstract)

Parameter: VEGF Serum Level

Setting: IL-2 Therapy - Metastatic Melanoma Sabatino M, Kim-Schulze S, Panelli MC, et al.: Serum vascular endothelial growth factor and fibronectin predict clinical response to high-dose interleukin-2 therapy. *J Clin Oncol* 27:2645-2652, 2009

Parameter: Treg Numbers

Setting: hTERT Pulsed DCs Therapy - Metastatic Solid Tumors Aloysius MM, Mc Kechnie AJ, Robins RA, et al.: Generation in vivo of peptide-specific cytotoxic T cells and presence of regulatory T cells during vaccination with hTERT (class I and II) peptide-pulsed DCs. *J Transl Med* 7, 2009

Parameter: CD54 Expression by APC

Setting: Sipuleucel-T Cellular Immunotherapy - Metastatic Prostate Cancer Higano CS, Schellhammer PF, Small EJ, et al.: Integrated data from 2 randomized, double-blind, placebo-controlled, phase 3 trials of active cellular immunotherapy with sipuleucel-T in advanced prostate cancer. *Cancer* 115:3670-3679, 2009

Parameter: Delta32 CCR5 Polymorphism

Setting: Immunotherapy (IL2/IFN α \pm Chemotherapy/Vaccination) - Metastatic Melanoma Ugurel S, Schrama D, Keller G, et al.: Impact of the CCR5 gene polymorphism on the survival of metastatic melanoma patients receiving immunotherapy. *Cancer Immunol Immunother* 57:685-691, 2008

Parameter: Telomere Length

Setting: Adoptive Therapy - Metastatic Melanoma Dudley ME, Yang JC, Sherry R, et al.: Adoptive cell therapy for patients with metastatic melanoma: evaluation of intensive myeloablative chemoradiation preparative regimens. *J Clin Oncol* 26:5233-5239, 2008

Parameter: CTLA-4 Polymorphisms

Setting: Anti-CTLA-4 Therapy - Metastatic Melanoma Breunis WB, Tarazona-Santos E, Chen R, et al.: Influence of cytotoxic T lymphocyte-associated antigen 4 (CTLA4) common polymorphisms on outcome in treatment of melanoma patients with CTLA-4 blockade. *J Immunother* 31:586-9, 2008

Parameter: CCL5, CCL11, IFN- γ , ICOS, CD20

Setting: MAGEA3-Based Vaccination - Metastatic Melanoma Louahed J, Gruselle O, Gaulis S, et al.: Expression of defined genes identified by pretreatment tumor profiling: Association with clinical responses to the GSK MAGE-A3 immunotherapeutic in metastatic melanoma patients (EORTC 16032-18031). *J Clin Oncol* 26:9045, 2008 (Meeting Abstract)

Parameter: Fc Gamma Receptor Polymorphisms

Setting: Immuno (Trastuzumab) Chemotherapy - Metastatic Breast Cancer Musolino A, Naldi N, Bortesi B, et al.: Immunoglobulin G fragment C receptor polymorphisms and clinical efficacy of trastuzumab-based therapy in patients with HER-2/neu-positive metastatic breast cancer. *J Clin Oncol* 26:1789-1796, 2008

Parameter: Phosphorylated - STAT-1/STAT-3 Ratio

Setting: Neoadjuvant IFN- α Therapy - Stage IIIB Melanoma Wang WJ, Edington HD, Rao UNM, et al.: Modulation of signal transducers and activators of transcription 1 and 3 signaling in melanoma by high-dose IFN alpha 2b. *Clin Cancer Res* 13:1523-1531, 2007

Parameter: IL 1 β , IL-1 α , IL-6, TNF- α , CCL3, CCL4

Setting: Adjuvant IFN- α Therapy - High Risk Melanoma Yurkovetsky ZR, Kirkwood JM, Edington HD, et al. Multiplex analysis of serum cytokines in melanoma patients treated with interferon-alpha 2b. *Clin Cancer Res* 13:2422-2428, 2007

Parameter: Autoantibodies and Clinical Manifestations of Autoimmunity

Setting: Adjuvant IFN- α Therapy - Melanoma Gogas H, Ioannovich J, Dafni U, et al.: Prognostic significance of autoimmunity during treatment of melanoma with interferon. *N Engl J Med*: 354:709-18, 2006

Parameter: IFN- γ Polymorphism

Setting: Immuno (IL-2) Chemotherapy - Metastatic Melanoma Liu DX, O'Day SJ, Yang DY, et al.: Impact of gene polymorphisms on clinical outcome for stage IV melanoma patients treated with biochemotherapy: An exploratory study. *Clin Cancer Res* 11:1237-1246, 2005

Parameter: IL-6, PPAR γ

Setting: BCG - Bladder Cancer Leibovici D, Grossman HB, Dinney CP, et al.: Polymorphisms in inflammation genes and bladder cancer: From initiation to recurrence, progression, and survival. *J Clin Oncol* 23:5746-5756, 2005

C. Correlation between Immune Response and Clinical Outcome in Cancer Patients; Positive Studies

Examples

Parameter: Melan-A-tetramer+ Immunity

Setting: High Dose Poly-Epitope Vaccine - Metastatic Melanoma Dangoor A, Lorigan P, Keilholz U, et al.: Clinical and immunological responses in metastatic melanoma patients vaccinated with a high-dose poly-epitope vaccine. *Cancer Immunol Immunother* 59:863-73, 2010

Parameter: High Interferon-gamma-associated Proliferative CD4+ T-cell Response and Broad Response of CD8+ Interferon-gamma T Cells

Setting: HPV-16 Vaccine Therapy - Vulvar Intraepithelial Neoplasia Welters MJ, Kenter GG, de Vos van Steenwijk PJ, et al.: Success or failure of vaccination for HPV16-positive vulvar lesions correlates with kinetics and phenotype of induced T-cell responses. *Proc Natl Acad Sci U S A.* 107:11895-9, 2010

Parameter: High Interferon-gamma-associated Proliferative CD4+ T-cell Response and Broad Response of CD8+ Interferon-gamma T Cells

Setting: HPV-16 Vaccine Therap - Vulvar Intraepithelial Neoplasia Kenter GG, Welters MJ, Valentijn AR, et al.: Vaccination against HPV-16 oncoproteins for vulvar intraepithelial neoplasia. *N Engl J Med* 361:1838-47, 2009

Parameter: Tumor Specific Immune Response (ELISPOT)

Setting: Tumor Multi-Epitope Vaccine - Metastatic Melanoma Kirkwood JM, Lee S, Moschos SJ, et al.: Immunogenicity and antitumor effects of vaccination with peptide vaccine +/- granulocyte-monocyte colony-stimulating factor and/or IFN-alpha2b in advanced metastatic melanoma: Eastern Cooperative Oncology Group Phase II Trial E1696. *Clin Canc Res* 15:1443-1451, 2009

Parameter: NY-ESO-1-specific B and T Cell Responses

Setting: Anti CTLA-4 Immunotherapy - Metastatic Melanoma Yuan J, Gnjatich S, Li H, Powel S, et al.: CTLA-4 blockade enhances polyfunctional NY-ESO-1 specific T cell responses in metastatic melanoma patients with clinical benefit. *Proc Natl Acad Sci U S A.* 105:20410-5, 2008

Parameter: Epitope Spreading

Setting: Adenovirus MART-1 Dendritic Cell Vaccination - Metastatic Melanoma Butterfield LH, Comin-Anduix B, Vujanovic L, et al.: Adenovirus MART-1-engineered autologous dendritic cell vaccine for metastatic melanoma. *J immunother* 31:294-309, 2008

Parameter: Epitope Spreading

Setting: Dendritic Cell Based Vaccination - Stage II-IV Melanoma Ribas A, Glaspy JA, Lee Y, et al.: Role of dendritic cell phenotype, determinant spreading, and negative costimulatory blockade in dendritic cell-based melanoma immunotherapy. *J Immunother* 27:354-367, 2004

Parameter: Epitope Spreading

Setting: Dendritic Cell Based Vaccination - Stage III-IV Melanoma Butterfield LH, Ribas A, Dissette VB, et al.: Determinant spreading associated with clinical response in dendritic cell-based immunotherapy for malignant melanoma. *Clin Canc Res* 9:998-1008, 2003

Parameter: Epitope Spreading

Setting: Peptide-Pulsed Dendritic Cells Vaccination - Metastatic Renal Cell Carcinoma Wiernecky J, Müller MR, Wirths S, et al.: Immunologic and clinical responses after vaccinations with peptide-pulsed dendritic cells in metastatic renal cancer patients. *Cancer Res* 66:5910-8, 2006

Parameter: Epitope Spreading

Setting: Her2 Specific Vaccination - Stage III-IV Breast Cancer Salazar LG, Goodell V, O'Meara M, et al.: Persistent immunity and survival after immunization with a HER2/neu (HER2) vaccine. *J Clin Oncol* 27 (15S):2010 (Meeting Abstract)

Parameter: Overall Immunity to MelAgS

Setting: Dendritic Cell Based Vaccination - Metastatic Melanoma Banchereau J, Palucka AK, Dhodapkar M, et al.: Immune and clinical responses in patients with metastatic melanoma to CD34(+) progenitor-derived dendritic cell vaccine. *Cancer Res* 64:51-8, 2001

Parameter: T-cell Response (ELISPOT)

Setting: 12-Peptide Vaccine - Stage IIB-IV - Melanoma Slingluff CL, Jr., Petroni GR, Chianese-Bullock KA, et al.: Immunologic and clinical outcomes of a randomized phase II trial of two multi-peptide vaccines for melanoma in the adjuvant setting. *Clin Canc Res* 13:6386-6395, 2007

Parameter: Tumor specific IFN- γ ELISPOT Response

Setting: Adjuvant Autologous Tumor Lysate Vaccine - Colon Cancer Barth RJ Jr, Fisher DA, Wallace PK, et al.: A randomized trial of ex vivo CD40L activation of a dendritic cell vaccine in colorectal cancer patients: tumor-specific immune responses are associated with improved survival. *Clin Cancer Res* 16:5548-56, 2010

Parameter: Delayed Type IV Hypersensitivity

Setting: Dendritic Cell Vaccine - Stage III-IV Melanoma Patients López MN, Pereda C, Segal G, et al:

Prolonged survival of dendritic cell-vaccinated melanoma patients correlates with tumor-specific delayed type IV hypersensitivity response and reduction of tumor growth factor beta-expressing T cells. *J Clin Oncol* 27:945-52, 2009

Parameter: Delayed Type IV Hypersensitivity

Setting: Adjuvant Autologous Tumor Cell Vaccination - Stage III-IV Melanoma Patients Baars A, Claessen AM, van den Eertwegh AJ et al.: Skin tests predict survival after autologous tumor cell vaccination in metastatic melanoma: experience in 81 patients. *Ann Oncol* 11:965-70, 2000

Parameter: Delayed Type IV Hypersensitivity

Setting: Allogeneic GM-CSF - Stage I-III Pancreatic Cancer Jaffee EM, Hruban RH, Biedrzycki B, et al.: Novel allogeneic granulocyte-macrophage colony-stimulating factor-secreting tumor vaccine for pancreatic cancer: a phase I trial of safety and immune activation. *J Clin Oncol* 19: 45-56, 2009

Parameter: Delayed Type IV Hypersensitivity

Setting: Dendritic Cell Vaccination - Stage IV Melanoma de Vries IJ, Bernsen MR, Lesterhuis WJ, et al.: Immunomonitoring tumor-specific T cells in delayed-type hypersensitivity skin biopsies after dendritic cell vaccination correlates with clinical outcome. *J Clin Oncol* 23:5779-87, 2005

3. Gene Expression Profiling in Cancer Immunotherapy

Examples

Gajewski TF, Fuertes M, Spaapen R et al.: Molecular profiling to identify relevant immune resistance mechanisms in the tumor microenvironment. *Curr Opin Immunol* 23:286-92, 2011

Bedognetti D, Wang E, Sertoli MR, Marincola FM: Gene-expression profiling in vaccine therapy and immunotherapy for cancer. *Expert Rev Vaccines* 6:555-65, 2010

Worschech A, Chen NH, Yu YA, et al.: Systemic treatment of xenografts with vaccinia virus GLV-1h68 reveals the immunologic facet of oncolytic therapy. *BMC Genomics* 10, 2009

Harlin H, Meng Y, Peterson AC, et al.: Chemokine expression in melanoma metastases associated with CD8 (+) T-cell recruitment. *Cancer Res* 69:3077-3085, 2009

Wang E, Miller LD, Ohnmacht GA, et al.: Prospective molecular profiling of melanoma metastases suggests classifiers of immune responsiveness. *Cancer Res* 62:3581-3586, 2002

Panelli MC, Stashower ME, Slade HB, et al.: Sequential gene profiling of basal cell carcinomas treated with imiquimod in a placebo-controlled study defines the requirements for tissue rejection. *Genome Biol* 8:R8, 2007

Sullivan RJ, Hoshida Y, Brunet J, et al.: A single center experience with high-dose (HD) IL-2 treatment for

patients with advanced melanoma and pilot investigation of a novel gene expression signature as a predictor of response. *J Clin Oncol* 27:9003, 2009 (Meeting Abstract)

Gajewski TF, Zha Y, Thurner B, et al.: Association of gene expression profile in metastatic melanoma and survival to a dendritic cell-based vaccine. *J Clin Oncol* 27:9002, 2009 (Meeting Abstract)

Louahed J, Gruselle O, Gaulis S, et al.: Expression of defined genes identified by pretreatment tumor profiling: Association with clinical responses to the GSK MAGE-A3 immunotherapeutic in metastatic melanoma patients (EORTC 16032-18031). *J Clin Oncol* 26:9045, 2008 (Meeting Abstract)

Vansteenkiste JF, Zielinski M, Dahabreh IJ, et al.: Association of gene expression signature and clinical efficacy of MAGE-A3 antigen-specific cancer immunotherapeutic (ASCI) as adjuvant therapy in resected stage IB/II non-small cell lung cancer (NSCLC). *J Clin Oncol* 26:7501, 2008 (Meeting Abstract)

Mandruzzato S, Callegaro A, Turcatel G, et al.: A gene expression signature associated with survival in metastatic melanoma. *J Transl Med.* 4:50, 2006

Panelli MC, Wang E, Phan G, et al.: Gene-expression profiling of the response of peripheral blood mononuclear cells and melanoma metastases to systemic IL-2 administration. *Genome Biol* 3, 2002

4. Useful Reviews

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16. [Epub ahead of print]

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Simon R: Translational research in oncology: key bottlenecks and new paradigms. *Expert Rev Mol Med* 12:e32, 2010

Kirkwood JM, Tarhini AA, Panelli MC, Moschos SJ, Zarour HM, Butterfield LH,

Gogas HJ: Next generation of immunotherapy for melanoma. *J Clin Oncol* 26:3445-55, 2008

Freidlin B, McShane LM, Korn EL: Randomized clinical trials with biomarkers: design issues. *J Natl Cancer Inst* 3;102:152-60, 2011

5. Bioinformatics for Biomarkers Discovery

Zhao Y, Simon R: Development and validation of predictive indices for a continuous outcome using gene expression profiles. *Cancer Inform* 9:105-114, 2010

Chaussabel D: Data management: it starts at the bench. *Nat Immunol.* 10:1225-7, 2009

Chaussabel D, Quinn C, Shen J, et al.: A modular analysis framework for blood genomics studies: Application to systemic lupus erythematosus. *Immunity* 29:150-164, 2008

Bredel M, Scholtens D, Harsh GR, et al.: Model of a cooperative genetic landscape in gliomas. *Neuro-Oncology* 11:606, 2009

Simon R: The use of genomics in clinical trial design. *Clin Cancer Res* 14:5984-5993, 2008

Simon R: Development and validation of biomarker classifiers for treatment selection. *J Stat Plan Infer* 138:308-320, 2008

Nacu S, Critchley-Thorne R, Lee P, et al.: Gene expression network analysis and applications to immunology. *Bioinformatics* 23:850-858, 2007

Dupuy A, Simon RM: Critical review of published microarray studies for cancer outcome and guidelines on statistical analysis and reporting. *J Natl Cancer Inst* 99:147-57, 2007

6. New Technologies for Biomarkers Discovery

Linnarsson S: Recent advances in DNA sequencing methods - general principles of sample preparation. *Exp Cell Res* 316:1339-1343, 2010

Metzker ML: Sequencing technologies - the next generation. *Nat Rev Genet* 11:31-46, 2010

Maecker HT, Nolan GP, Fathman CG: New technologies for autoimmune disease monitoring. *Curr Opin Endocrinol Diabetes Obes* 17:322-328, 2010

Ornatsky O, Bandura D, Baranov V, et al.: Highly multiparametric analysis by mass cytometry. *J Immunol Method* 361:1-20, 2010

Newell EW, Klein LO, Yu W, Davis MM.: Simultaneous detection of many T-cell specificities using combinatorial tetramer staining. *Nat Methods* 6:497-9, 2009

Hadrup SR, Bakker AH, Shu CJ, et al.: Parallel detection of antigen-specific T-cell responses by

multidimensional encoding of MHC multimers. *Nat Methods* 6:520-6, 2009

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Park PJ: ChIP-seq: advantages and challenges of a maturing technology. *Nat Rev Genet* 10:669-680, 2009

Hanash SM, Pitteri SJ, Faca VM: Mining the plasma proteome for cancer biomarkers. *Nature* 452:571-579, 2008

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Gilad Y, Borevitz J: Using DNA microarrays to study natural variation. *Curr Opin Genet Dev* 16:553-558, 2006

Speer R, Wulfschuhle JD, Liotta LA, et al.: Reverse-phase protein microarrays for tissue-based analysis. *Curr Opin Mol Ther* 7:240-245, 2005

Perfetto SP, Chattopadhyay PK, Roederer M: Seventeen-colour flow cytometry: unravelling the immune system. *Nat Rev Immunol* 4:648-655, 2004

Hanash S: Disease proteomics. *Nature* 422:226-232, 2003

7. Standardization and Harmonization of Sample Collection and Use of Immunological Assays

A. Processing, Storage and Shipping of Blood Samples and Serum for Immunological Studies - Technical Considerations Examples

Disis ML, dela Rosa C, Goodell V, et al.: Maximizing the retention of antigen specific lymphocyte function after cryopreservation. *J Immunol Methods* 308:13-18, 2006

Bull M, Lee D, Stucky J, et al.: Defining blood processing parameters for optimal detection of cryopreserved antigen-specific responses for HIV vaccine trials. *J Immunol Methods* 322:57-69, 2007

Kierstead LS, Dubey S, Meyer B, et al.: Enhanced rates and magnitude of immune responses detected against an HIV vaccine: effect of using an optimized process for isolating PBMC. *AIDS Res Human Retroviruses* 23:86-92, 2007

Ruitenbergh JJ, Mulder CB, Maino VC, et al.: VACU-TAINER CPT and Ficoll density gradient separation perform equivalently in maintaining the quality and function of PBMC from HIV seropositive blood samples. *BMC Immunol* 7:11, 2006

Tree TI, Roep BO, Peakman M.: Enhancing the sensitivity of assays to detect T cell reactivity: the effect of cell separation and cryopreservation media. *Ann N Y Acad Sci* 1037:26-32, 2004

Smith JG, Joseph HR, Green T, et al.: Establishing acceptance criteria for cell-mediated immunity assays using frozen peripheral blood mononuclear cells stored under optimal and suboptimal conditions. *Clin Vaccine Immunol* 14:527-37, 2007

McKenna KC, Beatty KM, Vicetti Miguel R, Bilonick RA: Delayed processing of blood increases the frequency of activated CD11b+ CD15+ granulocytes which inhibit T cell function. *J Immunol Method* 341:68-75, 2009

B. Cellular Immunotherapy: Characterization of Cellular Products

Examples

Jin P, Han TH, Ren J, Saunders S, et al.: Molecular signatures of maturing dendritic cells: implications for testing the quality of dendritic cell therapies. *J Transl Med* 8:4, 2010

Sheikh NA, Jones LA: CD54 is a surrogate marker of antigen presenting cell activation. *Immunol Immunother* 57:1381-1390, 2008

Higano CS, Schellhammer PF, Small EJ, et al.: Integrated data from 2 randomized, double-blind, placebo-controlled, phase 3 trials of active cellular immunotherapy with Sipuleucel-T in advanced prostate cancer. *Cancer* 115:3670-3679, 2009

Butterfield LH, Gooding W, Whiteside TL: Development of a potency assay for human dendritic cells: IL-12p70 production. *J Immunother* 31:89-100, 2008

Ayache S, Panelli M, Marincola FM, et al.: Effects of storage time and exogenous protease inhibitors on plasma protein levels. *American Journal of Clinical Pathology* 126:174-184, 2006

C. Assay Standardization and Harmonization

Examples

Clinical Laboratory Improvement Amendments (CLIA) Guideline: <http://www.cms.hhs.gov/CLIA/>:

International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) - common platform of Europe, Japan and United States authorities: <http://www.ich.org/products/guidelines.html>

Attig S, Price L, Janetzki S, Kalos M, Pride M, McNeil L, Clay T, Yuan J, Odunsi K, Hoos A, Romero P, Britten CM, Assay Working Group CC. A critical assessment for the value of markers to gate-out undesired events in HLA-peptide multimer staining protocols. *J Transl Med*, 9:108, 2011

Maecker HT, Hassler J, Payne JK, et al.: Precision and linearity targets for validation of an IFN-gamma ELISPOT, cytokine flow cytometry, and tetramer assay using CMV peptides. *BMC Immunol* 9:9, 2008

Nomura L, Maino VC, Maecker HT: Standardization and optimization of multiparameter intracellular cytokine staining. *Cytometry A* 73:984-991, 2008

Afonso G, Scotto M, Renand A, et al.: Critical parameters in blood processing for T-cell assays: validation on ELISpot and tetramer platforms. *J Immunol Methods* 359:28-36, 2010

Britten CM, Gouttefangeas C, Welters MJ, et al.: The CIMT-monitoring panel: a two-step approach to harmonize the enumeration of antigen-specific CD8+ T lymphocytes by structural and functional assays. *Cancer Immunol Immunother* 57:289-302, 2008

Britten CM, Janetzki S, Ben Porat L, et al.: Harmonization guidelines for HLA-peptide multimer assays derived from results of a large scale international proficiency panel of the Cancer Vaccine Consortium. *Cancer Immunol Immunother* 2009:1701-1713, 2009

Britten CM, Janetzki S, van der Burg SH, et al.: Toward the harmonization of immune monitoring in clinical trials: quo vadis? *Cancer Immunol Immunother* 57:285-288, 2008

Mander A, Gouttefangeas C, Ottensmeier C, et al.: Serum is not required for ex vivo IFN-gamma ELISPOT: a collaborative study of different protocols from the European CIMT Immunoguiding Program. *Cancer Immunol Immunother* 59:619-627

Fahey JL, Aziz N, Spritzler J, et al.: Need for an external proficiency testing program for cytokines, chemokines, and plasma markers of immune activation. *Clin Diagn Lab Immunol* 7:540-548, 2000

Denny TN, Gelman R, Bergeron M, et al.: A North American multilaboratory study of CD4 counts using flow cytometric panLeukogating (PLG): a NIAID-DAIDS Immunology Quality Assessment Program Study. *Cytometry B Clin Cytom* 74 Suppl 1: S52-S64, 2008

Boaz MJ, Hayes P, Tarragona T, et al.: Concordant proficiency in measurement of T-cell immunity in human immunodeficiency virus vaccine clinical trials by

peripheral blood mononuclear cell and enzyme-linked immunospot assays in laboratories from three continents. *Clin Vaccine Immunol* 16:147-155, 2009

Smith SG, Joosten SA, Verscheure V, et al.: Identification of major factors influencing ELISpot-based monitoring of cellular responses to antigens from *Mycobacterium tuberculosis*. *PLoS One* 4:e7972, 2009

Hanekom WA, Dockrell HM, Ottenhoff TH, et al.: Immunological outcomes of new tuberculosis vaccine trials: WHO panel recommendations. *PLoS Med* 5:e145, 2008

Schlott NC, Meierhoff G, Karlsson Faresjö M, et al.: Comparison of cytokine ELISpot assay formats for the detection of islet antigen autoreactive T cells. Report of the third immunology of diabetes society T-cell workshop. *J Autoimmun* 21:365-76, 2003

Moodie Z, Price L, Gouttefangeas C, et al.: Response definition criteria for ELISPOT assays revisited. *Cancer Immunol Immunother* 59:1489-501, 2010

D. Assays for Determination of Antitumor Immune-Response in Clinical Trials

Examples

ELISPOT Quast S, Zhang W, Shive C, et al.: IL-2 absorption affects IFN-gamma and IL-5, but not IL-4 producing memory T cells in double color cytokine ELISPOT assays. *Cell Immunol* 237:28-36, 2005

Granzyme B ELISPOT Shafer-Weaver K, Rosenberg S, Strobl S, et al.: Application of the granzyme B ELISPOT assay for monitoring cancer vaccine trials. *J Immunother* 29:328-335, 2006

Modified ELISPOT Malyguine A, Strobl SL, Shafer-Weaver KA, et al.: A modified human ELISPOT assay to detect specific responses to primary tumor cell targets. *J Trans Med* 2:9, 2004

FACS-based Cytotoxicity Assay Kim GG, Donnenberg VS, Donnenberg AD, et al.: A novel multiparametric flow cytometry-based cytotoxicity assay simultaneously immunophenotypes effector cells: comparisons to a 4 h ⁵¹Cr-release assay. *J Immunol Methods* 325:51-66, 2007

Devevre E, Romero P, Mahnke YD: LiveCount Assay: concomitant measurement of cytolytic activity and phenotypic characterisation of CD8(+) T-cells by flow cytometry. *J Immunol Methods* 311:31-46, 2006

Zaritskaya L, Shafer-Weaver KA, Gregory MK, et al.: Application of a flow cytometric cytotoxicity assay for monitoring cancer vaccine trials. *J Immunother* 32:186-194, 2009

Maecker HT, Dunn HS, Suni MA, et al.: Use of overlapping peptide mixtures as antigens for cytokine flow cytometry. *J Immunol Methods* 255:27-40, 2001

FACS/ELISPOT/TETRAMERS Whiteside TL, Zhao Y, Tsukishiro T, Elder EM, Gooding W, Baar J: Enzyme-linked immunospot, cytokine flow cytometry, and

tetramers in the detection of T-cell responses to a dendritic cell-based multi-peptide vaccine in patients with melanoma. *Clin Cancer Res*, 2003

8. Reporting Biomarkers Data in Publications

Examples

REMARK Recommendations

(accepted by most major journal included those published by the American Society of Clinical Oncology and the American Association for Cancer Research)

McShane LM, Altman DG, Sauerbrei W, et al.: Reporting recommendations for tumor marker prognostic studies (REMARK). *J Natl Canc Inst* 97:1180-1184, 2005

MIBBI Project - Guidelines Promotion

<http://www.mibbi.org>

Taylor CF, Field D, Sansone SA, et al.: Promoting coherent minimum reporting guidelines for biological and biomedical investigations: the MIBBI project. *Nat Biotechnol* 26:889-896, 2008

MIAME - Microarray Experiment

Brazma A, Hingamp P, Quackenbush J, et al.: Minimum information about a microarray experiment (MIAME)-toward standards for microarray data. *Nat Genet* 29:365-371, 2001

MIATA - T Cell Assays

<http://www.miataproject.org>

Janetzki S, Britten CM, Kalos M, et al.: "MIATA"-minimal information about T cell assays. *Immunity* 31:527-528, 2009

Britten CM, Janetzki S, van der Burg SH, et al.: Minimal information about T cell assays: the process of reaching the community of T cell immunologists in cancer and beyond. *Cancer Immunol Immunother* 60:15-22, 2011

MIFlowCyt - Flow Cytometry

Lee JA, Spidlen J, Boyce K, et al.: MIFlowCyt: the minimum information about a Flow Cytometry Experiment. *Cytometry A* 73:926-930, 2008

Data File for Flow Cytometry

Spidlen J, Moore W, Parks D, et al.: Data file standard for flow cytometry, version FCS 3.1. *Cytometry A* 77:97-100, 2010

Part II. High Throughput and New Technologies for Biomarker Discovery: Arrays, Platforms, Tools for The Bench and Online Resources

1. Genomic Biomarkers Discovery

A. Single Nucleotide Polymorphisms (SNPs) Arrays

Examples

Name: *Affymetrix Genome-Wide Human SNP Array 6.0*

Comment: 1.8 million genetic markers, including more than 906,600 single nucleotide polymorphisms (SNPs)

and more than 946,000 probes for the detection of copy number variation

Website:http://www.affymetrix.com/browse/products.jsp?productId=131533&navMode=34000&navAction=jump&aId=productsNav#1_1

Name: *Affymetrix DMET Plus Premier Pack*

Comment: Drug metabolism studies. Coverage of a wide range of genetic variations, including common and rare SNPs, insertions, deletions, tri-alleles, and copy number. 1,936 drug metabolism markers in 225 genes

Website:http://www.affymetrix.com/browse/products.jsp?productId=131412&navMode=34000&navAction=jump&aId=productsNav#1_1

Name: *Illumina Omni Microarray*

Comment: Next generation genome-wide association studies. The Omni family of microarrays will soon allow researchers to assay up to 5 million markers per sample, including comprehensive coverage of both common and rare variants identified by 1000 Genomes Project. As novel SNP sets are released into the public database, researchers using Omni products will have exclusive access to supplemental arrays that build up to the full 5 million variants

Website:<http://www.illumina.com/landing/click/gwas.ilmn?scid=2011110PPC1&gclid=Cjij6ffe26kCFQPc4AodcmxEZQ>

Name: *Fluidigm Dynamic Array for SNP Genotyping*

Comment: The Fluidigm Dynamic Arrays allow you to use your existing TaqMan[®] SNP Genotyping assays in a flexible and cost effective fashion. Each dynamic array allows you to setup up to 9,216 individual TaqMan reactions in a single experiment

Website:http://www.expressionanalysis.com/services/category/fluidigm_dna_focused_set_snp

Name: *TaqMan[®] Pre-Designed SNP Genotyping Assays*

Comment: Includes over 4.5 million SNP assays, including 3.5 million HapMap, and ~70,000 coding SNP assays. This collection now includes ~160,000 validated assays with associated minor allele frequency data available

Website:<https://products.appliedbiosystems.com/ab/en/US/adirect/ab?cmd=catNavigate2&catID=600769&tab=TechSpec>

B. Comparative Genomic Hybridization (CGH) Arrays

Examples

Name: *Agilent Human Genome CGH Microarrays*

Comment: High-resolution tool for genome-wide DNA copy number variation profiling. Comprehensive probe coverage is enhanced with emphasis on known genes, promoters, miRNAs, pseudoautosomal, and telomeric regions

Website:<http://www.genomics.agilent.com/Collection-Subpage.aspx?PageType=Product&SubPageType=ProductDetail&PageID=1463>

Name: *Nimblegen Cytogenetics (CGX) Arrays*

Comment: Genome-wide analysis of DNA copy number changes with a subset of probes focused in disease-associated regions

Website: <http://www.nimblegen.com/products/cgh/human.html#cyto>

C. Mitochondrial Genome Arrays

Example

Name: *Affymetrix GeneChip[®] Human Mitochondrial Resequencing Array 2.0*

Comment: Detection of germ line and heteroplasmic mutations by delivering the complete mitochondrial genome with minimal PCR in only 48 hours

Website:<http://www.affymetrix.com/support/technical/byproduct.aff?product=humitoreseq>

2. Epigenomic Biomarkers Discovery

A. Methylation Arrays

Examples

Name: *Agilent Human DNA Methylation Microarrays*

Comment: The array is specifically designed for analysis of methylated DNA derived from affinity-based isolation methods such as methylated DNA immunoprecipitation (MDIP)

Website:<http://www.genomics.agilent.com/Collection-Subpage.aspx?PageType=Product&SubPageType=ProductDetail&PageID=2157>

Name: *Nimblegen 2.1 M Whole-Genome Tiling Set*

Comment: Whole-genome formats are available in two versions: a 10-array set at 100 bp probe interval or a 4-array set at > 200 bp probe interval

Website:http://www.nimblegen.com/products/methylation/whole_genome.html

Name: *Illumina 450 K Infinium Methylation Bead-Chip Kit*

Comment: This assay allow to interrogate > 450,000 methylation sites per sample at single-nucleotide resolution

Website:http://www.illumina.com/products/methylation_450_beadchip_kits.ilmn

B. microRNA (miRNA) Arrays

Examples

Name: *Illumina MicroRNA Universal Array Matrix*

Comment: miRNA analysis

Website:http://www.illumina.com/products/micro-rna_universal_array_matrix.ilmn

Name: *Affymetrix GeneChip miRNA Array*

Comment: miRNA analysis

Website:<http://www.affymetrix.com/estore/browse/products.jsp?sessionid=7386716AB0BD679701B518044A-DA58F5?navMode=34000&productId=131473&navAction=jump&aId=productsNav>

Name: *Exiqon's miRCURY LNA microRNA Array*

Comment: miRNA analysis

Website:<http://www.exiqon.com/micrna-microarray-analysis>

C. Chromatin Immunoprecipitation (ChIP) Arrays (ChIP on chip)

[ChIP-on-chip (chromatin immunoprecipitation-on-chip), also known as Location Analysis (LA), is a high throughput (genome-wide) identification and analysis of DNA fragments that are bound by specific proteins such as histones, transcriptional factors and polymerases]

Examples

Name: Nimblegen 2.1 M ChIP-Chip Array

Comment:ChIP-chip analysis

Website:<http://www.nimblegen.com/products/chip/index.html>

Name: Agilent SurePrint G3 Human Promoter Microarray

Comment:ChIP-chip analysis

Website:<http://www.genomics.agilent.com/Collection-Subpage.aspx?PageType=Product&SubPageType=ProductDetail&PageID=1487>

Name: Affymetrix GeneChip Tiling Arrays

Comment:ChIP-chip analysis

Website:http://www.affymetrix.com/estore/browse/level_three_category_and_children.jsp?parent=35808&expand=true&category=35818&fromAccordionMenu=true&subCategory=35818

3. Transcriptomic Biomarkers Discovery

A. Expression Arrays

Examples

Name: Affymetrix Gene Chip Human Exon 1.0 ST Array

Comment:Whole genome microarray. With approximately four probes per exon and roughly 40 probes per gene, the GeneChip Human Exon 1.0 ST Array enables two complementary levels of analysis—gene expression and alternative splicing

Website:http://www.affymetrix.com/estore/browse/products.jsp?navMode=34000&productId=131453&navAction=jump&aId=productsNav#1_1

Name: Agilent SurePrint G3 Human GE 8 × 60 K Kit

Comment:Whole genome microarray. The SurePrint G3 Human GE 8 × 60 K Microarrays and Human GE 4 × 44 K v2 Microarrays are based on updated transcriptome databases for mRNA targets, while the SurePrint G3 arrays also include probes for lincRNAs (long intergenic non-coding RNAs). With the combination of mRNA and lincRNAs, it is possible to perform two experiments on a single microarray, confidently predicting lincRNA function

Website:<http://www.genomics.agilent.com/Collection-Subpage.aspx?PageType=Product&SubPageType=ProductDetail&PageID=1515>

Name: Illumina HumanHT-12 v4 Expression BeadChip Kits

Comment:The HumanHT-12 v4 Expression BeadChip provides high throughput processing of 12 samples per BeadChip. Each array on the HumanHT-12 v4 Expression BeadChip targets more than 31,000 annotated genes with more than 47,000 probes

Website:http://www.illumina.com/products/humanht_12_expression_beadchip_kits_v4.ilmn

B. Quantitative Assays

Examples

Name: QuantiGene[®] Plex 2.0 Reagent System (Luminex Assay)

Comment:Quantitatively measure 3-36 RNA targets simultaneously

Website:http://www.panomics.com/index.php?id=product_6

Name: Procarta Transcription Factor Profiling Kits (Luminex Assay)

Comment:Quantitate the DNA binding activity of up to 44 different transcription factors (TFs) in a single well using nuclear extracts or whole cell lysates

Website:http://www.panomics.com/index.php?id=product_17

Name: TaqMan[®] Probe-Based Gene Expression Analysis

Comment:Quantitatively measure 1-384 RNA targets simultaneously

Website:<http://www.appliedbiosystems.com/absite/us/en/home/applications-technologies/qpcr-real-time-pcr/taqman-probe-based-gene-expression-analysis.html>

Name: Fluidigm Dynamic Array for Single Cell Gene Expression

Comment:The Fluidigm Dynamic Array enables you to test up to 96 individual cells against 96 genes in a single experiment. The dynamic array assembles the cDNA material from individual cells and reagents to create individual qPCR reactions

Website:http://www.fluidigm.com/docs/Application_Note_Dynamic_Array_for_Single-Cell_GE_Analysis.pdf

Name: Nanostring nCounter Analysis System for digital gene expression analysis

Comment: The nCounter Analysis System utilizes a novel digital technology that is based on direct multiplexed measurement of gene expression and offers high levels of precision and sensitivity (< 1 copy per cell). The technology uses molecular “barcodes” and single molecule imaging to detect and count hundreds of unique transcripts in a single reaction.

Website: <http://www.nanostring.com/applications/technology/>

4. Proteomic Biomarkers Discovery

A. Protein and Phosphoprotein Assays

Examples

Name: Nodality

Comment:Single-cells network profiling (SCNP): advanced multiparametric quantitative flow cytometry to measure compound effects on multiple signaling cascades in a cell-type-specific manner

Website:<http://www.nodalityinc.com/>

Name: *NanoPro Immunoassay*

Comment:Characterization of proteins in extremely small and precious samples. Unlike traditional protein analysis techniques which require thousands to millions of cells, these assays require as few as 25 cells per assay

Website:<http://www.cellbiosciences.com/nanopro.html>

Name: *Invitrogen Immunoassays*

Comment:Wide range of immunoassays using antibody pairs for single-analyte (ELISA kits) as well as multi-analyte (Lumibex assays) analysis or accurate quantitation of intracellular or extracellular proteins

Website:<http://www.invitrogen.com/site/us/en/home/Products-and-Services/Applications/Cell-and-Tissue-Analysis/Immunoassays.html>

Name: *Procarta Cytokine Profiling Assays (Luminex Assay)*

Comment:Quantitatively measure 3-30 cytokine proteins simultaneously from a variety of matrices including cell culture supernatants, serum or plasma

Website:http://www.panomics.com/index.php?id=products_luminexAssays

Name: *SearchLight Multiplex Immunoassay Kits*

Comment:As many as 16 proteins (4 × 4 array in each well) can be measured per well simultaneously with each 50 µl sample

Website:<http://www.aushon.com/Products-and-Services.php>

Name: *MSD Multiplex Cytokines and Chemokines*

Comment:96-well; multiplex cytokine/chemokine kits for up to ten (10) analytes per well.

384-well: Order multiplex cytokine/chemokine kits for up to four (4) analytes per well

Website:<http://www.mesoscale.com>

Name: *Zeptomark*

Comment:Protein profiling for analysis of cell signaling pathways

Website:<http://www.zeptosens.com/en/>

Name: *Signaling Protein Luminex Assays*

Comment:Multiplex assays for measuring signal transduction, post-translational modification & cleaved proteins in multiple research areas, using Luminex bead-based technology

Website:<http://www.invitrogen.com/site/us/en/home/Products-and-Services/Applications/Cell-and-Tissue-Analysis/Immunoassays/Immunoassays-misc/Multiplex-Bead-Based-Luminex-Technology/Luminex-Assays.html>

Name: *VeraCode Carboxyl Bead Sets and BeadXpress Reader*

Comment:Multiplexed protein arrays (up to 48 immunoassays in a single reaction in a standard 96-well microplate: detection of protein as low as 10 pg/ml)

Website:http://www.illumina.com/products/veracode_carboxyl_bead_sets.ilmn

Name: *Procarta SH2 Domain Plex Profiling Kits (Luminex Assay)*

Comment:Profile phosphotyrosine protein interactions against 30 SH2 binding in a single well using treated and untreated cell lysates. Protein-Protein interaction

Website:http://www.panomics.com/downloads/13_4_SH2UM_2_V1.pdf

Name: *Proto-Array*

Comment:Advanced, high-content, functional protein microarray enables to scan thousands of proteins (> 9000) in as little as one day. Highly sensitive results for protein-protein interaction, kinase substrate identification, and serum profiling studies

Website:<http://www.invitrogen.com/protoarray>

Name: *Metal Nanoparticle Probes and Dynamic Light Scattering*

Comment:This assay is based on the use of gold nanoparticle probes combined with dynamic light scattering (DLS) technique, named nanoDLSAY, a highly sensitive, fast and convenient one-step homogeneous immunoassay for monitoring and detecting biotargets, including cancer biomarkers

Website:<http://tt.research.ucf.edu/LinkClick.aspx?fileticket=6p5A2nGSyZA%3d&tabid=119>

B. Multicolor Cytometric Systems

Examples

Name: *BD LSRFortessa™ Cell Analyzer*

Comment:Up to 4 lasers to detect up to 18 colors simultaneously

Website:<http://wwwbdbiosciences.com/instruments/lsr/index.jsp>

Name: *The MACSQuant® Analyzer*

Comment: Three lasers, up to 8 colors, compact bench-top flow cytometer that is small in size; *MACSQuant® Analyzer* allows researchers to perform sensitive rare cell analysis using magnetic separation

Website:http://www.miltenyibiotec.com/downloads/6760/6764/18602/MQ_brochure.pdf

Name: *CyAn ADP Analyzer*

Comment:Up to 11 standard parameters and 9 colors

Website:<http://www.coulterflow.com/bciflow/instrumentsus.php>

Name: *Partec CyFlow® ML*

Comment:3-laser configuration to detect up to 16 colors

Website:http://www.partec.com/cms/front_content.php?idcat=13

Name: *Accuri C6*

Comment:Standard 2-laser configuration, 4- color system

Website:<http://www.accuricytometers.com/products/>

5. Next Generation Sequencing: Whole Genomic and Transcriptomic Sequencing Systems

Next generation sequencing, possible applications: Whole Genome and Transcriptome Sequencing-Based analysis, Gene Regulation Analysis, SNP Discovery and Structural Variation Analysis, Cytogenetic Analysis, DNA-Protein Interaction Analysis (ChIP-Seq), Sequencing-Based Methylation Analysis, Small RNA Discovery and Analysis

Examples

Name: *Illumina* - several systems, examples: HiSeq 2000 (Sequencing); HiScanSQ (Sequencing + Arrays) and MiSeq

Comment:Next generation sequencing

Website:<http://www.illumina.com/systems.ilmn>

Name: *Roche* - several systems, examples: Genome Sequencer FLX System and GS Junior System (454 Sequencing)

Comment:Next generation sequencing

Website:<http://www.454.com/>

Name: *Applied Biosystems* - several systems, examples: Solid 4 System and Solid PI System (Solid System)

Comment:Next generation sequencing

Website:<http://www.appliedbiosystems.com/absite/us/en/home/applications-technologies/solid-next-generation-sequencing.html>

Name: *Ion Torrent PGM*

Comment:Next generation sequencing: semiconductor technology allows runs in about 2 hours.

Website:<http://www.iontorrent.com/products-ion-pgm/>

Name: *PacBio RS*

Comment:Third generation sequencing: Long read-lengths, single molecule sequencing

Website:<http://www.pacificbiosciences.com/products>

6. Software and Tools for Data Analysis

A. Microarrays/Sequencing Data Analysis

Examples:

Name: *BRB - ArrayTools*

Comment:Microarray/array CGH analysis. BRB-ArrayTools was developed by Dr. Richard Simon and BRB-ArrayTools Development Team, NCI, NIH. The program can be used for non-commercial purposes free-of-charge

Website:<http://linus.nci.nih.gov/pilot/index.htm>

License needed:No

Name: *Partek*

Comment:Next generation sequencing technologies, including gene expression and DGE, RNA-seq and

alternative splicing, copy number and association, ChIP-chip, ChIP-seq, microRNA and SNP association study

Website:<http://www.partek.com/>

License needed:Yes

Name: *Nexus Copy Number*

Comment:aCGH and SNP copy number analysis

Website:<http://www.biodiscovery.com/index/nexus>

License needed:Yes

Name: *Nexus Expression*

Comment:Microarray gene expression analysis

Website:<http://www.biodiscovery.com/index/nexus-expression>

License needed:Yes

Name: *mAdb (aka Mad Bee)*

Comment:Microarray gene expression analysis

Website:<http://madb.nci.nih.gov/>

License needed:No

Name: *Bioconductor*

Comment:Tools for the analysis and comprehension of high-throughput genomic data. Bioconductor uses the R statistical programming language

Website:<http://www.bioconductor.org/>

License needed:No

Name: *MATLAB 7.11*

Comment:MATLAB is a high-level technical computing language and interactive environment for algorithm development, data visualization, data analysis, and numeric computation

Website:<http://www.mathworks.com/products/matlab/description1.html>

License needed:Yes

Name: *GeneSifter[®] Analysis*

Comment:Software microarray and next generation sequencing analysis

Website:http://www.geospiza.com/Contact/genesifter-trial_ng.shtml

License needed:Yes

Name: *ADaCGH*

Comment:Web tool for the analysis of aCGH data sets

Website:<http://adacgh.bioinfo.cnio.es/>

License needed:No

Name: *ArrayStar*

Comment:Gene expression analysis software package that includes visualization tools to help analyze microarray data, including Venn diagrams, a scatter plot, heat maps and line graphs for clustering, and a gene ontology tree

Website:<http://www.dnastar.com/t-products-arraystar.aspx>

License needed:Yes

Name: *GeneSpring GX*

Comment:Statistical tools for fast visualization and analysis of expression and genomic structural variation data

Website:<http://www.chem.agilent.com/en-US/products/software/lifesciencesinformatics/genespringgx/pages/gp34525.aspx>

License needed:Yes

Name: ANAIS

Comment:User-friendly web-based tool for the processing of NimbleGen expression data

Website:<http://anais.versailles.inra.fr/>

License needed:No

Name: JMP

Comment:Software for Microarray, SNP and Proteomics Expression Analysis

Website:http://www.jmp.com/software/webinars/jmp_microarray.shtml

License needed:Yes

Name: SAM: Significance Analysis of Microarrays

Comment:Supervised learning software for genomic expression data mining

Website:<http://www-stat.stanford.edu/~tibs/SAM/>

License needed:No

Name: GenePublisher

Comment:Gene expression analysis

Website:<http://www.cbs.dtu.dk/services/GenePublisher/>

License needed:No

B. Next Generation Sequencing Data Analysis

Blog

Name: SEQanswers

Comment:SEQanswers is a blog founded to be an information resource and user-driven community focused on all aspects of next-generation genomics. A reasonably thorough table of next-gen-seq software available in the commercial and public domain is provided

Website:<http://seqanswers.com/forums/showthread.php?t=43>

Note:

Examples

Name: Cufflinks

Comment:Cufflinks assembles transcripts, estimates their abundances, and tests for differential expression and regulation in RNA-Seq samples. It accepts aligned RNA-Seq reads and assembles the alignments into a parsimonious set of transcripts, free of charge

Website:<http://cufflinks.cbcb.umd.edu/>

License needed:No

Name: Bowtie

Comment:Bowtie is an ultrafast, memory-efficient short read aligner. It aligns short DNA sequences (reads) to the human genome at a rate of over 25 million 35-bp reads per hour. Bowtie indexes the genome with a Burrows-Wheeler index to keep its memory footprint small: typically about 2.2 GB for the human genome (2.9 GB for paired-end).

Website:<http://bowtie-bio.sourceforge.net/index.shtml>

License needed:No

Name: Tophat

Comment:TopHat is a program that aligns RNA-Seq reads to a genome in order to identify exon-exon splice junctions. It is built on the ultrafast short read mapping program Bowtie. TopHat runs on Linux and OS X.

Website:<http://tophat.cbcb.umd.edu/>

License needed:No

Name: Oases

Comment:Oases is a *de novo* transcriptome assembler designed to produce transcripts from short read sequencing technologies, such as Illumina, SOLiD, or 454 in the absence of any genomic assembly

Website:<http://www.ebi.ac.uk/~zerbino/oases/>

License needed:No

Name: Genomatix Genome Analyzer (GGA) and Genomatix Software Suite (GSS)

Comment:GGA comprehensive second-level analysis of Next Generation Sequencing (NGS) data from CHIP-Seq, RNA-Seq or genotyping experiments. GSS conducts a scientific analysis of genomic data in gene regulation, networks, pathways and genome annotation visualization.

Website:<http://www.genomatix.de/en/index.html>

License needed:yes

Name: ALLPATHS-LG

Comment:genome assembly algorithms recommended by the Broad Institute

Website:<http://www.broadinstitute.org/science/programs/genome-biology/computational-rd/computational-research-and-development>

License needed:no

C. Multicolor Cytometric Data-Analysis

Examples

Name: FlowJo

Comment:FlowJo is designed around the structure of flow data and the researcher's experiments. Through FlowJo's patent-pending Groups structure, for example, gates can be applied to many samples as easily as one

Website:<http://www.treestar.com>

License needed:Yes

Name: WinList/FCOM

Comment:Load FCS files from instruments and do FACS data analysis with a full set of region tools and gates. The algorithms allow for rapid generation of registers with frequencies/numbers of all possible suphenotype combinations. Can be used to input data for cluster analysis and heat maps, allowing rapid visualization of numerous complex data sets.

Website:<http://www.vsh.com/>

License needed:Yes

Name: FCSPress

Comment:FCSPress is an easy-to-use Macintosh program that produces presentation quality graphics and generates statistics from flow-cytometric data

Website:<http://www.fcspress.com/>

License needed:Yes

Name: FCS EXpress

Comment:FCS Express is designed to bring the power sophisticated analysis protocols to users in an intuitive, easy to grasp manner

Website:<http://www.denovosoftware.com/>

License needed:Yes

Name: GemStone

Comment:GemStone is software for analysis of high-dimensional, flow cytometry data. Based on patented Probability State Modeling technology, GemStone eliminates some problems that have faced flow cytometry. Subjective gating and associated errors are eliminated. Population overlaps in multidimensional data are accounted for. Multiple samples may be combined into one coherent analysis

Website:<http://www.vsh.com/>

License needed:Yes

Name: SPICE

Comment:SPICE is a data mining software application that analyzes large FLOWJO data sets from polychromatic flow cytometry and organizes the normalized data graphically. SPICE enables users to discover potential correlations in their experimental data within complex data sets.

Website:<http://exon.niaid.nih.gov/spice/>

License needed:Yes

7. Software and Tools for Function and Pathway Analysis

Examples

Name: Ingenuity Pathway Analysis (IPA)

Comment:User friendly software that allows analysis of biological and chemical systems

Website:<http://www.ingenuity.com/>

License needed:Yes

Name: GeneGo MetaCore

Comment:User friendly software that allows analysis of biological and chemical systems

Website:<http://www.genego.com/about.php>

License needed:Yes

Name: Ariadne Pathway Studio

Comment:Analysis of biological system with an interactive software interface and the computational approach to generating database content from the literature

Website:<http://www.ariadnegenomics.com/>

License needed:Yes

Name: DAVID

Comment:Analysis of biological system

Website:http://david.abcc.ncifcrf.gov/content.jsp?file=-functional_annotation.html#intro

License needed:No

Name: KEGG Pathway

Comment:Collection of manually drawn pathway maps representing knowledge on the molecular interaction and reaction networks, free of charge

Website:<http://www.genome.jp/kegg/pathway.html>

License needed:No

Name: BioCarta pathway

Comment:BioCarta pathway provides displays of gene interactions within pathways for human cellular processes, such as apoptosis and signal transduction

Website:http://cgap.nci.nih.gov/Pathways/BioCarta_Pathways

License needed:No

Name: Interactive Genomics Viewer

Comment:The Integrative Genomics Viewer (IGV) is a high-performance visualization tool for interactive exploration of large, integrated datasets. It supports a wide variety of data types including sequence alignments, microarrays, and genomic annotations

Website:<http://www.broadinstitute.org/igv/home>

License needed:No

Name: GSEA

Comment:Gene Set Enrichment Analysis (GSEA) is a computational method that determines whether an a priori defined set of genes shows statistically significant, concordant differences between two biological states (e.g., phenotypes)

Website:<http://www.broadinstitute.org/gsea/>

License needed:No

Name: GOMiner

Comment:A tool for biological interpretation of 'omic' data - including data from gene expression microarrays. Omic experiments often generate lists of dozens or hundreds of genes that differ in expression between samples

Website:<http://discover.nci.nih.gov/gominer/index.jsp>

License needed:No

Name: MatchMiner

Comment:Set of tools that enables the user to translate between disparate ids for the same gene. It uses data from the UCSC, LocusLink, Unigene, OMIM, Affymetrix and Jackson data sources to determine how different ids relate. Supported id types include, gene symbols and names, IMAGE and FISH clones, GenBank accession numbers and UniGene cluster ids

Website:<http://discover.nci.nih.gov/matchminer/index.jsp>

License needed:No

Name: PANTHER Classification System

Comment:Panther is a resource that classifies genes by their functions, using published scientific experimental

evidence and evolutionary relationships to predict function even in the absence of direct experimental evidence. Proteins are also classified

Website:<http://www.pantherdb.org/>

License needed: Yes

8. siRNA Libraries

Examples

Name: *Ambion siRNA Libraries*

Comment: siRNA Libraries

Website:<http://www.ambion.com/techlib/tn/116/11.html>

Name: *Thermo Scientific siRNA Libraries*

Comment: siRNA Libraries

Website:<http://www.dharmacon.com/catalog/catalog-temtemplate.aspx?id=1435&imageid=1882>

Name: *siRNA Libraries*

Comment: siRNA Libraries

Website:<http://genome.duke.edu/cores/rnai/libraries/qiagen/>

9. Public Databases

Name: *NCBI Databases*

Comment: Gene/Protein/SNP/Nucleotide and several other databases

Website:<http://www.ncbi.nlm.nih.gov/gquery/>

Name: *Gene Expression Omnibus (GEO)*

Comment: Public functional genomics data repository supporting MIAME-compliant (Minimum information about a microarray experiment) data submissions. Array- and sequence-based data are accepted. Tools are provided to help users query and download experiments and curated gene expression profiles

Website:<http://www.ncbi.nlm.nih.gov/geo/>

Name: *Catalogue Of Somatic Mutations In Cancer (COSMIC)*

Comment: COSMIC is designed to store and display somatic mutation information and related details and contains information related to human cancers

Website:<http://www.sanger.ac.uk/genetics/CGP/cosmic/>

Name: *The Cancer Genome Atlas (TCGA)*

Comment: The Cancer Genome Atlas (TCGA) Data Portal provides a platform for researchers to search, download, and analyze data sets generated by TCGA. Launched in 2006 as a partnership between the National Cancer Institute and the National Human Genome Research Institute, both NIH components, The Cancer Genome Atlas (TCGA) has developed a comprehensive strategy for comparing the genome of cancer cells to the genome of normal cells from the same patient

Website:<http://cancergenome.nih.gov/>

Name: *The Human Protein Atlas*

Comment: The Human Protein Atlas portal is a publicly available database with millions of high-resolution images showing the spatial distribution of proteins in 46 different normal human tissues and 20 different cancer types, as well as 47 different human cell lines. The data is released together with application-specific validation performed for each antibody. The database was developed in a gene-centric manner with the inclusion of all human genes predicted from genome efforts

Website:<http://www.proteinatlas.org>

Name: DIP (Database of Interacting Proteins)

Comment: The DIP database catalogs experimentally determined interactions between proteins. It combines information from a variety of sources to create a single, consistent set of protein-protein interactions

Website:<http://dip.doe-mbi.ucla.edu/dip/Main.cgi>

Name: MINT (Molecular INTeraction Database)

Comment: MINT focuses on experimentally verified protein-protein interactions mined from the scientific literature by expert curators. The curated data can be analyzed in the context of the high throughput data and viewed graphically with the 'MINT Viewer'

Website:<http://mint.bio.uniroma2.it/mint/Welcome.do>

Name: *Human Protein Reference Database*

Comment: The Human Protein Reference Database represents a centralized platform to visually depict and integrate information pertaining to domain architecture, post-translational modifications, interaction networks and disease association for each protein in the human proteome

Website:<http://www.hprd.org>

Name: *Cancer Genome Anatomy Project (CGAP)*

Comment: The NCI's Cancer Genome Anatomy Project sought to determine the gene expression profiles of normal, precancer, and cancer cells, leading eventually to improved detection, diagnosis, and treatment for the patient. Resources generated by the CGAP initiative are available to the broad cancer community

Website:<http://cgap.nci.nih.gov/cgap.html>

Name: *caBIG*

Comment: caBIG stands for the cancer Biomedical Informatics Grid. caBIG is an information network enabling members of the cancer community - researchers, physicians, and patients - to share data and knowledge

Website:<https://cabig.nci.nih.gov/>

Name: *mirbase*

Comment: Searchable database of published miRNA sequences and annotation

Website:<http://www.mirbase.org>

Name: *deepBase*

Comment: deepBase is a database for annotating and discovering small and long ncRNAs (microRNAs,

siRNAs and piRNAs) from high-throughput deep sequencing data

Website:<http://deepbase.sysu.edu.cn/miRDeep.php>

Name: *Gene Ontology Annotation (UniProtKB-GOA) Database*

Comment:The UniProtKB-GOA project aims to provide high-quality Gene Ontology (GO) annotations to proteins in the UniProt Knowledgebase (UniProtKB) and International Protein Index (IPI) and is a central dataset for other major multi-species databases; such as Ensembl and NCBI

Website:<http://www.ebi.ac.uk/GOA/>

Name: *ProtFun*

Comment:The ProtFun 2.2 server produces *ab initio* predictions of protein function from sequence

Website:<http://www.cbs.dtu.dk/services/ProtFun/>

Name: *TRANSFAC 7.0 Public 2005*

Comment:Data on transcription factors, their experimentally-proven binding sites, and regulated genes. Its broad compilation of binding sites allows the derivation of positional weight matrices

Website:<http://www.gene-regulation.com/pub/databases.html>

Name: *OptiTope*

Comment:OptiTope aims at assisting immunologists in designing epitope-based vaccines. It is an easy-to-use tool to determine a provably optimal set of epitopes with respect to overall immunogenicity in a specific individual or a target population, free of charge

Website:<http://www.epitoolkit.org/optitope>

Name: *Cancer Central Clinical Database (C3D)*

Comment:Cancer Central Clinical Database (C3D) is a clinical trials data management system. C3D collects clinical trial data using standard case report forms (CRFs) based on common data elements (CDEs)

Website:<https://cabig.nci.nih.gov/tools/c3d>

Name: *UCSC Genome Browser*

Comment:Provides a large database of publicly available sequence and annotation data along with an integrated tool set for examining and comparing the genomes of organisms, aligning sequence to genomes, and displaying and sharing users' own annotation data

Website:<http://genome.ucsc.edu/>

Name: *Next Bio*

Comment:Exhaustive collection of public microarray data. NextBio's platform combines powerful tools with unique correlated content. With NextBio you can search tens of thousands of studies containing billions of data points spanning different experimental platforms, organisms and data types

Website:<http://www.nextbio.com/b/corp/faq.nb>

Name: *SYFPEITHI*

Comment:SYFPEITHI is a database comprising more than 7,000 peptide sequences known to bind class I and

class II MHC molecules. The entries are compiled from published reports only

Website:<http://www.syfpeithi.de/>

Name: *Melanoma Molecular Map Project*

Comment:MMMP Databases, putting together the pieces of the melanoma puzzle. Seven interconnected databanks for the interactive collection, update and consultation of the translational and clinical information on melanoma biology and treatment.

Website:<http://www.mmmp.org>

Name: *The Targeted Therapy Database (TTD)*

Comment:Systematic collection of the scientific knowledge regarding the development of targeted therapy for melanoma

Website:<http://www.mmmp.org>

10. Tools for the Bench and Other Useful Websites

A. Primer Design Software

Examples

Name: *Primer3*

Comment:Primer3 is a free online tool to design and analyze primers for PCR and real time PCR experiments. Primer3 can also select single primers for sequencing reactions and can design oligonucleotide hybridization probes

Website:<http://frodo.wi.mit.edu/primer3>

Name: *Oligo Analyzer 3.0*

Comment:Software developed by IDT (Integrated DNA Technologies) that analyzes physical properties of a specific oligo sequence. The results show a complementary sequence, oligo length, GC content, melting temperature, extinction coefficient, molecular weight, $\mu\text{g}/\text{OD}$, and nmoles/OD .

Website:<http://www.idtdna.com/analyzer/applications/oligoanalyzer>

Name: *MethPrimer*

Comment:MethPrimer is a program for designing bisulfite-conversion-based methylation PCR Primers. It can design primers for Methylation-Specific PCR (MSP), Bisulfite-Sequencing PCR (BSP) and Bisulfite-Restriction PCR

Website:<http://www.urogene.org/methprimer/index1.html>

B. Transcription Factors Binding Sites Prediction Software

Examples

Name: *TFSEARCH*

Comment:TFSEARCH predicts transcription factors binding sites from a given sequence. It does simple correlation calculation with binding site profile matrices

Website:<http://www.cbrc.jp/research/db/TFSEARCH.html>

C. Design of Antisense Oligonucleotides, Nucleic Acid Probes, siRNA Software

Name: *Sfold*

Comment:Sfold predicts RNA duplex thermodynamics for rational siRNA design. It supports target accessibility prediction and rational design of antisense oligonucleotides (ASO) and nucleic acid probes. It can design an ASO for a gene of interest based on the mRNA sequence

Website:<http://sfold.wadsworth.org/cgi-bin/soligo.pl>

D. miRNA Prediction

Examples

Name: *TargetScan*

Comment:Prediction of biological targets of miRNAs

Website:<http://www.targetscan.org/>

License needed:No

Name:<http://microRNA.org>

Comment:A resource for predicted microRNA targets and expression, free of charge

Website:<http://www.microrna.org/microrna/home.do>

License needed:No

Name: *DIANA LAB*

Comment:miRNA computational predictive models. Experimental supported databases

Website:<http://diana.cslab.ece.ntua.gr/?sec=home>

License needed:No

Name: *RNA22*

Comment:This software first finds putative microRNA binding sites in the sequence of interest, then identifies the targeting microRNA

Website:<http://cbcsrv.watson.ibm.com/rna22.html>

Name: *PicTar*

Comment:PicTar provides combinatorial microRNA target predictions

Website:<http://pictar.mdc-berlin.de>

Name: *miRANDA*

Comment:miRanda is an algorithm for finding genomic targets for microRNAs

Website:<http://www.microrna.org/microrna/home.do>

E. Alternative Splicing Analysis

Example

Name: *SpliceCenter*

Comment:The SpliceCenter applications are user-friendly tools that provide information on the target location of probesets, primers, or siRNAs within the known splice variants of a gene

Website:<http://www.tigerteamconsulting.com/SpliceCenter/>

License needed:No

F. Linkage Disequilibrium Analysis

Examples

Name: *PhAT*

Comment:It analyzes SNP data, showing pairwise Linkage Disequilibrium of various types (r^2 , r , D' and $\text{abs}(D)$) and producing graphical matrices of obtained results

Website:<http://pharmgat.org/Tools/pbtoldplatform>

G. Analysis Support, Laboratory Optimization and Other Useful Websites

Examples

Name: *Immuneering*

Comment: *Computer Model.* Development of computer models that aim to predict the response to biological therapies in cancer patients

Website:<http://www.immuneering.com/>

Name: *Cellumen CellCiphr Patient Sample Profiling*

Comment:To use the cellular systems biology approach to improve patient stratification for clinical trial enrollments. Cellumen is collaborating with the Mayo Clinic and Foundation to create panels of cellular biomarkers using multiplexed fluorescence to apply to patient cells and tissues, starting with breast cancer

Website:<http://www.cellumen.com/solutions/patient.php>

Name: *Biotracker*

Comment: *Lab Management.* Biotracker is a specialty Lab Information Management Solution (LIMS) for enhancing productivity and effectiveness in life sciences research laboratories

Website:<http://www.ocimumbio.com/lims2/products/>

Name: *Gene's Logic Expression Array Analysis*

Comment:Array analysis support; team dedicated to statistical and bioinformatic analyses of microarray data, ranging from basic quality control and differential gene expression analysis

Website:<http://www.genelogic.com/services/bioinformatic-analysis>

Name: *Microsoft Word Add-In for the GenePattern Reproducible Research Document (GRRD)*

Comment:To facilitate publishing reproducible results, GenePattern automatically captures the history of any computational work being done, allowing scientists to easily generate pipelines to reproduce computational methods

Website:<http://www.broadinstitute.org/cancer/software/genepattern/grrd/AddIn.html>

License needed:No

Name: *IUBio Archive*

Comment:Archive of biology data and software, established in 1989 to promote public access to freely available information, primarily in the field of molecular biology and bioinformatics

Website:<http://iubio.bio.indiana.edu/>

Name: *Labome*

Comment:Tools for searching antibodies, siRNA/shRNA, ELISA, cDNA clones, proteins/peptides, microRNA, and biochemicals from all suppliers

Website:<http://www.labome.com/>

License needed:No

H. Nanotechnology

Examples

Name: *Inno.CNT*

Comment: The Inno.CNT website gives an updated and wide overview of the research status of carbon nanotubes as one of the most promising nanomaterial to open up completely new dimensions in biomedical applications

Website: <http://www.inno-cnt.de/en/uebercnt.php>

Name: *ObservatoryNano*

Comment: The ObservatoryNano provides wide-ranging analysis focusing on nanoscience and nanotechnology developments

Website: <http://www.observatory-nano.eu/project/>

Name: *Nanofutures*

Comment: Nanofutures is an integration platform aimed at becoming a long-lasting nanotechnology hub, connecting the most relevant nanotechnology stakeholders

Website: <http://www.nanofutures.eu/>

Name: *Nanotechnology Medicine*

Comment: Nanotechnology Medicine brings you informative nanomedicine articles, educational nanomedicine videos, and lively nanomedicine dialogue and chatter.

Website: <http://nanotechnologymedicine.org/>

I. Clinical Trials Registries

Example

Name: *Clinical Trial Registries*

Comment: The five clinical trials registries approved by the International Committee of Medical Journal Editors (ICMJE)

Website: <http://ClinicalTrials.gov>, <http://www.actr.org.au>, <http://www.ISRCTN.org>, <http://www.umin.ac.jp/ctr/index/htm>, <http://www.trialregister.nl>

Conclusion

Immune biomarkers are playing an increasingly important role in the successful development, clinical evaluation, and immune monitoring of cancer immunotherapies. The references, products and online resources in this Cancer Immunotherapy Biomarkers Resource Document were identified by the authors and the SITC/iSBTC Taskforce on Immunotherapy Biomarkers to support the discovery, evaluation and application of biomarkers for cancer immunotherapy. These selected references and links serve as a compass to point investigators to useful resources in this ever growing, and important field of cancer immunotherapy biomarkers. Emerging issues surrounding cancer immunotherapy biomarker discovery and clinical application will continue to be addressed in upcoming SITC Annual Meetings and Associated Programs [13].

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Authors' contributions

DB and JB prepared the manuscript. EW, MLD, CMB, LGD, ST, BAF, TFG, FMM and LHB provided substantive editing and critical review. All authors read and approved the final manuscript.

Competing interests

DB, JB, EW, MLD, CMB, LGD, ST, TFG, LHB and FFM declare that they have no competing interests. BAF is co-founder of UbiVac, LLC and serves on the scientific advisory boards of Micromet, Inc. and MannKind Corporation.

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