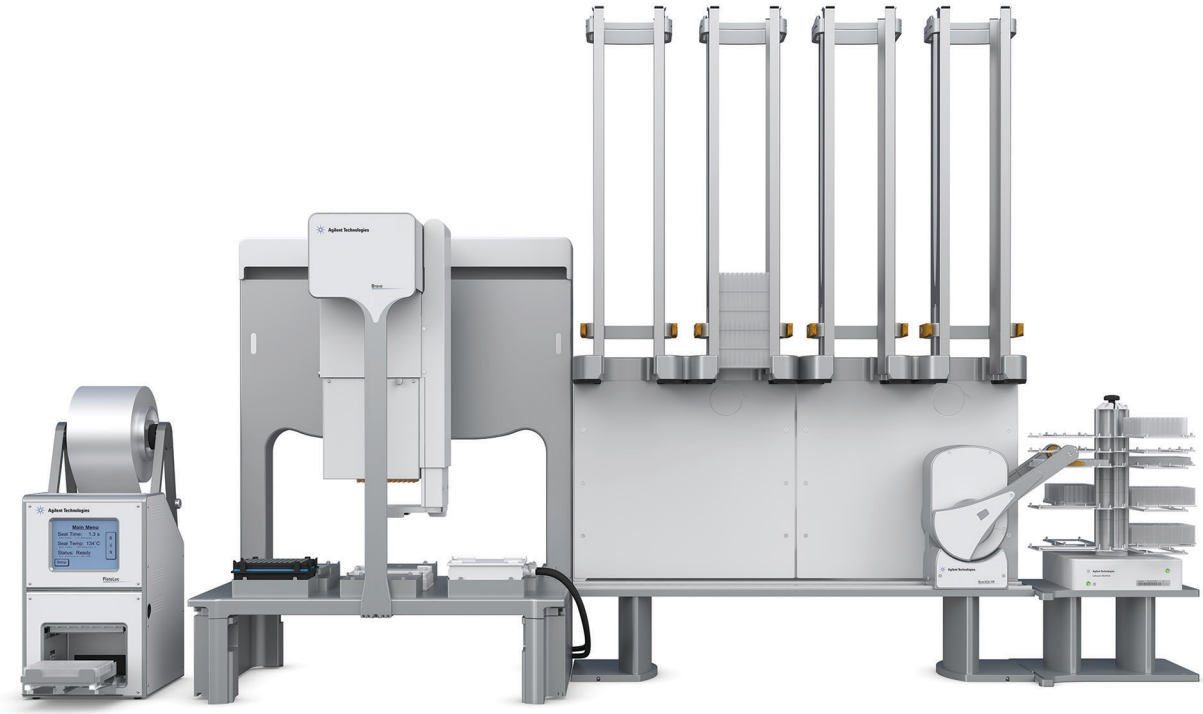
Agilent

Trusted Answers

Agilent NGS Automation

Citation Index



Agilent NGS Automation

In this Citation Index, you'll gain fast access to the bibliographic information you need to research emerging data, review trends and stay up-to-date with the latest **Agilent NGS Automation** application being performed by genomic researchers around the world. It contains a variety of research areas that are utilizing the value of automation to increase throughput and maintain consistent data quality.

Cost-effective, high-throughput DNA sequencing libraries for multiplexed targetcapture

Nadin Rohland, David Reich

Genome Research, Volume 22, Pages 939-946, 2012

Improvements in technology have reduced the cost of DNA sequencing to the point that the limiting factor for many experiments is the time and reagent cost of sample preparation. We present an approach in which 192 sequencing libraries can be produced in a single day of technician time at a cost of about $15 per sample. These libraries are effective not only for low-pass whole-genome sequencing, but also for simultaneously enriching them in pools of approximately 100 individually barcoded samples for a subset of the genome without substantial loss in efficiency of target capture. We illustrate the power and effectiveness of this approach on about 2000 samples from a prostate cancer study.

Pre-capture multiplexing improves efficiency and cost-effectiveness of targeted genomic enrichment

A Eliot Shearer, Michael S Hildebrand, Harini Ravi, Swati Joshi, Angelica C Guiffre, Barbara Novak, Scott Happe, Emily M LeProust, Richard JH Smith

BMC Genomics, Volume 13, Page 618, 2012

Targeted genomic enrichment (TGE) is a widely used method for isolating and enriching specific genomic regions prior to massively parallel sequencing. To make effective use of sequencer output, barcoding and sample pooling (multiplexing) after TGE and prior to sequencing (post-capture multiplexing) has become routine. While previous reports have indicated that multiplexing prior to capture (pre-capture multiplexing) is feasible, no thorough examination of the effect of this method has been completed on a large number of samples. Here we compare standard post-capture TGE to two levels of pre-capture multiplexing: 12 or 16 samples per pool. We evaluated these methods using standard TGE metrics and determined the ability to identify several classes of genetic mutations in three sets of 96 samples, including 48 controls. Our overall goal was to maximize cost reduction and minimize experimental time while maintaining a high percentage of reads on target and a high depth of coverage at thresholds required for variant detection.

A High-Throughput Chromatin Immunoprecipitation Approach Reveals Principles of Dynamic Gene Regulation in Mammals

Manuel Garber, Nir Yosef, Alon Goren, Raktima Raychowdhury, Anne Thielke, Mitchell Guttman, James Robinson, Brian Minie, Nicolas Chevrier, Zohar Itzhaki, Ronnie Blecher-Gonen, Chamutal Bornstein, Daniela Amann-Zalcenstein, Assaf Weiner, Dennis Friedrich, James Meldrim, Oren Ram, Christine Cheng, Andreas Gnirke, Sheila Fisher, Nir Friedman, Bang Wong, Bradley E. Bernstein, Chad Nusbaum, Nir Hacohen, Aviv Regev, Ido Amit

Molecular Cell, Volume 47, Pages 810-822, 2012

Understanding the principles governing mammalian gene regulation has been hampered by the difficulty in measuring in vivo binding dynamics of large numbers of transcription factors (TF) to DNA. Here, we develop a high-throughput Chromatin ImmunoPrecipitation (HT-ChIP) method to systematically map protein-DNA interactions. HT-ChIP was applied to define the dynamics of DNA binding by 25 TFs and 4 chromatin marks at 4 time-points following pathogen stimulus of dendritic cells. Analyzing over 180,000 TF-DNA interactions we find that TFs vary substantially in their temporal binding landscapes. This data suggests a model for transcription regulation whereby TF networks are hierarchically organized into cell differentiation factors, factors that bind targets prior to stimulus to prime them for induction, and factors that regulate specific gene programs. Overlaying HT-ChIP data on gene-expression dynamics shows that many TF-DNA interactions are established prior to the stimuli, predominantly at immediate-early genes, and identified specific TF ensembles that coordinately regulate gene-induction.

A scalable, fully automated process for construction of sequence-ready human exome targeted capture libraries

Sheila Fisher, Andrew Barry, Justin Abreu, Brian Minie, Jillian Nolan, Toni M Delorey, Geneva Young, Timothy J Fennell, Alexander Allen, Lauren Ambrogio, Aaron M Berlin, Brendan Blumenstiel, Kristian Cibulskis, Dennis Friedrich, Ryan Johnson, Frank Juhn, Brian Reilly, Ramy Shammas, John Stalker, Sean M Sykes, Jon Thompson, John Walsh, Andrew Zimmer, Zac Zwirko, Stacey Gabriel, Robert Nicol and Chad Nusbaum

Genome Biology, Volume 12, Page R1, 2011

Genome targeting methods enable cost-effective capture of specific subsets of the genome for sequencing. We present here an automated, highly scalable method for carrying out the Solution Hybrid Selection capture approach that provides a dramatic increase in scale and throughput of sequence-readylibraries produced. Significant process improvements and a series of in-process quality control checkpoints are also added. These process improvements can also be used in a manual version of the protocol.

AHT-ChIP-seq: a completely automated robotic protocol for high-throughput chromatin immunoprecipitation

Sarah Aldridge, Stephen Watt, Michael A Quail, Tim Rayner, Margus Lukk, Michael F Bimson, Daniel Gaffney and Duncan T Odom

Genome Biology, Volume 14, Page R124, 2013

ChIP-seq is an established manually-performed method for identifying DNA-protein interactions genome-wide. Here, we describe a protocol for automated high-throughput (AHT) ChIP-seq. To demonstrate the quality of data obtained using AHT-ChIP-seq, we applied it to five proteins in mouse livers using a single 96-well plate, demonstrating an extremely high degree of qualitative and quantitative reproducibility among biological and technical replicates. We estimated the optimum and minimum recommended cell numbers required to perform AHT-ChIP-seq by running an additional plate using HepG2 and MCF7 cells. With this protocol, commercially available robotics can perform four hundred experiments in five days.

The Genetic Landscape of Clinical Resistance to RAF Inhibition in Metastatic Melanoma

Eliezer M. Van Allen, Nikhil Wagle, Antje Sucker, Daniel J. Treacy, Cory M. Johannessen, Eva M. Goetz, Chelsea S. Place, Amaro Taylor-Weiner, Steven Whittaker, Gregory V. Kryukov, Eran Hodis, Mara Rosenberg, Aaron McKenna, Kristian Cibulskis, Deborah Farlow, Lisa Zimmer, Uwe Hillen, Ralf Gutzmer, Simone M. Goldinger, Selma Ugurel, Helen J. Gogas, Friederike Egberts, Carola Berking, Uwe Trefzer, Carmen Loquai, Benjamin Weide, Jessica C. Hassel, Stacey B. Gabriel, Scott L. Carter, Gad Getz, Levi A. Garraway and Dirk Schadendorf

Cancer Discovery, Volume 4, Page 94-109, 2014

Most patients with BRAF(V600)-mutant metastatic melanoma develop resistance to selective RAF kinase inhibitors. The spectrum of clinical genetic resistance mechanisms to RAF inhibitors and options for salvage therapy are incompletely understood. We performed whole-exome sequencing on formalin-fixed, paraffin-embedded tumors from 45 patients with BRAF(V600)-mutant metastatic melanoma who received vemurafenib or dabrafenib monotherapy. Genetic alterations in known or putative RAF inhibitor resistancegenes were observed in 23 of 45 patients (51%). Besides previously characterized alterations, we discovered a “long tail” of new mitogen-activated protein kinase (MAPK) pathway alterations (MAP2K2, MITF) that confer RAF inhibitor resistance. In three cases, multiple resistance gene alterations were observed within the same tumor biopsy. Overall, RAF inhibitor therapy leads to diverse clinical geneticresistance mechanisms, mostly involving MAPK pathway reactivation. Novel therapeutic combinations may be needed to achieve durable clinical control of BRAF(V600)-mutant melanoma. Integrating clinicalgenomics with preclinical screens may model subsequent resistance studies.

Automation of PacBio SMRTbell NGS library preparation for bacterial genome sequencing

Nguyet Kong, Whitney Ng, Kao Thao, Regina Agulto, Allison Weis, Kristi Spittle Kim, Jonas Korlach, Luke Hickey, Lenore Kelly, Stephen Lappin and Bart C. Weimer

Standards in Genomic Sciences, Volume 12, Page 27, 2017

BACKGROUND: The PacBio RS II provides for single molecule, real-time DNA technology to sequence genomes and detect DNA modifications. The starting point for high-quality sequence production is high molecular weight genomic DNA. To automate the library preparation process, there must be high-throughput methods in place to assess the genomic DNA, to ensure the size and amounts of the sheared DNA fragments and final library.

FINDINGS: The library construction automation was accomplished using the Agilent NGS workstation with Bravo accessories for heating, shaking, cooling, and magnetic bead manipulations for template purification. The quality control methods from gDNA input to final library using the Agilent Bioanalyzer System and Agilent TapeStation System were evaluated.

CONCLUSIONS: Automated protocols of PacBio 10 kb library preparation produced libraries with similar technical performance to those generated manually. The TapeStation System proved to be a reliable method that could be used in a 96-well plate format to QC the DNA equivalent to the standard Bioanalyzer System results. The DNA Integrity Number that is calculated in the TapeStation System software upon analysis of genomic DNA is quite helpful to assure that the starting genomic DNA is not degraded. In this respect, the gDNA assay on the TapeStation System is preferable to the DNA 12000 assay on the Bioanalyzer System, which cannot run genomic DNA, nor can the Bioanalyzer work directly from the 96-well plates.

Efficient method to optimize antibodies using avian leukosis virus display and eukaryotic cells

Changming Yua, Gennett M. Pikea, Tommy A. Rinkoskia, Cristina Correiab, Scott H. Kaufmannb, and Mark J. Federspiel

Proceedings of the National Academy of Sciences of the United States of America, Volume 112, Pages 9860-9865, 2015

Antibody-based therapeutics have now had success in the clinic. The affinity and specificity of the antibody for the target ligand determines the specificity of therapeutic delivery and off-target side effects. The discovery and optimization of high-affinity antibodies to important therapeutic targets could be significantly improved by the availability of a robust, eukaryotic display technology comparable to phage display that would overcome the protein translation limitations of microorganisms. The use of eukaryotic cells would improve the diversity of the displayed antibodies that can be screened and optimized as well as more seamlessly transition into a large-scale mammalian expression system for clinical production. In this study, we demonstrate that the replication and polypeptide display characteristics of a eukaryotic retrovirus, avianleukosis virus (ALV), offers a robust, eukaryotic version of bacteriophage display. The binding affinity of a model single-chain Fv antibody was optimized by using ALV display, improving affinity >2,000-fold, from micromolar to picomolar levels. We believe ALV display provides an extension to antibody display on microorganisms and offers virus and cell display platforms in a eukaryotic expression system. ALV displayshould enable an improvement in the diversity of properly processed and functional antibody variants that can be screened and affinity-optimized to improve promising antibody candidates.

Development of a high-resolution NGS-based HLA-typing and analysis pipeline

Michael Wittig, Jarl A. Anmarkrud, Jan C. Kässens, Simon Koch, Michael Forster, Eva Ellinghaus, Johannes R. Hov, Sascha Sauer, Manfred Schimmler, Malte Ziemann, Siegfried Görg, Frank Jacob, Tom H. Karlsen, Andre Franke

Nucleic Acid Research, Volume 43, Page e70, 2015

The human leukocyte antigen (HLA) complex contains the most polymorphic genes in the human genome. The classical HLA class I and II genes define the specificity of adaptive immune responses. Genetic variation at the HLA genes is associated with susceptibility to autoimmune and infectious diseases and plays a major role in transplantation medicine and immunology. Currently, the HLA genes are characterized using Sanger- or next-generation sequencing (NGS) of a limited amplicon repertoire or labeled oligonucleotides for allele-specific sequences. High-quality NGS-based methods are in proprietary use and not publicly available. Here, we introduce the first highly automated open-kit/open-source HLA-typing method for NGS. The method employs in-solution targeted capturing of the classical class I (HLA-A, HLA-B, HLA-C) and class II HLA genes (HLA-DRB1, HLA-DQA1, HLA-DQB1, HLA-DPA1, HLA-DPB1). The calling algorithm allows for highly confident allele-calling to three-field resolution (cDNA nucleotide variants). The method was validated on 357 commercially available DNA samples with known HLA alleles obtained by classical typing. Our results showed on average an accurate allele call rate of 0.99 in a fully automated manner, identifying also errors in the reference data. Finally, our method provides the flexibility to add further enrichment target regions.

Genomic Comparison of Campylobacter spp. and Their Potential for Zoonotic Transmission between Birds, Primates, and Livestock

Allison M. Weis, Dylan B. Storey, Conor C. Taff, Andrea K. Townsend, Bihua C. Huang, Nguyet T. Kong, Kristin A. Clothier, Abigail Spinner, Barbara A. Byrne, and Bart C. Weimer

Applied and Environmental Microbiology, Volume 82, Pages 7165-7175, 2016

Campylobacter is the leading cause of human gastroenteritis worldwide. Wild birds, including American crows, are abundant in urban, suburban, and agricultural settings and are likely zoonotic vectors of Campylobacter Their proximity to humans and livestock increases the potential spreading of Campylobacter via crows between the environment, livestock, and humans. However, no studies have definitively demonstrated that crows are a vector for pathogenic Campylobacter We used genomics to evaluate the zoonotic and pathogenic potential of Campylobacter from crows to other animals with 184 isolates obtained from crows, chickens, cows, sheep, goats, humans, and nonhuman primates. Whole-genome analysis uncovered two distinct clades of Campylobacter jejuni genotypes; the first contained genotypes found only in crows, while a second genotype contained "generalist" genomes that were isolated from multiple host species, including isolates implicated in human disease, primate gastroenteritis, and livestock abortion. Two major β-lactamase genes were observed frequently in these genomes (oxa-184, 55%, and oxa-61, 29%), where oxa-184 was associated only with crows and oxa-61 was associated with generalists. Mutations in gyrA, indicative of fluoroquinolone resistance, were observed in 14% of the isolates. Tetracycline resistance (tetO) was present in 22% of the isolates, yet it occurred in 91% of the abortion isolates. Virulence genes were distributed throughout the genomes; however, cdtC alleles recapitulated the crow-only and generalist clades. A specific cdtC allele was associated with abortion in livestock and was concomitant with tetO These findings indicate that crows harboring a generalist C. jejuni genotype may act as a vector for the zoonotic transmission of Campylobacter IMPORTANCE: This study examined the link between public health and the genomic variation of Campylobacter in relation to disease in humans, primates, and livestock. Use of large-scale whole-genome sequencing enabled population-level assessment to find new genes that are linked to livestock disease. With 184 Campylobactergenomes, we assessed virulence traits, antibiotic resistance susceptibility, and the potential for zoonotictransfer to observe that there is a “generalist” genotype that may move between host species.

Spatial reconstruction of single-cell gene expression

Rahul Satija, Jeffrey A. Farrell, David Gennert, Alexander F. Schier, and Aviv Regev

Nature Biotechnology, Volume 33, Pages 495-502, 2015

Spatial localization is a key determinant of cellular fate and behavior, but methods for spatially resolved, transcriptome-wide gene expression profiling across complex tissues are lacking. RNA staining methods assay only a small number of transcripts, whereas single-cell RNA-seq, which measures global gene expression, separates cells from their native spatial context. Here we present Seurat, a computational strategy to infer cellular localization by integrating single-cell RNA-seq data with in situ RNA patterns. We applied Seurat to spatially map 851 single cells from dissociated zebrafish (Danio rerio) embryos and generated a transcriptome-wide map of spatial patterning. We confirmed Seurat's accuracy using several experimental approaches, then used the strategy to identify a set of archetypal expression patterns and spatial markers. Seurat correctly localizes rare subpopulations, accurately mapping both spatially restricted and scattered groups. Seurat will be applicable to mapping cellular localization within complex patterned tissues in diverse systems.

Massively parallel single cell RNA-Seq for marker-free decomposition of tissues into cell types

Diego Adhemar Jaitin, Ephraim Kenigsberg, Hadas Keren-Shaul, Naama Elefant, Franziska Paul, Irina Zaretsky, Alexander Mildner, Nadav Cohen, Steffen Jung, Amos Tanay, and Ido Amit

Science, Volume 343, Pages 776-779, 2014

In multicellular organisms, biological function emerges when heterogeneous cell types form complex organs. Nevertheless, dissection of tissues into mixtures of cellular subpopulations is currently challenging. We introduce an automated massively parallel single-cell RNA sequencing (RNA-seq) approach for analyzing in vivo transcriptional states in thousands of single cells. Combined with unsupervised classification algorithms, this facilitates ab initio cell-type characterization of splenic tissues. Modeling single-cell transcriptional states in dendritic cells and additional hematopoietic cell types uncovers rich cell-type heterogeneity and gene-modules activity in steady state and after pathogen activation. Cellular diversity is thereby approached through inference of variable and dynamic pathway activity rather than a fixed preprogrammed cell-type hierarchy. These data demonstrate single-cell RNA-seq as an effective tool for comprehensive cellular decomposition of complex tissues.

Full-length RNA-seq from single cells using Smart-seq2.

Simone Picelli, Omid R Faridani, Åsa K Björklund, Gösta Winberg, Sven Sagasser, and Rickard Sandberg

Nature Protocols, Volume 9, Pages 171-181, 2014

Emerging methods for the accurate quantification of gene expression in individual cells hold promise for revealing the extent, function and origins of cell-to-cell variability. Different high-throughput methods for single-cell RNA-seq have been introduced that vary in coverage, sensitivity and multiplexing ability. We recently introduced Smart-seq for transcriptome analysis from single cells, and we subsequently optimized the method for improved sensitivity, accuracy and full-length coverage across transcripts. Here we present a detailed protocol for Smart-seq2 that allows the generation of full-length cDNA and sequencing libraries by using standard reagents. The entire protocol takes ~2 d from cell picking to having a final library ready for sequencing; sequencing will require an additional 1-3 d depending on the strategy and sequencer. The current limitations are the lack of strand specificity and the inability to detect nonpolyadenylated (polyA(-)) RNA.

Detection of Low Frequency Multi-Drug Resistance and Novel Putative Maribavir Resistance in Immunocompromised Pediatric Patients with Cytomegalovirus

Charlotte J. Houldcroft, Josephine M. Bryant, Daniel P. Depledge, Ben K. Margetts, Jacob Simmonds, Stephanos Nicolaou, Helena J. Tutill, Rachel Williams, Austen J. J. Worth, Stephen D. Marks, Paul Veys, Elizabeth Whittaker, and Judith Breuer, the PATHSEEK Consortium

Frontiers in Microbiology, Volume 7, Page 1317, 2016

Human cytomegalovirus (HCMV) is a significant pathogen in immunocompromised individuals, with the potential to cause fatal pneumonitis and colitis, as well as increasing the risk of organ rejection in transplant patients. With the advent of new anti-HCMV drugs there is therefore considerable interest in using virus sequence data to monitor emerging resistance to antiviral drugs in HCMV viraemia and disease, including the identification of putative new mutations. We used target-enrichment to deep sequence HCMV DNA from 11 immunosuppressed pediatric patients receiving single or combination anti-HCMV treatment, serially sampled over 1-27 weeks. Changes in consensus sequence and resistancemutations were analyzed for three ORFs targeted by anti-HCMV drugs and the frequencies of drug resistance mutations monitored. Targeted-enriched sequencing of clinical material detected mutations occurring at frequencies of 2%. Seven patients showed no evidence of drug resistance mutations. Four patients developed drug resistance mutations a mean of 16 weeks after starting treatment. In two patients, multiple resistance mutations accumulated at frequencies of 20% or less, including putative maribavir and ganciclovir resistance mutations P522Q (UL54) and C480F (UL97). In one patient, resistance was detected 14 days earlier than by PCR. Phylogenetic analysis suggested recombination or superinfection in one patient. Deep sequencing of HCMV enriched from clinical samples excluded resistance in 7 of 11 subjects and identified resistance mutations earlier than conventional PCR-based resistance testing in 2 patients. Detection of multiple low level resistance mutations was associated with poor outcome.

Elucidation of *MRAS*-mediated Noonan syndrome with cardiac hypertrophy

Erin M. Higgins, J. Martijn Bos, Heather Mason-Suares, David J. Tester, Jaeger P. Ackerman, Calum A. MacRae, Katia Sol-Church, Karen W. Gripp, Raul Urrutia, and Michael J. Ackerman

JCI Insight, Volume 2, Page e91225, 2017

Noonan syndrome (NS; MIM 163950) is an autosomal dominant disorder and a member of a family of developmental disorders termed "RASopathies," which are caused mainly by gain-of-function mutations in genes encoding RAS/MAPK signaling pathway proteins. Whole exome sequencing (WES) and trio-based genomic triangulation of a 15-year-old female with a clinical diagnosis of NS and concomitant cardiachypertrophy and her unaffected parents identified a de novo variant in *MRAS*-encoded RAS-related protein 3 as the cause of her disease. Mutation analysis using in silico mutation prediction tools and molecular dynamics simulations predicted the identified variant, p.Gly23Val-MRAS, to be damaging to normal protein function and adversely affect effector interaction regions and the GTP-binding site. Subsequent ectopic expression experiments revealed a 40-fold increase in MRAS activation for p.Gly23Val-MRAS compared with WT-MRAS. Additional biochemical assays demonstrated enhanced activation of both RAS/MAPK pathway signaling and downstream gene expression in cells expressing p.Gly23Val-MRAS. Mutational analysis of *MRAS* in a cohort of 109 unrelated patients with phenotype-positive/genotype-negative NS and cardiac hypertrophy yielded another patient with a sporadic de novo MRAS variant (p.Thr68Ile, c.203C>T). Herein, we describe the discovery of mutations in MRAS in patients with NS and cardiac hypertrophy, establishing *MRAS* as the newest NS with cardiac hypertrophy-susceptibility gene.

Mapping the Hallmarks of Lung Adenocarcinoma with Massively Parallel Sequencing

Marcin Imielinski, Alice H. Berger, Peter S. Hammerman, Bryan Hernandez, Trevor J. Pugh, Eran Hodis, Jeonghee Cho, James Suh, Marzia Capelletti, Andrey Sivachenko, Carrie Sougnez, Daniel Auclair, Michael S. Lawrence, Petar Stojanov, Kristian Cibulskis, Kyusam Choi, Luc de Waal, Tanaz Sharifnia, Angela Brooks, Heidi Greulich, Shantanu Banerji, Thomas Zander, Danila Seidel, Frauke Leenders, Sascha Ansén, Corinna Ludwig, Walburga Engel-Riedel, Erich Stoelben, Jürgen Wolf, Chandra Goparju, Kristin Thompson, Wendy Winckler, David Kwiatkowski, Bruce E. Johnson, Pasi A. Jänne, Vincent A. Miller, William Pao, William D. Travis, Harvey I. Pass, Stacey B. Gabriel, Eric S. Lander, Roman K. Thomas, Levi A. Garraway, Gad Getz, Matthew Meyerson

Cell, Volume 150, Pages 1107-1120, 2012

Lung adenocarcinoma, the most common subtype of non-small cell lung cancer, is responsible for more than 500,000 deaths per year worldwide. Here, we report exome and genome sequences of 183 lungadenocarcinoma tumor/normal DNA pairs. These analyses revealed a mean exonic somatic mutation rate of 12.0 events/megabase and identified the majority of genes previously reported as significantly mutated in lung adenocarcinoma. In addition, we identified statistically recurrent somatic mutations in the splicing factor gene U2AF1 and truncating mutations affecting RBM10 and ARID1A. Analysis of nucleotide context-specific mutation signatures grouped the sample set into distinct clusters that correlated with smoking history and alterations of reported lung adenocarcinoma genes. Whole-genome sequence analysis revealed frequent structural rearrangements, including in-frame exonic alterations within EGFR and SIK2 kinases. The candidate genes identified in this study are attractive targets for biological characterization and therapeutic targeting of lung adenocarcinoma.

Whole exome analysis identifies frequent CNGA1 mutations in Japanese population with autosomal recessive retinitis pigmentosa

Satoshi Katagiri, Masakazu Akahori, Yuri Sergeev, Kazutoshi Yoshitake, Kazuho Ikeo, Masaaki Furuno, Takaaki Hayashi, Mineo Kondo, Shinji Ueno, Kazushige Tsunoda, Kei Shinoda, Kazuki Kuniyoshi, Yohinori Tsurusaki, Naomichi Matsumoto, Hiroshi Tsuneoka, Takeshi Iwata

PLoS One, Volume 9, Page e108721, 2014

OBJECTIVE:

The purpose of this study was to investigate frequent disease-causing gene mutations in autosomal recessive retinitis pigmentosa (arRP) in the Japanese population.

METHODS:

In total, 99 Japanese patients with non-syndromic and unrelated arRP or sporadic RP (spRP) were recruited in this study and ophthalmic examinations were conducted for the diagnosis of RP. Among these patients, whole exome sequencing analysis of 30 RP patients and direct sequencing screening of all CNGA1 exons of the other 69 RP patients were performed.

RESULTS:

Whole exome sequencing of 30 arRP/spRP patients identified disease-causing gene mutationsof CNGA1 (four patients), EYS (three patients) and SAG (one patient) in eight patients and potential disease-causing gene variants of USH2A (two patients), EYS (one patient), TULP1 (one patient) and C2orf71 (one patient) in five patients. Screening of an additional 69 arRP/spRP patients for the CNGA1gene mutation revealed one patient with a homozygous mutation.

CONCLUSIONS:

This is the first identification of CNGA1 mutations in arRP Japanese patients. The frequency of CNGA1 gene mutation was 5.1% (5/99 patients). CNGA1 mutations are one of the most frequent arRP-causing mutations in Japanese patients.

Determining the frequency of pathogenic germline variants from exome sequencing in patients with castrate-resistant prostate cancer

Steven N Hart, Marissa S Ellingson, Kim Schahl, Peter T Vedell, Rachel E Carlson, Jason P Sinnwell, Poulami Barman, Hugues Sicotte, Jeanette E Eckel-Passow, Liguo Wang, Krishna R Kalari, Rui Qin, Teresa M Kruisselbrink, Rafael E Jimenez, Alan H Bryce, Winston Tan, Richard Weinshilboum, Liewei Wang, Manish Kohli

BMJ Open, Volume 6, Page e010332, 2016

OBJECTIVES:

To determine the frequency of pathogenic inherited mutations in 157 select genes from patients with metastatic castrate-resistant prostate cancer (mCRPC).

DESIGN:

Observational.

SETTING:

Multisite US-based cohort.

PARTICIPANTS:

Seventy-one adult male patients with histological confirmation of prostate cancer, and had progressive disease while on androgen deprivation therapy.

RESULTS:

Twelve patients (17.4%) showed evidence of carrying pathogenic or likely pathogenic germlinevariants in the ATM, ATR, BRCA2, FANCL, MSR1, MUTYH, RB1, TSHR and WRN genes. All but one patient opted in to receive clinically actionable results at the time of study initiation. We also found that pathogenic germline BRCA2 variants appear to be enriched in mCRPC compared to familial prostatecancers.

CONCLUSIONS:

Pathogenic variants in cancer-susceptibility genes are frequently observed in patientswith mCRPC. A substantial proportion of patients with mCRPC or their family members would derive clinical utility from mutation screening.

Prognostic interaction between *ASXL1* and *TET2* mutations in chronic myelomonocytic leukemia

M M Patnaik, T L Lasho, P Vijayvargiya, C M Finke, C A Hanson, R P Ketterling, N Gangat, and A Tefferi

Blood Cancer Journal, Volume 6, Page e385, 2016

Mutations involving epigenetic regulators (TET2~60% and ASXL1~40%) and splicing components (SRSF2~50%) are frequent in chronic myelomonocytic leukemia (CMML). On a 27-gene targeted capture panel performed on 175 CMML patients (66% males, median age 70 years), common mutations included: TET2 46%, ASXL1 47%, SRSF2 45% and SETBP1 19%. A total of 172 (98%) patients had at least one mutation, 21 (12%) had 2, 24 (14%) had 3 and 30 (17%) had >3 mutations. In a univariate analysis, the presence of ASXL1 mutations (P=0.02) and the absence of TET2 mutations (P=0.03), adversely impacted survival; while the number of concurrent mutations had no impact (P=0.3). In a multivariable analysis that included hemoglobin, platelet count, absolute monocyte count and circulating immature myeloid cells (Mayo model), the presence of ASXL1 mutations (P=0.01) and absence of TET2 mutations (P=0.003) retained prognostic significance. Patients were stratified into four categories: ASXL1wt/TET2wt (n=56), ASXL1mut/TET2wt (n=31), ASXL1mut/TET2mut (n=50) and ASXL1wt/TET2mut (n=38). Survival data demonstrated a significant difference in favor of ASXL1wt/TET2mut (38 months; P=0.016), compared with those with ASXL1wt/TET2wt (19 months), ASXL1mut/TET2wt (21 months) and ASXL1mut/TET2mut (16 months) (P=0.3). We confirm the negative prognostic impact imparted by ASXL1 mutations and suggest a favorable impact from TET2 mutations in the absence of ASXL1 mutations.

Targeted deep sequencing in primary myelofibrosis

Ayalew Tefferi, Terra L. Lasho, Christy M. Finke, Yoseph Elala, Curtis A. Hanson, Rhett P. Ketterling, Naseema Gangat, and Animesh Pardanani

Blood Advances, Volume 1, Pages 105-111, 2016

A myeloid neoplasm–relevant 27-gene panel was used for next-generation sequencing of bone marrow or whole blood DNA in 182 patients with primary myelofibrosis (PMF). DNA sequence variants/mutations other than *JAK2/CALR/MPL* were detected in 147 patients (81%), with the most frequent being *ASXL1* (36%), *TET2* (18%), *SRSF2* (18%), and *U2AF1* (16%); furthermore, 35%, 26%, 10%, and 9% of the patients harbored 1, 2, 3, or 4 or more such variants/mutations, respectively. Adverse variants/mutations were identified by age-adjusted multivariable analysis of impact on overall survival or leukemia-free survival and included *ASXL1*, *SRSF2*, *CBL*, *KIT*, *RUNX1*, *SH2B3*, and *CEBPA*; their combined prevalence was 56%. Adverse variants/mutations were associated with inferior overall survival (median, 3.6 vs 8.5 years; P < .001) and leukemia-free survival (7-year risk, 25% vs 4%; P < .001), and the effect on survival was independent of both the Dynamic International Prognostic Scoring System Plus and *JAK2/CALR/MPL* mutational status, with respective hazard ratios of 2.0 (95% confidence interval [CI], 1.3-3.1) and 2.9 (95% CI, 1.9-4.4). Additional prognostic information was obtained by considering the number of adverse variants/mutations; median survivals in patients with zero (n = 80), 1 or 2 (n = 93), or 3 or more (n = 9) adverse variants/mutations were 8.5, 4, and 0.7 years, respectively (*P* < .001). Additional data were obtained on pattern of mutation co-segregation and phenotypic correlation, including significant associations between *U2AF1* and *JAK2* mutations (*P* = .04) and U2AF1 mutations and anemia (*P* = .003) and thrombocytopenia (*P* = .006). We conclude that DNA variants/mutations other than *JAK2/CALR/MPL* are prevalent in PMF and are qualitatively and quantitatively relevant in predicting overall and leukemia-free survival.

Comprehensive Assessment of Genetic Variants Within *TCF4* in Fuchs' Endothelial Corneal Dystrophy

Eric D. Wieben; Ross A. Aleff; Bruce W. Eckloff; Elizabeth J. Atkinson; Saurabh Baheti; Sumit Middha; William L. Brown; Sanjay V. Patel; Jean-Pierre A. Kocher; Keith H. Baratz

Investigative Ophthalmology & Visual Science, Volume 55, Pages 6101-6107, 2014

PURPOSE:

The single nucleotide variant (SNV), rs613872, in the transcription factor 4 (TCF4) gene was previously found to be strongly associated (P = 6 × 10(-26)) with Fuchs' endothelial corneal dystrophy(FECD). Subsequently, an intronic expansion of the repeating trinucleotides, TGC, was found to be even more predictive of disease. We performed comprehensive sequencing of the TCF4 gene region in order to identify the best marker for FECD within TCF4 and to identify other novel variants that may be associated with FECD.

METHODS:

Leukocyte DNA was isolated from 68 subjects with FECD and 16 unaffected individuals. A custom capture panel was used to isolate the region surrounding the two previously validated markers of FECD. Sequencing of the TCF4 coding region, introns and flanking sequence, spanning 465 kb was performed at >1000× average coverage using the Illumina HiSequation 2000.

RESULTS:

TGC expansion (>50 repeats) was present in 46 (68%) FECD-affected subjects and one (6%) normal subject. A total of 1866 variants, including 1540 SNVs, were identified. Only two previously reported SNVs resided in the TCF4 coding region, neither of which segregated with disease. No variant, including TGC expansion, correlated perfectly with disease status. Trinucleotide repeat expansion was a better predictor of disease than any other variant.

CONCLUSIONS:

Complete sequencing of the TCF4 genomic region revealed no single causative variant for FECD. The intronic trinucleotide repeat expansion within TCF4 continues to be more strongly associated with FECD than any other genetic variant.

Hereditary hemorrhagic telangiectasia: genetics and molecular diagnostics in a new era

Jamie McDonald, Whitney Wooderchak-Donahue, Chad VanSant Webb, Kevin Whitehead, David A. Stevenson, and Pinar Bayrak-Toydemir

Frontiers in Genetics, Volume 6, Page 1, 2015

Hereditary hemorrhagic telangiectasia (HHT) is a vascular dysplasia characterized by telangiectases and arteriovenous malformations (AVMs) in particular locations described in consensus clinical diagnostic criteria published in 2000. Two genes in the transforming growth factor-beta (TGF-β) signaling pathway, ENG and ACVRL1, were discovered almost two decades ago, and mutations in these genes have been reported to cause up to 85% of HHT. In our experience, approximately 96% of individuals with HHT have a mutation in these two genes, when published (Curaçao) diagnostic criteria for HHT are strictly applied. More recently, two additional genes in the same pathway, SMAD4 and GDF2, have been identified in a much smaller number of patients with a similar or overlapping phenotype to HHT. Yet families still exist with compelling evidence of a hereditary telangiectasia disorder, but no identifiable mutation in a known gene. Recent availability of whole exome and genome testing has created new opportunities to facilitate gene discovery, identify genetic modifiers to explain clinical variability, and potentially define an increased spectrum of hereditary telangiectasia disorders. An expanded approach to molecular diagnostics for inherited telangiectasia disorders that incorporates a multi-gene next generation sequencing (NGS) HHT panel is proposed.

BMP9 Mutations Cause a Vascular-Anomaly Syndrome with Phenotypic Overlap with Hereditary Hemorrhagic Telangiectasia

Whitney L. Wooderchak-Donahue, Jamie McDonald, Brendan O'Fallon, Paul D. Upton, Wei Li, Beth L. Roman, Sarah Young, Parker Plant, Gyula T. Fülöp, Carmen Langa, Nicholas W. Morrell, Luisa M. Botella, Carmelo Bernabeu, David A. Stevenson, James R. Runo, Pinar Bayrak-Toydemir

American Journal of Human Genetics, Volume 93, Pages 530-537, 2013

Hereditary hemorrhagic telangiectasia (HHT), the most common inherited vascular disorder, is caused by mutations in genes involved in the transforming growth factor beta (TGF-β) signaling pathway (ENG, ACVRL1, and SMAD4). Yet, approximately 15% of individuals with clinical features of HHT do not have mutations in these genes, suggesting that there are undiscovered mutations in other genes for HHT and possibly vascular disorders with overlapping phenotypes.

The genetic etiology for 191 unrelated individuals clinically suspected to have HHT was investigated with the use of exome and Sanger sequencing; these individuals had no mutations in ENG, ACVRL1, and SMAD4. Mutations in BMP9 (also known as GDF2) were identified in three unrelated probands. These three individuals had epistaxis and dermal lesions that were described as telangiectases but whose location and appearance resembled lesions described in some individuals with RASA1-related disorders (capillary malformation-arteriovenous malformation syndrome). Analyses of the variant proteins suggested that mutations negatively affect protein processing and/or function, and a bmp9-deficient zebrafish model demonstrated that BMP9 is involved in angiogenesis. These data confirm a genetic cause of a vascular-anomaly syndrome that has phenotypicoverlap with HHT.

Genetic Variants Associated with Port-Wine Stains

Alice Frigerio, Karol Wright, Whitney Wooderchak-Donahue, Oon T. Tan, Rebecca Margraf, David A. Stevenson, J. Fredrik Grimmer, Pinar Bayrak-Toydemir

PLoS One, Volume 10, Page e0133158, 2015

BACKGROUND:

Port-wine stains (PWS) are capillary malformations, typically located in the dermis of the head and neck, affecting 0.3% of the population. Current theories suggest that port-wine stains are caused by somatic mutations that disrupt vascular development.

OBJECTIVES:

Understanding PWS genetic determinants could provide insight into new treatments.

METHODS:

Our study used a custom next generation sequencing (NGS) panel and digital polymerase chain reaction to investigate genetic variants in 12 individuals with isolated port-wine stains. Importantly, affected and healthy skin tissue from the same individual were compared. A subtractive correction method was developed to eliminate background noise from NGS data. This allowed the detection of a very low level of mosaicism.

RESULTS:

A novel somatic variant GNAQ, c.547C>G, p.Arg183Gly was found in one case with 4% allele frequency. The previously reported GNAQ c.548G>A, p.Arg183Gln was confirmed in 9 of 12 cases with an allele frequency ranging from 1.73 to 7.42%. Digital polymerase chain reaction confirmed novel variantsdetected by next generation sequencing. Two novel somatic variants were also found in RASA1, although neither was predicted to be deleterious.

CONCLUSIONS:

This is the second largest study on isolated, non-syndromic PWS. Our data suggest that GNAQ is the main genetic determinant in this condition. Moreover, isolated port-wine stains are distinct from capillary malformations seen in RASA1 disorders, which will be helpful in clinical evaluation.

RASA1 somatic mutation and variable expressivity in capillary malformation/arteriovenous malformation (CM/AVM) syndrome

Colleen F. Macmurdo, Whitney Wooderchak-Donahue, Pinar Bayrak-Toydemir, Jenny Le, Matthew B. Wallenstein, Carlos Milla, Joyce M. C. Teng, Jonathan A. Bernstein, David A. Stevenson

American Journal of Medical Genetics, Volume 170, Pages 1450-1454, 2016

Germline mutations in RASA1 are associated with capillary malformation-arteriovenous malformation (CM-AVM) syndrome. CM-AVM syndrome is characterized by multi-focal capillary malformations and arteriovenous malformations. Lymphatic anomalies have been proposed as part of the phenotype. Intrafamilial variability has been reported, suggesting modifiers and somatic events. The objective of the study was to identify somatic RASA1 "second hits" from vascular malformations associated with CM-AVMsyndrome, and describe phenotypic variability. Participants were examined and phenotyped. Genomic DNA was extracted from peripheral blood on all participants. Whole-exome sequencing was performed on the proband. Using Sanger sequencing, RASA1 exon 8 was PCR-amplified to track the c.1248T>G, p.Tyr416X germline variant through the family. A skin biopsy of a capillary malformation from the proband's mother was also obtained, and next-generation sequencing was performed on DNA from the affected tissue. A familial germline heterozygous novel pathogenic RASA1 variant, c.1248T>G (p.Tyr416X), was identified in the proband and her mother. The proband had capillary malformations, chylothorax, lymphedema, and overgrowth, while her affected mother had only isolated capillary malformations. Sequence analysis of DNA extracted from a skin biopsy of a capillary malformation of the affected mother showed a second RASA1 somatic mutation (c.2245C>T, p.Arg749X). These results and the extreme variable expressivity support the hypothesis that somatic "second hits" are required for the development of vascular anomalies associated with CM-AVM syndrome. In addition, the phenotypes of the affected individuals further clarify that lymphatic manifestations are also part of the phenotypic spectrum of RASA1-related disorders.

www.agilent.com/chem

For Research Use Only. Not for use in diagnostic procedures.

This information is subject to change without notice.

Printed in the USA, October 25, 2017
5991-8612EN

Agilent
Trusted Answers