Applied & Translational Genomics 2 (2013) 28–33

Contents lists available at ScienceDirect

Applied & Translational Genomics

journal homepage: www.elsevier.com/locate/atg

Review

Incorporate gene signature profiling into routine molecular testing☆,☆☆

Neng Chen *

Pathology and Laboratory Medicine, Beaumont Health System, Royal Oak, MI, USA

ARTICLE INFO

Article history:
Received 25 December 2012
Accepted 16 March 2013

Keywords:
Gene signature profiling
Personalized genomic medicine
Clinical diagnosis

ABSTRACT

The success of gene and gene expression profiling, such as the Onco*type* DX® test for breast cancer patients, demonstrates that as technology becomes more sophisticated molecular diagnostics will continue to play a more important role in disease management in the future. Such promises have been and continue to be enabled by advances in real-time PCR, microarray detection platforms and next generation sequencing technologies. Practical adoption of new technologies into routine clinical care, however, has not always been a smooth ride. Challenges lie on several fronts: establishment of clinical validity in large scale patient population, mechanisms of incorporating molecular tests into standard care, and keeping up with the pace of ever changing technologies in regulated clinical laboratories, just to name a few. This review's goals are to educate, to stimulate discussion and to provoke efforts to build consensus, share resources, and establish standards in order to realize the promises of genomic technologies for routine patient care.

Contents

1. Introduction

An integrated RNA expression pattern or transcriptional profile of a set of genes can elucidate disease mechanisms, regulatory pathways, and gene functions (Schena et al., 1995). During normal development, the expression pattern of the genes (i.e., gene signature profiles) can be unique or similar between different cell and tissue types or different locations within the same tissue type (Rinn et al., 2008). The same can also be true in different cancer types and even in different regions of the same tumor (Gerlinger et al., 2012). Importantly, gene signature profiling can be used to distinguish fundamentally different disease types. For example, diagnosis of Burkitt's lymphoma (BL), a rare and highly aggressive B-cell lymphoma, is based on integration of morphologic, immunophenotypic and cytogenetic data (Jaffe, 2009). Diffuse large B-cell lymphoma (DLBCL) is more common but a less aggressive disease, yet the two types of lymphoma can share overlapping morphologic and immunophenotypic features. The t(8;14) translocation, a characteristic cytogenetic feature of BL (Gerbitz et al., 1999; Hecht and Aster, 2000; Neri et al., 1988) is present in 5 to 10% of DLBCL (Kramer et al., 1998). To determine appropriate therapeutic intervention, it is critical to distinguish BL from DLBCL, as BL requires a more rigorous regimen of chemotherapy, as opposed to relatively low-dose chemotherapy typically employed for DLBCL. Thus, gene expression profiling can, quite practically, inform diagnosis and choice of therapy.

A retrospective study (Dave et al., 2006) indicated that a classifier based on gene expression profile correctly identified all 25 pathologically verified cases of classic BL. The typical gene signature for BL was also present in eight cases of pathologically diagnosed DLBCL. Further analysis was done with 28 of those patients with complete clinical information and a molecular diagnosis of BL. The authors found that the overall survival was markedly longer among those who received intensive chemotherapy than those treated with the lower dose regimens. Among seven of the eight patients pathologically diagnosed with DLBCL yet who had the

☆ Source of support: Funded by the Department of Pathology and Laboratory Medicine, Beaumont Health System.

* Pathology and Laboratory Medicine, Beaumont Health System, 3601 West 13 Mile Rd, Royal Oak, MI 48073, USA. Tel.: +1 248 551 1635; fax: +1 248 551 0557.
E-mail address: neng.chen@beaumont.edu.

2212-0661/$ – see front matter
http://dx.doi.org/10.1016/j.atg.2013.03.002

gene signature of BL, five received lower doses of chemotherapy. None survived more than two years. For the remaining two patients who received BL chemotherapy instead, one survived more than five years and one died after nine months of the therapy. Overall, those observations suggest that gene signature profiling can be used to distinguish the two types of lymphomas, and thus tailor more rational therapy choices for a given patient's disease.

t would be highly beneficial if molecular methods can be developed to facilitate the diagnosis of morphologically similar yet molecularly different types of diseases. Such capability could also yield diagnostic information that in turn guides treatment with targeted therapy, especially for cancer patients. As we saw in the above example, molecular techniques certainly can be very effective. Given the various considerations of the different technological platforms, however, is the diagnostic community ready to adopt gene signature profiling into *routine* diagnostic practice? This article focuses on the technologies used for gene signature profiling and how to implement gene signature profiling for routine molecular diagnosis.

2. Technologies

The completion of the human genome project (Lander et al., 2001; Venter et al., 2001) about a decade ago revolutionized the way genomic research is conducted. It stimulated groundbreaking ideas on technology development in molecular biology and has since led to fundamental advances in biomedical research. Classical approaches required researchers to "fish out" the sequence of the gene of their interest first in order to study the gene function. Discovery of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which when mutated causes cystic fibrosis, is one classical example in that regard. It took years and many different laboratories to map, clone and finally sequence the gene (Beaudet et al., 1989; Duncan et al., 1988; Eiberg et al., 1985; Estivill et al., 1987; Kerem et al., 1989; Klinger et al., 1986; Knowlton et al., 1985; Mayo et al., 1980; Riordan et al., 1989; Rommens et al., 1989; Scambler et al., 1985; Tsui et al., 1985; Wainwright et al., 1986, 1985; Watkins et al., 1986; Zielenski et al., 1991) before the information became available for routine patient care. Upon completion of the human genome project with the resultant public dissemination of vast amounts of generated data, it became possible to imagine interrogating tens of thousands of genes for mutation detection or gene expression at the same time in an individual laboratory. Experimentally, however, conventional mutation detection, genotyping and gene expression methods were ill suited to high throughput studies. Around that time, early in the century, newly developed microarray technologies enabled the dream of high throughput gene studies (Lashkari et al., 1997; Maskos and Southern, 1992; Schena et al., 1995). Implementation of real-time PCR technologies (Becker-Andre and Hahlbrock, 1989; Chiang et al., 1996; Gibson et al., 1996; Gilliland et al., 1990; Heid et al., 1996; Higuchi et al., 1993; Wang et al., 1989) simplifies the bench-level workflow and, in turn, is a technology platform even more accessible to majority of research and clinical laboratories. With the advent of next generation sequencing (NGS) technologies (Brenner et al., 2000; Margulies et al., 2005), the practical possibility on the simultaneous interrogation of both mutation profiles and gene expression could enable clinical investigators to "capitalize" on both technological advances and individual genetic information on a grander scale. These representative technologies will be explored below, as they pertain to clinical use.

Microarray analysis is a nucleic acid hybridization based detection technology. Tens of thousands, even millions of oligonucleotide sequences (i.e., probes) derived from genes of interest are synthesized and covalently immobilized on a solid phase, constituting a high density detection array, commonly referred to as a microarray. The labeled complementary DNA (cDNA) molecules derived from the RNA of a test sample are hybridized to the microarray. Within the appropriately calibrated linear dynamic range, the amount of the labeled fluorescent signal hybridized on probes correlates to the extent of the RNA expression of a particular gene. With the ability of microarray platforms that accommodates large numbers of probes from many genes, detection of the gene expression of tens to hundreds of thousands of genes can be achieved in parallel. By comparing a cancerous tissue with its normal control sample, microarray technology can be used to identify the patterns of differential gene expression and genes responsible for the regulation of the observed expression pattern(s). A smaller pool of genes that determine a particular expression pattern can be identified and is colloquially known as a gene signature, the process being called gene signature profiling. Such signatures (i.e., gene signature profile) can further be verified in subsequent microarray-based experiments using a large number of samples with similar phenotypic features.

Agendia's MammaPrint®, a breast cancer gene signature profiling test, was developed by screening the gene expression of 25,000 human genes in lymph node negative, primary breast cancer patients who either developed distant metastasis within five years or were disease-free after a period of at least five years (Mook et al., 2007; van't Veer et al., 2002). About 5000 significant genes were selected initially based on the gene expression level. After application of the cancer disease outcome and supervised mathematical classification methods, a 70-gene signature was identified. With the 70-gene signature profiling, lymph node negative early stage breast cancer patients with low risk score would only need the hormonal therapy to reduce the risk of distant metastasis, while patients with a high risk score would need more aggressive therapy, such as chemotherapy. Further studies indicated that the 70-gene prognosis signature outperformed the clinical and histologic criteria as an independent prognostic factor in lymph node-negative young breast cancer patients (Bueno-de-Mesquita et al., 2009; Buyse et al., 2006; van de Vijver et al., 2002).

The complexity of microarray technology, however, requires a rigorous design (Churchill, 2002). Continuing with the MammaPrint® example, for clinical testing each MammaPrint® microarray chip contains eight sub-array areas for eight different samples. Each sub-array contains 1900 features, including 232 probes in triplicate for the targeted 70-gene, additional 915 genes for normalization and 289 spots for hybridization and array manufacture quality controls. Depending on the platform used for each microarray technology, most of the customized microarrays are produced by commercial manufacturers. In addition, the labeling, hybridization and scanning steps require extensive development and optimization. Therefore, to ease the lab workflow, other technologies, such as real-time PCR have been favorably implemented in gene signature profiling after initial large scale screening using microarray.

Real-time PCR technologies are able to quantify a target of interest during PCR amplification. For gene expression, a one-step reverse transcription and PCR can be accomplished in a same reaction. Empirically, it is most efficient when the number of target genes are less than 100 (VanGuilder et al., 2008). Genomic Health developed Onco*type* DX® breast cancer test, a 21-gene signature profile employing real-time PCR. The gene signature was based on the study of the clinical outcome for lymph node negative and estrogen receptor (ER) positive patients, who received hormonal treatment (Cobleigh et al., 2005; Habel et al., 2006; Paik, 2007; Paik et al., 2004). The signature profiling stratifies the patients into three subgroups: low, medium and high risk. The high risk group has the worst clinical outcome and a more aggressive treatment like chemotherapy is expected to benefit these patients.

Although real-time PCR has the simplest workflow for generation of gene signature profiles, we may not be able to use it to uncover specific gene signature profiles in some diseases. The microarray method could be more practical in such cases in searching for abnormal gene expression patterns within the entire transcriptome (i.e., expression of all the RNA in a sample). With recent advances in NGS technologies, clinical laboratories are eager to adapt this newest of molecular tools into routine clinical practice. Similar to microarray technology, NGS is a high throughput method that generates results of a large number of genes simultaneously. The difference is that NGS produces "digital" information in that sequence of each base pair of target genes is discrete, whereas microarray-derived signals from target genes are hybridization based readout which is "analog". Furthermore, NGS is a more diverse platform than microarrays, with its

ability to detect alternative splice variants and novel transcripts (Marioni et al., 2008) without prior knowledge of their existence. For example, sequencing the entire RNA content of a sample by NGS (i.e., RNAseq) provides all of above information as opposed to only signal intensity of the probes by microarray. Therefore, NGS platform generates a comprehensive and holistic picture of the gene expression profile of that sample (Morin et al., 2008; Wang et al., 2009). Although NGS is comparable in throughput to microarray technology for studying gene expression, microarray technology is generally less expensive than NGS technologies. For initial high throughput gene expression studies, microarray technology may be a better choice. Once a short list of gene signatures is identified, real-time PCR would be an easier method to implement in clinical laboratories. Certainly, NGS expands capabilities and provides more insights into gene expression (Trapnell et al., 2010). For difficult cases, NGS may be a better choice to find clinically relevant gene expression patterns, just as the current whole genome sequencing is applied in clinical practices (Worthey et al., 2011).

3. Clinical laboratory implementation

Although clinical research has revealed great potential using gene signature profiling to stratify patients and treat patients accordingly (Alizadeh et al., 2000; Dave et al., 2006; Mori et al., 2008; Rosenwald et al., 2002; Schmitz et al., 2012; Zhang et al., 2012), gene signature profiling still does not represent high volume testing or indeed even a common molecular test in diagnostic laboratories. This may be due to the complexity during assay development and test validation. Currently, Laboratory-Developed Tests (LDTs) account for the majority of test results generated in molecular pathology laboratories. In the U.S., based on the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88), a laboratory must perform a full analytical validation before an LDT is launched. Verification of accuracy, precision (repeatability), analytical sensitivity, analytical specificity, reference range (normal value), and reportable range of the system is required (Code of Federal Regulations, 42CFR493.1253, http://www.gpo.gov/fdsys/pkg/CFR-2011-title42-vol5/pdf/CFR-2011-title42-vol5-sec493-1253.pdf accessed 12/01/2012). Furthermore, every test offered for clinical testing must demonstrate how consistently and accurately the result detects or predicts the intermediate or final clinical outcomes (clinical validity), and how likely the test result is to significantly improve patient outcomes (clinical utility) (http://www.cdc.gov/genomics/gtesting/ACCE/index.htm, accessed on 12/01/2012). Analytical validation is relatively straightforward for single target gene testing, which currently comprises the majority of the assays on the test menu in a molecular laboratory. However, demonstration of clinical validity and utility for LDTs is more problematic for majority of individual laboratories that do not have sufficient resources and are not equipped to undertake large clinical studies. Most often, laboratories rely on referencing peer-reviewed publications to demonstrate clinical validity and utility.

Given these realities, developing and validating a much more complex gene signature profiling test would take significant time, resources and technical sophistication, more than is generally available in most clinical laboratories. First, a gene signature profiling test targets a number of genes instead of a single gene, therefore more effort on assay development is required relative to a single gene testing. Moreover, data directly generated from the microarray, real-time PCR and NGS platforms (i.e., "wet bench" data) for each assay are typically not the final results of a test.

Further statistical and mathematical analysis ("dry bench") to combine all the individual wet bench data is necessary to generate a meaningful result for interpretation. "Dry bench" analysis certainly would heavily depend on efforts from statisticians and bioinformaticians. Most molecular pathology laboratories, however, do not have sufficient "dry bench" expertise and it is a relatively new concept that clinical lab personnel would need to work closely with statisticians or bioinformaticians to make sense of the wet bench data before generating test results or case reports. To make the scenario even more difficult, developing a new gene signature profiling test requires a large data set to perform the statistical analysis and build the algorithm (Kratz et al., 2012; Mook et al., 2007; Paik et al., 2004; van't Veer et al., 2002). Therefore, establishing a new gene signature today is truly a clinical research project that most likely is carried out in laboratories or institutions whose mandates are not on diagnostic operations.

Even if a gene signature profiling test is based on published literature, clinical laboratories would still need to develop the "wet bench" gene signature assays and "dry bench" analysis in house for validation, and eventual offering as an LDT. In many published research works (Alizadeh et al., 2000; Kratz et al., 2012; Paik et al., 2004; van't Veer et al., 2002), only names of the signature genes and rudimentary bioinformatics, insufficient for true reproduction, are provided; often primer or probe sequences are missing and this can be related to proprietary intellectual property. Given the above complexities, building a gene signature profiling test is clearly not a simple undertaking for clinical laboratories, as opposed to the single gene tests.

Perhaps not surprisingly, clinical laboratories traditionally rely on in vitro diagnostic (IVD) manufacturers to develop this type of tests. Under such circumstances, labs don't need to conduct large clinical studies by themselves. They would only need to perform analytical verification to offer the test clinically. Unfortunately, only one such test, Agendia's MammaPrint®, is currently cleared by FDA. Furthermore, it is an instrument-based FDA clearance, meaning that only a number of instruments with specific serial numbers at Agendia are included in the clearance. Such practice in effect makes Agendia the only lab that is qualified to perform the MammaPrint® test.

With the success of the Genomic Health's Onco*type* DX® breast cancer test, it is clear that gene signature profiling can help to distinguish morphologically similar, but pathologically different diseases for clinical diagnosis and lead to different treatments. Onco*type* DX® breast cancer test is an LDT and performs at Genomic Health. It may be the most recognized gene signature profiling test at the current time. The Onco*type* DX® breast cancer test was launched in early 2004. In 2006, Medicare agreed to cover the test for lymph node negative and ER positive breast cancer patients. In 2007 and 2008, the American Society of Clinical Oncology (ASCO) (Harris et al., 2007) and the National Comprehensive Cancer Network (NCCN) (http://www.nccn.org/) added the 21-gene signature to the breast cancer practice and treatment guidelines, respectively. At this point, Onco*type* DX® breast cancer test is widely used in daily clinical practices.

What have we learned from the Onco*type* DX® breast cancer test? Obviously, clinical utility is the driving force for test implementation. Onco*type* DX® breast cancer test targets lymph node-negative, estrogen-receptor-positive breast cancer patients. The majority of these patients can be cured with only hormone therapy, but about 15% of them would have distant recurrence, who should go through chemotherapy. Onco*type* DX® breast cancer test is to stratify these breast cancer patients into two groups, low and high risk for distant metastasis. Based on the risk factor, only patients with high risk would undergo chemotherapy (Paik et al., 2004). Without stratification, all the patients would be likely to go through the aggressive chemotherapy.

In addition, Onco*type* DX® was developed to streamline the physician ordering and lab workflow. Although the initial study was based on frozen tissues (Golub et al., 1999; Perou et al., 2000; Sorlie et al., 2001; van't Veer et al., 2002), Genomic Health further developed the gene signature using formalin-fixed, paraffin-embedded (FFPE) tissues, which is commonly used for surgical specimens. Real-time PCR, the most simplified molecular method for gene signature profiling, was developed as the platform for Onco*type* DX®.

After the large clinical studies to determine the clinical validity of the gene signature profile assay (Paik et al., 2004), Onco*type* DX® test was offered as, and remains, an LDT. Arguably, it would be sensible for a commercial laboratory like Genomic Health to seek FDA clearance or approval on the relatively complex gene signature profiling test, thereby boosting the confidence of patients and ordering physicians. This is

what Agendia did with MammaPrint®. However, after the first FDA clearance of the Agendia test in 2007, there were five additional modifications (i.e., five submissions and approvals from FDA) for the same test (http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm accessed on 12/02/2012). All these processes require extra resources and implementation of the modifications represented delays for Agendia. With a single lab performing tests and a fast changing molecular field, Genomic Health chose the LDT practice, which is regulated by CLIA and inspected by College of American Pathologist (CAP), to manage any modifications of the test. After the first launch of the Onco*type* DX® test to predict the likelihood of the distant breast cancer recurrence, Genomic Health has validated Onco*type* DX® breast cancer test for ductal carcinoma in situ (DCIS) patients. Indication for DCIS was launched in 2011. Recently, Genomic Health launched a 12-gene Onco*type* DX® colon cancer test to predict the risk of disease recurrence in patients with newly diagnosed Stage II disease. They are working on gene signature profiling for prostate, renal and non-small cell lung cancer (http://www.genomichealth.com/en-US/Pipeline.aspx#.UMlVHYMmeRQ, accessed on 12/12/2012). Evidently, Genomic Health has made gene signature profiling a success in molecular diagnostics without embarking on the FDA clearance/approval path. Suffice it to say that an LDT is as good as an FDA approved or cleared test as long as analytical and clinical validation is performed properly, as in the case of Onco*type* DX ®.

Furthermore, long-term evaluation of the clinical validity and utility of a test offers a more complete picture to both laboratories and physicians. After the Onco*type* DX® breast cancer test was made available, continuing clinical studies on the 21-gene signature further proved the clinical validity and utility for breast cancer patients as what was initially stated (Cobleigh et al., 2005; Habel et al., 2006; Paik, 2007; Paik et al., 2004). There is no doubt that Medicare coverage, and recognition by ASCO and NCCN also play significant roles in the use of Onco*type* DX® breast cancer test in daily oncology practice.

4. Perspectives

The success of Onco*type* DX® has been a very encouraging development for the implementation of gene signature profiling in molecular diagnostic laboratories. Very recently, Pinpoint Genomics and University of California at San Francisco developed a 14-gene signature for non-squamous, non-small-cell lung cancer (Kratz et al., 2012). Pinpoint Genomics (now part of Life Technologies) has already started to offer the test to physicians.

Most service laboratories, however, do not have adequate resources to develop a new gene signature profiling test like venture funded companies like Genomic Health and Pinpoint Genomics. It is difficult to imagine how individual academic or hospital-based service laboratories can develop and implement similar gene signature tests simply from a clinical validity point of view, let alone a commercialization point of view. With these realities in mind, what can service laboratories do to mimic successful routes for implementation of personalized genomic medicine into daily practice?

The clearest route, it seems, is based on real-time PCR methodology coupled to FFPE samples; this dyad is very familiar to clinical laboratories and thus provides at least some measure of a competitive advantage. After evaluating the clinical utility of the test, initial clinical studies to select signature genes and build dry bench classifiers may then need collaboration among multiple clinical labs and/or researchers from within and outside the institution. Such a strategy would enable the institution to more easily accumulate a dataset that is large enough for statistical data analysis. Once the classifier is built, multiple sites would validate the test and verify the clinical validity and utility at their own institution. If the gene signature test is shown to be beneficial to patients, multi-center studies would also positively boost confidence in the test and possibly lead to inclusion in ASCO and NCCN guidelines for standard practice. Test reimbursement is also another important part of the equation. Tests that become a standard of clinical practice stand a better chance of Medicare reimbursement and coverage by other insurers.

The scope of clinical study needed to verify clinical validity and utility of a test is large. To successfully develop a gene signature test, therefore, may well require building a collaborative network of clinical laboratories and researchers. Such a "divide and conquer" approach allows individual labs to perform only part of a clinical study and not put a huge burden on any one site. This may be especially suitable for medium sized laboratories that have the capability to do more than just testing a single gene target, yet are not so well resourced as to undertake large clinical studies. While it is beneficial to combine resources from different labs and centers, communication among them is especially important to coordinate the execution and data integration to generate a meaningful result. Consequently, it is important to build standards on how to collect and process data and share resources in order to enable the proper communication among laboratories and institutions. The willingness to collaborate and share will expedite the adoption of personalized genomic medicine, such as gene signature profiling, into routine practice.

Gene signature profiling is complex and demands significant resources to develop as clinical testing. Thus, the proverbial questions are: (i) is a signature necessary (i.e., can the result improve patient care?), and (ii), how many genes are enough? One study indicated that the widely performed four immunohistochemical (IHC) markers, estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER2) and Ki-67, can provide the same information as Genomic Health's 21-gene signature profile test (Cuzick et al., 2011). These four markers are included (at the genomic, not protein, level) in the Onco*type* DX® breast cancer test (Paik et al., 2004). However, additional studies may be necessary to determine whether this can be generalized. Nevertheless, it indicates that gene signature profiling could start from a set of known genes that are associated with the same or different disease pathways. If the small group of genes cannot stratify patients, expanding to a larger gene set should next be considered. Additionally, RNAseq is a good candidate technology to facilitate diagnosis and treatment of clinically difficult cases as the whole genome sequencing for rare inherited disorders.

5. Conclusion

Overall, gene signature profiling is one of the great ways to enact personalized genomic medicine in this post-human genomic project era. Development of the high-throughput technologies, such as microarray, real-time PCR and NGS enables studying gene expression of many genes, even the entire transcriptome of a specimen simultaneously. The possibility that gene signature profiling can stratify patients into different molecular subgroups prompted the effort to develop gene signature profiling for clinical diagnosis and treatment. Such test, however, is much more complex than single gene testing and will change the way molecular laboratories function now. Statistical and bioinformatics support will be an important part of the "dry bench" development in clinical laboratories. To facilitate large-scale clinical studies for a new test, a network of clinical laboratories and researchers must work together and share responsibilities. Building standards on data collection and analysis methods, as well as proper communication protocols would also enhance the chance of success. Furthermore, continued assessment of the gene signature tests would yield more evidence and insights on the performance of the test and help determine the type of improvement needed to benefit diagnosis and patient treatment. Thus, while the individual ingredients for developing gene signature based tests are all available now, bringing them together and exploiting the required synergies is the challenge for clinical laboratories who have aspirations for such an undertaking. Given the differences in culture and practice among institutions, laboratories with the vision, complementing capabilities, staying power, and the collaborative spirit will have a better chance of taking full advantage of

the technological advances and bring the gene signature based tests to fruition.

Acknowledgment

The author would like to thank Daniel H. Farkas, PhD, HCLD for the critical review and comments on the manuscript.

References

Alizadeh, A.A., Eisen, M.B., Davis, R.E., Ma, C., Lossos, I.S., Rosenwald, A., Boldrick, J.C., Sabet, H., Tran, T., Yu, X., Powell, J.I., Yang, L., Marti, G.E., Moore, T., Hudson Jr., J., Lu, L., Lewis, D.B., Tibshirani, R., Sherlock, G., Chan, W.C., Greiner, T.C., Weisenburger, D.D., Armitage, J.O., Warnke, R., Levy, R., Wilson, W., Grever, M.R., Byrd, J.C., Botstein, D., Brown, P.O., Staudt, L.M., 2000. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 403, 503–511.

Beaudet, A.L., Feldman, G.L., Fernbach, S.D., Buffone, G.J., O'Brien, W.E., 1989. Linkage disequilibrium, cystic fibrosis, and genetic counseling. American Journal of Human Genetics 44, 319–326.

Becker-Andre, M., Hahlbrock, K., 1989. Absolute mRNA quantification using the polymerase chain reaction (PCR). A novel approach by a PCR aided transcript titration assay (PATTY). Nucleic Acids Research 17, 9437–9446.

Brenner, S., Johnson, M., Bridgham, J., Golda, G., Lloyd, D.H., Johnson, D., Luo, S., McCurdy, S., Foy, M., Ewan, M., Roth, R., George, D., Eletr, S., Albrecht, G., Vermaas, E., Williams, S.R., Moon, K., Burcham, T., Pallas, M., DuBridge, R.B., Kirchner, J., Fearon, K., Mao, J., Corcoran, K., 2000. Gene expression analysis by massively parallel signature sequencing (MPSS) on microbead arrays. Nature Biotechnology 18, 630–634.

Bueno-de-Mesquita, J.M., Linn, S.C., Keijzer, R., Wesseling, J., Nuyten, D.S., van Krimpen, C., Meijers, C., de Graaf, P.W., Bos, M.M., Hart, A.A., Rutgers, E.J., Peterse, J.L., Halfwerk, H., de Groot, R., Pronk, A., Floore, A.N., Glas, A.M., Van't Veer, L.J., van de Vijver, M.J., 2009. Validation of 70-gene prognosis signature in node-negative breast cancer. Breast Cancer Research and Treatment 117, 483–495.

Buyse, M., Loi, S., van't Veer, L., Viale, G., Delorenzi, M., Glas, A.M., d'Assignies, M.S., Bergh, J., Lidereau, R., Ellis, P., Harris, A., Bogaerts, J., Therasse, P., Floore, A., Amakrane, M., Piette, F., Rutgers, E., Sotiriou, C., Cardoso, F., Piccart, M.J., Consortium, T., 2006. Validation and clinical utility of a 70-gene prognostic signature for women with node-negative breast cancer. Journal of the National Cancer Institute 98, 1183–1192.

Chiang, P.W., Song, W.J., Wu, K.Y., Korenberg, J.R., Fogel, E.J., Van Keuren, M.L., Lashkari, D., Kurnit, D.M., 1996. Use of a fluorescent-PCR reaction to detect genomic sequence copy number and transcriptional abundance. Genome Research 6, 1013–1026.

Churchill, G.A., 2002. Fundamentals of experimental design for cDNA microarrays. Nature Genetics 32, 490–495 (Suppl.).

Cobleigh, M.A., Tabesh, B., Bitterman, P., Baker, J., Cronin, M., Liu, M.L., Borchik, R., Mosquera, J.M., Walker, M.G., Shak, S., 2005. Tumor gene expression and prognosis in breast cancer patients with 10 or more positive lymph nodes. Clinical Cancer Research 11, 8623–8631.

Cuzick, J., Dowsett, M., Pineda, S., Wale, C., Salter, J., Quinn, E., Zabaglo, L., Mallon, E., Green, A.R., Ellis, I.O., Howell, A., Buzdar, A.U., Forbes, J.F., 2011. Prognostic value of a combined estrogen receptor, progesterone receptor, Ki-67, and human epidermal growth factor receptor 2 immunohistochemical score and comparison with the Genomic Health recurrence score in early breast cancer. Journal of Clinical Oncology 29, 4273–4278.

Dave, S.S., Fu, K., Wright, G.W., Lam, L.T., Kluin, P., Boerma, E.J., Greiner, T.C., Weisenburger, D.D., Rosenwald, A., Ott, G., Muller-Hermelink, H.K., Gascoyne, R.D., Delabie, J., Rimsza, L.M., Braziel, R.M., Grogan, T.M., Campo, E., Jaffe, E.S., Dave, B.J., Sanger, W., Bast, M., Vose, J.M., Armitage, J.O., Connors, J.M., Smeland, E.B., Kvaloy, S., Holte, H., Fisher, R.I., Miller, T.P., Montserrat, E., Wilson, W.H., Bahl, M., Zhao, H., Yang, L., Powell, J., Simon, R., Chan, W.C., Staudt, L.M., Lymphoma/Leukemia Molecular Profiling, P., 2006. Molecular diagnosis of Burkitt's lymphoma. The New England Journal of Medicine 354, 2431–2442.

Duncan, A.M., Buchwald, M., Tsui, L.C., 1988. In situ hybridization of two cloned chromosome 7 sequences tightly linked to the cystic fibrosis locus. Cytogenetics and Cell Genetics 49, 309–310.

Eiberg, H., Mohr, J., Schmiegelow, K., Nielsen, L.S., Williamson, R., 1985. Linkage relationships of paraoxonase (PON) with other markers: indication of PON-cystic fibrosis synteny. Clinical Genetics 28, 265–271.

Estivill, X., Farrall, M., Scambler, P.J., Bell, G.M., Hawley, K.M., Lench, N.J., Bates, G.P., Kruyer, H.C., Frederick, P.A., Stanier, P., et al., 1987. A candidate for the cystic fibrosis locus isolated by selection for methylation-free islands. Nature 326, 840–845.

Gerbitz, A., Mautner, J., Geltinger, C., Hortnagel, K., Christoph, B., Asenbauer, H., Klobeck, G., Polack, A., Bornkamm, G.W., 1999. Deregulation of the proto-oncogene c-myc through t(8;22) translocation in Burkitt's lymphoma. Oncogene 18, 1745–1753.

Gerlinger, M., Rowan, A.J., Horswell, S., Larkin, J., Endesfelder, D., Gronroos, E., Martinez, P., Matthews, N., Stewart, A., Tarpey, P., Varela, I., Phillimore, B., Begum, S., McDonald, N.Q., Butler, A., Jones, D., Raine, K., Latimer, C., Santos, C.R., Nohadani, M., Eklund, A.C., Spencer-Dene, B., Clark, G., Pickering, L., Stamp, G., Gore, M., Szallasi, Z., Downward, J., Futreal, P.A., Swanton, C., 2012. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. The New England Journal of Medicine 366, 883–892.

Gibson, U.E., Heid, C.A., Williams, P.M., 1996. A novel method for real time quantitative RT-PCR. Genome Research 6, 995–1001.

Gilliland, G., Perrin, S., Blanchard, K., Bunn, H.F., 1990. Analysis of cytokine mRNA and DNA: detection and quantitation by competitive polymerase chain reaction. Proceedings of the National Academy of Sciences of the United States of America 87, 2725–2729.

Golub, T.R., Slonim, D.K., Tamayo, P., Huard, C., Gaasenbeek, M., Mesirov, J.P., Coller, H., Loh, M.L., Downing, J.R., Caligiuri, M.A., Bloomfield, C.D., Lander, E.S., 1999. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. Science 286, 531–537.

Habel, L.A., Shak, S., Jacobs, M.K., Capra, A., Alexander, C., Pho, M., Baker, J., Walker, M., Watson, D., Hackett, J., Blick, N.T., Greenberg, D., Fehrenbacher, L., Langholz, B., Quesenberry, C.P., 2006. A population-based study of tumor gene expression and risk of breast cancer death among lymph node-negative patients. Breast Cancer Research 8, R25.

Harris, L., Fritsche, H., Mennel, R., Norton, L., Ravdin, P., Taube, S., Somerfield, M.R., Hayes, D.F., Bast Jr., R.C., American Society of Clinical, O., 2007. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. Journal of Clinical Oncology 25, 5287–5312.

Hecht, J.L., Aster, J.C., 2000. Molecular biology of Burkitt's lymphoma. Journal of Clinical Oncology 18, 3707–3721.

Heid, C.A., Stevens, J., Livak, K.J., Williams, P.M., 1996. Real time quantitative PCR. Genome Research 6, 986–994.

Higuchi, R., Fockler, C., Dollinger, G., Watson, R., 1993. Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. Biotechnology (N Y) 11, 1026–1030.

Jaffe, E.S., 2009. The 2008 WHO classification of lymphomas: implications for clinical practice and translational research. American Society of Hematology Education Program 523–531.

Kerem, B., Rommens, J.M., Buchanan, J.A., Markiewicz, D., Cox, T.K., Chakravarti, A., Buchwald, M., Tsui, L.C., 1989. Identification of the cystic fibrosis gene: genetic analysis. Science 245, 1073–1080.

Klinger, K., Stanislovitis, P., Hoffman, N., Watkins, P.C., Schwartz, R., Doherty, R., Scambler, P., Farrall, M., Williamson, R., Wainwright, B., 1986. Genetic homogeneity of cystic fibrosis. Nucleic Acids Research 14, 8681–8686.

Knowlton, R.G., Cohen-Haguenauer, O., Van Cong, N., Frezal, J., Brown, V.A., Barker, D., Braman, J.C., Schumm, J.W., Tsui, L.C., Buchwald, M., et al., 1985. A polymorphic DNA marker linked to cystic fibrosis is located on chromosome 7. Nature 318, 380–382.

Kramer, M.H., Hermans, J., Wijburg, E., Philippo, K., Geelen, E., van Krieken, J.H., de Jong, D., Maartense, E., Schuuring, E., Kluin, P.M., 1998. Clinical relevance of BCL2, BCL6, and MYC rearrangements in diffuse large B-cell lymphoma. Blood 92, 3152–3162.

Kratz, J.R., He, J., Van Den Eeden, S.K., Zhu, Z.H., Gao, W., Pham, P.T., Mulvihill, M.S., Ziaei, F., Zhang, H., Su, B., Zhi, X., Quesenberry, C.P., Habel, L.A., Deng, Q., Wang, Z., Zhou, J., Li, H., Huang, M.C., Yeh, C.C., Segal, M.R., Ray, M.R., Jones, K.D., Raz, D.J., Xu, Z., Jahan, T.M., Berryman, D., He, B., Mann, M.J., Jablons, D.M., 2012. A practical molecular assay to predict survival in resected non-squamous, non-small-cell lung cancer: development and international validation studies. Lancet 379, 823–832.

Lander, E.S., Linton, L.M., Birren, B., Nusbaum, C., Zody, M.C., Baldwin, J., Devon, K., Dewar, K., Doyle, M., FitzHugh, W., Funke, R., Gage, D., Harris, K., Heaford, A., Howland, J., Kann, L., Lehoczky, J., LeVine, R., McEwan, P., McKernan, K., Meldrim, J., Mesirov, J.P., Miranda, C., Morris, W., Naylor, J., Raymond, C., Rosetti, M., Santos, R., Sheridan, A., Sougnez, C., Stange-Thomann, N., Stojanovic, N., Subramanian, A., Wyman, D., Rogers, J., Sulston, J., Ainscough, R., Beck, S., Bentley, D., Burton, J., Clee, C., Carter, N., Coulson, A., Deadman, R., Deloukas, P., Dunham, A., Dunham, I., Durbin, R., French, L., Grafham, D., Gregory, S., Hubbard, T., Humphray, S., Hunt, A., Jones, M., Lloyd, C., McMurray, A., Matthews, L., Mercer, S., Milne, S., Mullikin, J.C., Mungall, A., Plumb, R., Ross, M., Shownkeen, R., Sims, S., Waterston, R.H., Wilson, R.K., Hillier, L.W., McPherson, J.D., Marra, M.A., Mardis, E.R., Fulton, L.A., Chinwalla, A.T., Pepin, K.H., Gish, W.R., Chissoe, S.L., Wendl, M.C., Delehaunty, K.D., Miner, T.L., Delehaunty, A., Kramer, J.B., Cook, L.L., Fulton, R.S., Johnson, D.L., Minx, P.J., Clifton, S.W., Hawkins, T., Branscomb, E., Predki, P., Richardson, P., Wenning, S., Slezak, T., Doggett, N., Cheng, J.F., Olsen, A., Lucas, S., Elkin, C., Uberbacher, E., Frazier, M., Gibbs, R.A., Muzny, D.M., Scherer, S.E., Bouck, J.B., Sodergren, E.J., Worley, K.C., Rives, C.M., Gorrell, J.H., Metzker, M.L., Naylor, S.L., Kucherlapati, R.S., Nelson, D.L., Weinstock, G.M., Sakaki, Y., Fujiyama, A., Hattori, M., Yada, T., Toyoda, A., Itoh, T., Kawagoe, C., Watanabe, H., Totoki, Y., Taylor, T., Weissenbach, J., Heilig, R., Saurin, W., Artiguenave, F., Brottier, P., Bruls, T., Pelletier, E., Robert, C., Wincker, P., Smith, D.R., Doucette-Stamm, L., Rubenfield, M., Weinstock, K., Lee, H.M., Dubois, J., Rosenthal, A., Platzer, M., Nyakatura, G., Taudien, S., Rump, A., Yang, H., Yu, J., Wang, J., Huang, G., Gu, J., Hood, L., Rowen, L., Madan, A., Qin, S., Davis, R.W., Federspiel, N.A., Abola, A.P., Proctor, M.J., Myers, R.M., Schmutz, J., Dickson, M., Grimwood, J., Cox, D.R., Olson, M.V., Kaul, R., Raymond, C., Shimizu, N., Kawasaki, K., Minoshima, S., Evans, G.A., Athanasiou, M., Schultz, R., Roe, B.A., Chen, F., Pan, H., Ramser, J., Lehrach, H., Reinhardt, R., McCombie, W.R., de la Bastide, M., Dedhia, N., Blocker, H., Hornischer, K., Nordsiek, G., Agarwala, R., Aravind, L., Bailey, J.A., Bateman, A., Batzoglou, S., Birney, E., Bork, P., Brown, D.G., Burge, C.B., Cerutti, L., Chen, H.C., Church, D., Clamp, M., Copley, R.R., Doerks, T., Eddy, S.R., Eichler, E.E., Furey, T.S., Galagan, J., Gilbert, J.G., Harmon, C., Hayashizaki, Y., Haussler, D., Hermjakob, H., Hokamp, K., Jang, W., Johnson, L.S., Jones, T.A., Kasif, S., Kaspryzk, A., Kennedy, S., Kent, W.J., Kitts, P., Koonin, E.V., Korf, I., Kulp, D., Lancet, D., Lowe, T.M., McLysaght, A., Mikkelsen, T., Moran, J.V., Mulder, N., Pollara, V.J., Ponting, C.P., Schuler, G., Schultz, J., Slater, G., Smit, A.F., Stupka, E., Szustakowski, J., Thierry-Mieg, D., Thierry-Mieg, J., Wagner, L., Wallis, J., Wheeler, R., Williams, A., Wolf, Y.I., Wolfe, K.H., Yang, S.P., Yeh, R.F., Collins, F., Guyer, M.S., Peterson, J., Felsenfeld, A., Wetterstrand, K.A., Patrinos, A., Morgan, M.J., de Jong, P., Catanese, J.J., Osoegawa, K., Shizuya, H., Choi, S., Chen, Y.J., International Human Genome Sequencing, C., 2001. Initial sequencing and analysis of the human genome. Nature 409, 860–921.

Lashkari, D.A., DeRisi, J.L., McCusker, J.H., Namath, A.F., Gentile, C., Hwang, S.Y., Brown, P.O., Davis, R.W., 1997. Yeast microarrays for genome wide parallel genetic and gene expression analysis. Proceedings of the National Academy of Sciences of the United States of America 94, 13057–13062.

Margulies, M., Egholm, M., Altman, W.E., Attiya, S., Bader, J.S., Bemben, L.A., Berka, J., Braverman, M.S., Chen, Y.J., Chen, Z., Dewell, S.B., Du, L., Fierro, J.M., Gomes, X.V., Godwin, B.C., He, W., Helgesen, S., Ho, C.H., Irzyk, G.P., Jando, S.C., Alenquer, M.L., Jarvie, T.P., Jirage, K.B., Kim, J.B., Knight, J.R., Lanza, J.R., Leamon, J.H., Lefkowitz, S.M., Lei, M., Li, J., Lohman, K.L., Lu, H., Makhijani, V.B., McDade, K.E., McKenna, M.P., Myers, E.W., Nickerson, E., Nobile, J.R., Plant, R., Puc, B.P., Ronan, M.T., Roth, G.T., Sarkis, G.J., Simons, J.F., Simpson, J.W., Srinivasan, M., Tartaro, K.R., Tomasz, A., Vogt, K.A., Volkmer, G.A., Wang, S.H., Wang, Y., Weiner, M.P., Yu, P., Begley, R.F., Rothberg, J.M., 2005. Genome sequencing in microfabricated high-density picolitre reactors. Nature 437, 376–380.

Marioni, J.C., Mason, C.E., Mane, S.M., Stephens, M., Gilad, Y., 2008. RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. Genome Research 18, 1509–1517.

Maskos, U., Southern, E.M., 1992. Oligonucleotide hybridizations on glass supports: a novel linker for oligonucleotide synthesis and hybridization properties of oligonucleotides synthesised in situ. Nucleic Acids Research 20, 1679–1684.

Mayo, B.J., Klebe, R.J., Barnett, D.R., Lankford, B.J., Bowman, B.H., 1980. Somatic cell genetic studies of the cystic fibrosis mucociliary inhibitor. Clinical Genetics 18, 379–386.

Mook, S., Van't Veer, L.J., Rutgers, E.J., Piccart-Gebhart, M.J., Cardoso, F., 2007. Individualization of therapy using Mammaprint: from development to the MINDACT trial. Cancer Genomics & Proteomics 4, 147–155.

Mori, S., Rempel, R.E., Chang, J.T., Yao, G., Lagoo, A.S., Potti, A., Bild, A., Nevins, J.R., 2008. Utilization of pathway signatures to reveal distinct types of B lymphoma in the Emicro-myc model and human diffuse large B-cell lymphoma. Cancer Research 68, 8525–8534.

Morin, R., Bainbridge, M., Fejes, A., Hirst, M., Krzywinski, M., Pugh, T., McDonald, H., Varhol, R., Jones, S., Marra, M., 2008. Profiling the HeLa S3 transcriptome using randomly primed cDNA and massively parallel short-read sequencing. Biotechniques 45, 81–94.

Neri, A., Barriga, F., Knowles, D.M., Magrath, I.T., Dalla-Favera, R., 1988. Different regions of the immunoglobulin heavy-chain locus are involved in chromosomal translocations in distinct pathogenetic forms of Burkitt lymphoma. Proceedings of the National Academy of Sciences of the United States of America 85, 2748–2752.

Paik, S., 2007. Development and clinical utility of a 21-gene recurrence score prognostic assay in patients with early breast cancer treated with tamoxifen. The Oncologist 12, 631–635.

Paik, S., Shak, S., Tang, G., Kim, C., Baker, J., Cronin, M., Baehner, F.L., Walker, M.G., Watson, D., Park, T., Hiller, W., Fisher, E.R., Wickerham, D.L., Bryant, J., Wolmark, N., 2004. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. The New England Journal of Medicine 351, 2817–2826.

Perou, C.M., Sorlie, T., Eisen, M.B., van de Rijn, M., Jeffrey, S.S., Rees, C.A., Pollack, J.R., Ross, D.T., Johnsen, H., Akslen, L.A., Fluge, O., Pergamenschikov, A., Williams, C., Zhu, S.X., Lonning, P.E., Borresen-Dale, A.L., Brown, P.O., Botstein, D., 2000. Molecular portraits of human breast tumours. Nature 406, 747–752.

Rinn, J.L., Wang, J.K., Liu, H., Montgomery, K., van de Rijn, M., Chang, H.Y., 2008. A systems biology approach to anatomic diversity of skin. The Journal of Investigative Dermatology 128, 776–782.

Riordan, J.R., Rommens, J.M., Kerem, B., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenski, J., Lok, S., Plavsic, N., Chou, J.L., et al., 1989. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science 245, 1066–1073.

Rommens, J.M., Iannuzzi, M.C., Kerem, B., Drumm, M.L., Melmer, G., Dean, M., Rozmahel, R., Cole, J.L., Kennedy, D., Hidaka, N., et al., 1989. Identification of the cystic fibrosis gene: chromosome walking and jumping. Science 245, 1059–1065.

Rosenwald, A., Wright, G., Chan, W.C., Connors, J.M., Campo, E., Fisher, R.I., Gascoyne, R.D., Muller-Hermelink, H.K., Smeland, E.B., Giltnane, J.M., Hurt, E.M., Zhao, H., Averett, L., Yang, L., Wilson, W.H., Jaffe, E.S., Simon, R., Klausner, R.D., Powell, J., Duffey, P.L., Longo, D.L., Greiner, T.C., Weisenburger, D.D., Sanger, W.G., Dave, B.J., Lynch, J.C., Vose, J., Armitage, J.O., Montserrat, E., Lopez-Guillermo, A., Grogan, T.M., Miller, T.P., LeBlanc, M., Ott, G., Kvaloy, S., Delabie, J., Holte, H., Krajci, P., Stokke, T., Staudt, L.M., Lymphoma/Leukemia Molecular Profiling, 2002. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. The New England Journal of Medicine 346, 1937–1947.

Scambler, P., Robbins, T., Gilliam, C., Boylston, A., Tippett, P., Williamson, R., Davies, K.E., 1985. Linkage studies between polymorphic markers on chromosome 4 and cystic fibrosis. Human Genetics 69, 250–254.

Schena, M., Shalon, D., Davis, R.W., Brown, P.O., 1995. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 270, 467–470.

Schmitz, R., Young, R.M., Ceribelli, M., Jhavar, S., Xiao, W., Zhang, M., Wright, G., Shaffer, A.L., Hodson, D.J., Buras, E., Liu, X., Powell, J., Yang, Y., Xu, W., Zhao, H., Kohlhammer, H., Rosenwald, A., Kluin, P., Muller-Hermelink, H.K., Ott, G., Gascoyne, R.D., Connors, J.M., Rimsza, L.M., Campo, E., Jaffe, E.S., Delabie, J., Smeland, E.B., Ogwang, M.D., Reynolds, S.J., Fisher, R.I., Braziel, R.M., Tubbs, R.R., Cook, J.R., Weisenburger, D.D., Chan, W.C., Pittaluga, S., Wilson, W., Waldmann, T.A., Rowe, M., Mbulaiteye, S.M., Rickinson, A.B., Staudt, L.M., 2012. Burkitt lymphoma pathogenesis and therapeutic targets from structural and functional genomics. Nature 490, 116–120.

Sorlie, T., Perou, C.M., Tibshirani, R., Aas, T., Geisler, S., Johnsen, H., Hastie, T., Eisen, M.B., van de Rijn, M., Jeffrey, S.S., Thorsen, T., Quist, H., Matese, J.C., Brown, P.O., Botstein, D., Lonning, P.E., Borresen-Dale, A.L., 2001. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proceedings of the National Academy of Sciences of the United States of America 98, 10869–10874.

Trapnell, C., Williams, B.A., Pertea, G., Mortazavi, A., Kwan, G., van Baren, M.J., Salzberg, S.L., Wold, B.J., Pachter, L., 2010. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nature Biotechnology 28, 511–515.

Tsui, L.C., Buchwald, M., Barker, D., Braman, J.C., Knowlton, R., Schumm, J.W., Eiberg, H., Mohr, J., Kennedy, D., Plavsic, N., et al., 1985. Cystic fibrosis locus defined by a genetically linked polymorphic DNA marker. Science 230, 1054–1057.

van de Vijver, M.J., He, Y.D., van't Veer, L.J., Dai, H., Hart, A.A., Voskuil, D.W., Schreiber, G.J., Peterse, J.L., Roberts, C., Marton, M.J., Parrish, M., Atsma, D., Witteveen, A., Glas, A., Delahaye, L., van der Velde, T., Bartelink, H., Rodenhuis, S., Rutgers, E.T., Friend, S.H., Bernards, R., 2002. A gene-expression signature as a predictor of survival in breast cancer. The New England Journal of Medicine 347, 1999–2009.

van't Veer, L.J., Dai, H., van de Vijver, M.J., He, Y.D., Hart, A.A., Mao, M., Peterse, H.L., van der Kooy, K., Marton, M.J., Witteveen, A.T., Schreiber, G.J., Kerkhoven, R.M., Roberts, C., Linsley, P.S., Bernards, R., Friend, S.H., 2002. Gene expression profiling predicts clinical outcome of breast cancer. Nature 415, 530–536.

VanGuilder, H.D., Vrana, K.E., Freeman, W.M., 2008. Twenty-five years of quantitative PCR for gene expression analysis. Biotechniques 44, 619–626.

Venter, J.C., Adams, M.D., Myers, E.W., Li, P.W., Mural, R.J., Sutton, G.G., Smith, H.O., Yandell, M., Evans, C.A., Holt, R.A., Gocayne, J.D., Amanatides, P., Ballew, R.M., Huson, D.H., Wortman, J.R., Zhang, Q., Kodira, C.D., Zheng, X.H., Chen, L., Skupski, M., Subramanian, G., Thomas, P.D., Zhang, J., Gabor Miklos, G.L., Nelson, C., Broder, S., Clark, A.G., Nadeau, J., McKusick, V.A., Zinder, N., Levine, A.J., Roberts, R.J., Simon, M., Slayman, C., Hunkapiller, M., Bolanos, R., Delcher, A., Dew, I., Fasulo, D., Flanigan, M., Florea, L., Halpern, A., Hannenhalli, S., Kravitz, S., Levy, S., Mobarry, C., Reinert, K., Remington, K., Abu-Threideh, J., Beasley, E., Biddick, K., Bonazzi, V., Brandon, R., Cargill, M., Chandramouliswaran, I., Charlab, R., Chaturvedi, K., Deng, Z., Di Francesco, V., Dunn, P., Eilbeck, K., Evangelista, C., Gabrielian, A.E., Gan, W., Ge, W., Gong, F., Gu, Z., Guan, P., Heiman, T.J., Higgins, M.E., Ji, R.R., Ke, Z., Ketchum, K.A., Lai, Z., Lei, Y., Li, Z., Li, J., Liang, Y., Lin, X., Lu, F., Merkulov, G.V., Milshina, N., Moore, H.M., Naik, A.K., Narayan, V.A., Neelam, B., Nusskern, D., Rusch, D.B., Salzberg, S., Shao, W., Shue, B., Sun, J., Wang, Z., Wang, A., Wang, X., Wang, J., Wei, M., Wides, R., Xiao, C., Yan, C., Yao, A., Ye, J., Zhan, M., Zhang, W., Zhang, H., Zhao, Q., Zheng, L., Zhong, F., Zhong, W., Zhu, S., Zhao, S., Gilbert, D., Baumhueter, S., Spier, G., Carter, C., Cravchik, A., Woodage, T., Ali, F., An, H., Awe, A., Baldwin, D., Baden, H., Barnstead, M., Barrow, I., Beeson, K., Busam, D., Carver, A., Center, A., Cheng, M.L., Curry, L., Danaher, S., Davenport, L., Desilets, R., Dietz, S., Dodson, K., Doup, L., Ferriera, S., Garg, N., Gluecksmann, A., Hart, B., Haynes, J., Haynes, C., Heiner, C., Hladun, S., Hostin, D., Houck, J., Howland, T., Ibegwam, C., Johnson, J., Kalush, F., Kline, L., Koduru, S., Love, A., Mann, F., May, D., McCawley, S., McIntosh, T., McMullen, I., Moy, M., Moy, L., Murphy, B., Nelson, K., Pfannkoch, C., Pratts, E., Puri, V., Qureshi, H., Reardon, M., Rodriguez, R., Rogers, Y.H., Romblad, D., Ruhfel, B., Scott, R., Sitter, C., Smallwood, M., Stewart, E., Strong, R., Suh, E., Thomas, R., Tint, N.N., Tse, S., Vech, C., Wang, G., Wetter, J., Williams, S., Williams, M., Windsor, S., Winn-Deen, E., Wolfe, K., Zaveri, J., Zaveri, K., Abril, J.F., Guigo, R., Campbell, M.J., Sjolander, K.V., Karlak, B., Kejariwal, A., Mi, H., Lazareva, B., Hatton, T., Narechania, A., Diemer, K., Muruganujan, A., Guo, N., Sato, S., Bafna, V., Istrail, S., Lippert, R., Schwartz, R., Walenz, B., Yooseph, S., Allen, D., Basu, A., Baxendale, J., Blick, L., Caminha, M., Carnes-Stine, J., Caulk, P., Chiang, Y.H., Coyne, M., Dahlke, C., Mays, A., Dombroski, M., Donnelly, M., Ely, D., Esparham, S., Fosler, C., Gire, H., Glanowski, S., Glasser, K., Glodek, A., Gorokhov, M., Graham, K., Gropman, B., Harris, M., Heil, J., Henderson, S., Hoover, J., Jennings, D., Jordan, C., Jordan, J., Kasha, J., Kagan, L., Kraft, C., Levitsky, A., Lewis, M., Liu, X., Lopez, J., Ma, D., Majoros, W., McDaniel, J., Murphy, S., Newman, M., Nguyen, T., Nguyen, N., Nodell, M., Pan, S., Peck, J., Peterson, M., Rowe, W., Sanders, R., Scott, J., Simpson, M., Smith, T., Sprague, A., Stockwell, T., Turner, R., Venter, E., Wang, M., Wen, M., Wu, D., Wu, M., Xia, A., Zandieh, A., Zhu, X., 2001. The sequence of the human genome. Science 291, 1304–1351.

Wainwright, B.J., Scambler, P.J., Schmidtke, J., Watson, E.A., Law, H.Y., Farrall, M., Cooke, H.J., Eiberg, H., Williamson, R., 1985. Localization of cystic fibrosis locus to human chromosome 7cen-q22. Nature 318, 384–385.

Wainwright, B., Scambler, P., Farrall, M., Schwartz, M., Williamson, R., 1986. Linkage between the cystic fibrosis locus and markers on chromosome 7q. Cytogenetics and Cell Genetics 41, 191–192.

Wang, A.M., Doyle, M.V., Mark, D.F., 1989. Quantitation of mRNA by the polymerase chain reaction. Proceedings of the National Academy of Sciences of the United States of America 86, 9717–9721.

Wang, Z., Gerstein, M., Snyder, M., 2009. RNA-Seq: a revolutionary tool for transcriptomics. Nature Reviews Genetics 10, 57–63.

Watkins, P.C., Schwartz, R., Hoffman, N., Stanislovitis, P., Doherty, R., Klinger, K., 1986. A linkage study of cystic fibrosis in extended multigenerational pedigrees. American Journal of Human Genetics 39, 735–743.

Worthey, E.A., Mayer, A.N., Syverson, G.D., Helbling, D., Bonacci, B.B., Decker, B., Serpe, J.M., Dasu, T., Tschannen, M.R., Veith, R.L., Basehore, M.J., Broeckel, U., Tomita-Mitchell, A., Arca, M.J., Casper, J.T., Margolis, D.A., Bick, D.P., Hessner, M.J., Routes, J.M., Verbsky, J.W., Jacob, H.J., Dimmock, D.P., 2011. Making a definitive diagnosis: successful clinical application of whole exome sequencing in a child with intractable inflammatory bowel disease. Genetics in Medicine 13, 255–262.

Zhang, J., Ding, L., Holmfeldt, L., Wu, G., Heatley, S.L., Payne-Turner, D., Easton, J., Chen, X., Wang, J., Rusch, M., Lu, C., Chen, S.C., Wei, L., Collins-Underwood, J.R., Ma, J., Roberts, K.G., Pounds, S.B., Ulyanov, A., Becksfort, J., Gupta, P., Huether, R., Kriwacki, R.W., Parker, M., McGoldrick, D.J., Zhao, D., Alford, D., Espy, S., Bobba, K.C., Song, G., Pei, D., Cheng, C., Roberts, S., Barbato, M.I., Campana, D., Coustan-Smith, E., Shurtleff, S.A., Raimondi, S.C., Kleppe, M., Cools, J., Shimano, K.A., Hermiston, M.L., Doulatov, S., Eppert, K., Laurenti, E., Notta, F., Dick, J.E., Basso, G., Hunger, S.P., Loh, M.L., Devidas, M., Wood, B., Winter, S., Dunsmore, K.P., Fulton, R.S., Fulton, L.L., Hong, X., Harris, C.C., Dooling, D.J., Ochoa, K., Johnson, K.J., Obenauer, J.C., Evans, W.E., Pui, C.H., Naeve, C.W., Ley, T.J., Mardis, E.R., Wilson, R.K., Downing, J.R., Mullighan, C.G., 2012. The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. Nature 481, 157–163.

Zielenski, J., Rozmahel, R., Bozon, D., Kerem, B., Grzelczak, Z., Riordan, J.R., Rommens, J., Tsui, L.C., 1991. Genomic DNA sequence of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Genomics 10, 214–228.