

Company Overview

Rubicon Genomics is a pre-analytical platform company that has successfully developed and commercialized a unified approach for significantly improving the performance of DNA and RNA analytical instruments for research and diagnostics. The Rubicon platforms are a set of unique PCR-based processes that pre-amplify and standardize nucleic acids before genetic, expression or methylation analyses, thereby solving the common problems of insufficient or variable quantity and quality of the DNA/RNA. With standardized, pre-amplified nucleic acids the analytical instruments yield more accurate and reproducible results, and can be used to test for an unlimited number of analytes. These simple, low-cost pre-analytical solutions are proven to *increase sensitivity, specificity and robustness* of virtually all analytical platforms without modification of the platforms themselves. The performance of qPCR, microarray, and next-gen sequencing instruments are improved even for the most challenging samples such as single cells, plasma, serum, urine, circulating tumor cells and biopsies. Researchers are able to acquire more information from more samples, companies develop products faster, and clinicians better able to diagnose and manage disease using genetic, epigenetic and expression biomarkers.

The Rubicon pre-analytical processes have been used for the testing of many hundreds of thousands of samples in thousands of research laboratories, major pharmaceutical and diagnostics companies, and large international genomics initiatives. The results of those projects are numerous research publications, discovery of more than 50 genetic, epigenetic, and expression biomarkers, as well as a creation of a diverse set of diagnostic tests developed or in development. Rubicon is committed to playing a key role in nucleic acid research and clinical testing by improving the performance of the major research and diagnostic platforms, especially to enable more sensitive, specific, and robust clinical tests for cancer and other diseases.

Major Limitations of Nucleic Acid Analysis Platforms

DNA and RNA analysis platforms require that the sample nucleic acids meet specific requirements for quantity, quality, and purity. The platforms perform best when all samples have the same properties, sub-optimally when samples have variable properties even within the allowed range for the parameters, and fail for samples that fall outside the allowed range for the parameters. As a result, researchers sometimes have sample drop outs and incorrect results. Clinical applications have low tolerance for such problems and therefore testing must be limited to larger samples and fewer analytes in order that failures and false results do not occur. This precludes effective testing from plasma, serum, single cells, and many surgical and biopsy specimens. For over five years, customers and collaborators have employed Rubicon to process DNA and RNA to enable an expanding list of specific research and clinical applications to overcome practical limitations in nucleic acid quantity, quality, or purity.

Generic research and clinical examples of instrument-independent limitations include:

- Inability to test single cells (e.g., single blastomere or circulating tumor cell)
- Inability to assay DNA methylation with conventional qPCR or microarrays
- Erratic results or failures for small numbers of cells (e.g., circulating tumor cells, LCM)

- Inability to test multiple analytes or to profile rare or degraded DNA (e.g., plasma, serum)
- Inability to profile formalin-fixed DNA/RNA using microarrays or next-gen sequencing
- Poor quality data from many frozen surgical samples due to degradation
- Inability to use immunoprecipitation to profile DNA methylation or protein binding
- Variable test results from buccal swabs and blood cards
- Erratic detection of pathogens in environmental samples (air, soil)
- Depletion of valuable biospecimen resources

These generic limitations are important to all types of testing (mutations, genotypes, gene amplification/deletion/fusion, expression, and methylation) on all types of analytical platform (qPCR, microarrays, next-gen sequencing, and mismatch detection). Clearly, elimination of these limitations would enable new research and clinical applications. Although there are many pre-amplification methods that have been developed over the last 20 years to mitigate these limitations for specific applications, in the last three years the Rubicon platforms have been chosen by major government agencies, diagnostic companies, and institutions to give better sensitivity, representation and robustness as well as lower random background than other pre-analytical platforms such as MDA, LM-PCR and DOP PCR. In addition, Rubicon has the only platforms that are universally applicable across the full range of genetic, epigenetic, and expression analyses and instrumentation.

Rubicon Platforms as Unified Solutions to Major Analytical Limitations

Rubicon has successfully commercialized a series of PCR-based amplification platforms and stand-alone processes. Each platform synthesizes a molecular library that is amplified more than 1,000-fold to produce a standardized (constant concentration, molecular weight and purity) reamplifiable DNA library with minimal locus/allele bias and background. The company's GenomePlex™ platform is used to amplify and standardize total DNA for genetic analysis, its TransPlex™ platform amplifies and standardizes RNA sequences for expression analysis, and its MethylPlex™ platform selectively amplifies and standardizes the methylated portion of DNA for methylation analysis. These platforms increase the analytical sensitivity, specificity, reproducibility, and robustness of nucleic acid analyses for research and clinical applications. These improvements have been proven across virtually all the analytical platforms for analyzing genotypes, mutations, deletions and amplifications, aneuploidies, gene expression, presence of infectious agents, and DNA methylation. In fact, the ability to amplify and reamplify nucleic acids without introducing bias or background makes multi-analyte or genome-wide profiling routine even with very difficult clinical samples such as single cells, plasma/serum, and formalin-fixed tissue. Rubicon believes that all diagnostic and research platforms (including qPCR, microarray, and next-gen sequencing instruments) and most existing diagnostic assays can be significantly enhanced through the application of the Rubicon proprietary pre-analytical platforms.

In addition, Rubicon has an extensive portfolio of patented “derivative” pre-analytical processes that were originally developed for the platforms, but also have significant value as independent pre-analytical processes in their own right. Derivatives enable important improvements in conventional locus-specific qPCR, whole genome amplification of bisulfite converted DNA, simplification of dye labeling, novel hot-start PCR, complexity reduction for microarrays and next-gen sequencing, and single-tube preparation of molecules for next-gen sequencing

For research applications, these pre-analytical platforms and processes are simple and reproducible methods to achieve the highest quality results using very small samples. For diagnostics and pharmacogenomics, they enable discovery and validation of better biomarkers, shorter product development cycles, and patient tests that have higher performance.

Rubicon products have effectively addressed the needs of the nucleic acid market, achieving increasing revenue growth each year from kits, reagents, services, licenses, and partnerships. To date, the Rubicon pre-analytical platforms are accepted as standard operating procedure in many government and academic institutions, biorepositories, and diagnostic companies. The company's platforms have been used to improve the performance of many of the leading microarray, PCR, and sequencing platforms for specific applications in epidemiology, drug target discovery, and biomarker discovery and validation. In the field of diagnostics, an increasing number of Rubicon partners and customers are successfully incorporating Rubicon pre-analytical processes as integral parts in the most sensitive research and diagnostic products.

Rubicon Products

Rubicon has commercialized its pre-analytical products in multiple forms:

- Kits for research and diagnostics
- Contract services to biotech, pharma, and diagnostic companies and institutions
- OEM reagents for diagnostic manufacturing
- Partnerships to develop and commercialize cancer biomarkers and tests
- Out-licenses for cancer biomarkers
- Out-licenses for kits and processes

Research Products

Rubicon's strategy is to initially market its products in kit form to the research genetics and expression markets while providing premium pre-analytical and analytical services to pharmaceutical companies and large institutions. The first generation GenomePlex™ and TransPlex™ product lines have been exclusively licensed to Sigma-Aldrich for sale in the worldwide research market, specifically excluding all clinical applications. This approach has generated commercial validation of both methods, generated revenue and stimulated second-party development of advanced diagnostic applications. Recently, Rubicon developed a second generation GenomePlex™ platform for diagnostic applications; however, it too will find commercial application in the research markets, but under the Rubicon name. Simultaneously, the company has developed and commercialized the MethylPlex™ pre-analytical and analytical platforms for quantifying DNA methylation and is presently using this platform for in-house research as well as in fee-for-service projects with other companies. It is the company's intention to market the MethylPlex platform in kit format to the research market for discovery and testing of methylation biomarkers.

Diagnostic Products

Rubicon is bringing its GenomePlex, TransPlex, and MethylPlex pre-analytical platforms to the diagnostic markets for novel biomarker discovery and validation, and ultimately for clinical testing, using qPCR, microarrays or next-gen sequencing.

To improve existing diagnostic tests for genetic and expression abnormalities, Rubicon is collaborating with specific diagnostic companies to improve clinical tests for cancer and other

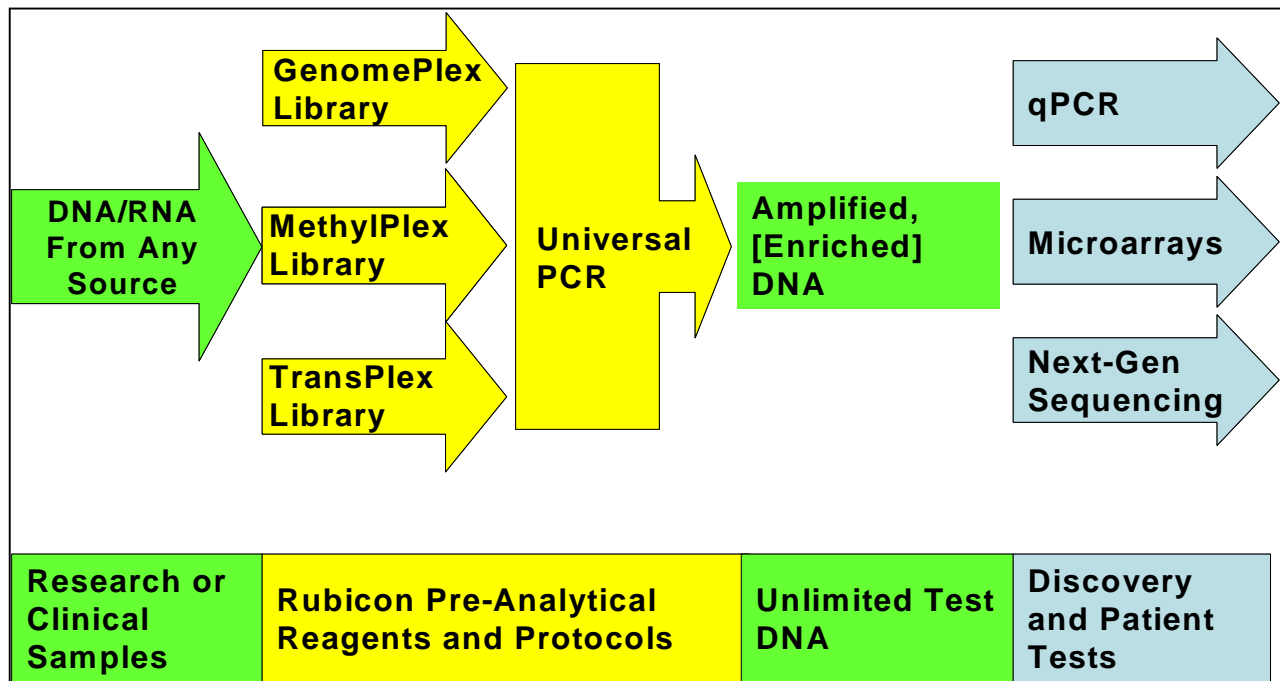
diseases. These diagnostic companies currently purchase or will purchase customized diagnostic GenomePlex or TransPlex kits from Rubicon that will be incorporated into their final tests to improve these DNA and RNA tests.

To improve existing and future clinical tests for abnormal DNA methylation, Rubicon has partnered with other diagnostic companies to use MethylPlex for the discovery and validation of new cancer biomarkers. These partnerships will result in ASR and IVD products that incorporate MethylPlex as the critical pre-analytical step. MethylPlex is a simpler method than bisulfite conversion to prepare DNA for methylation analysis and is the *only* method that enables multiple biomarkers to be analyzed from the same patient sample and that multiple biomarkers are essential to achieve the high sensitivity and specificity required for a commercially viable cancer test.

Technology

Rubicon's pre-analytical platforms substantially improve nucleic acids for downstream analysis by standardizing the quality and quantity of the nucleic acids, increasing the amount of nucleic acid that can be analyzed, and reducing the background from unwanted sequences. These improvements enable qPCR, microarray, and next-gen sequencing platforms to achieve higher sensitivity, specificity, and robustness. This has been validated for genotyping, aCGH, sequencing, and methylation analysis in research and diagnostic applications.

GenomePlex, TransPlex, and MethylPlex pre-analytical platforms are schematically displayed below. After conventional isolation of the DNA or RNA, the molecules are converted to amplifiable libraries consisting of total DNA, cDNA, or methylated DNA sequences. Each type of library is then amplified >1,000-fold using a universal-primer PCR reaction to produce DNA or cDNA of standardized concentration, molecular weight and quality, ready to be loaded onto any of the microarray, qPCR, or next-gen sequencing platforms for analysis. Any impurities such as oligonucleotides, unwanted salts, and PCR inhibitors are diluted >1,000-fold in the process. As a result of nucleic acid pre-amplification and standardization, the analytical platforms are operated under ideal conditions to produce more consistent results than could be produced from the unamplified DNA or RNA. For non-invasive testing, as little as 2 mL of plasma is sufficient for detection of an unlimited number of mutation or methylation biomarkers. For specialized analysis of specific parts of the genome, a proprietary method of sequence enrichment is used to reduce the sequence complexity of the amplified materials by ~100 times, which is especially important in microarray and next-gen sequencing applications. Synthesis, amplification, and enrichment of these libraries is rapid, inexpensive, and automatable on existing general-purpose instruments.



GenomePlex I (first-generation WGA product) is a patented method that employs quasi-random, non-self-complementary primers to make libraries for amplifying total genomic DNA with high fidelity. Highly representative and reproducible amplification can be achieved on intact or degraded DNA, as found in most clinical samples such as plasma, serum, urine, formalin-fixed, and necrotic frozen tissue. GenomePlex is more robust and reproducible than any of the other total DNA amplification products that have been brought to market over the years. Many investigators have published results showing that the GenomePlex amplified DNA performs exceptionally well for genotyping, sequencing, and gene copy number analysis using qPCR and microarray platforms.

GenomePlex II (second generation WGA product) is a patented method for simple, rapid, highly-efficient single-tube linker-mediated PCR for total genome amplification. It was initially developed to amplify highly fragmented DNA in plasma and serum for diagnostic applications; however, the method has been demonstrated to be superior to GenomePlex I in many research and diagnostic applications such as amplification and standardization of DNA from plasma, serum, and immunoprecipitation.

TransPlex is a patented method that uses quasi-random primers to make libraries for amplifying total cellular or viral RNA with high fidelity. Highly representative and reproducible amplification can be achieved from intact RNA, or degraded RNA as found in most clinical samples. TransPlex is very robust and reproducible and seems to be the only effective method to amplify RNA from fixed tissue. Several diagnostic companies and universities have chosen TransPlex as the standard pre-analytical platform for discovery of novel expression biomarkers for cancer.

MethylPlex represents several patented methods for selective amplification of the methylated portions of any genome utilizing very efficient quasi-random primers or ligation. MethylPlex is unique and elegant, and results in increasing the amount of DNA that is specific for the presence

and status of the tumor. MethylPlex is achieved in a simple, enzymatic process that completely avoids the bisulfite conversion chemical reactions used in other methylation assays. MethylPlex enables the discovery and validation of diagnostic biomarkers faster and more accurately than bisulfite methods and is the only method capable of measuring multiple methylated DNA analytes from a single sample of plasma or serum. MethylPlex has been chosen by multiple diagnostic companies to discover new cancer biomarkers and will be used as a pre-analytical step in patient tests. Not only is MethylPlex processing simple, rapid, and inexpensive, but it has also been automated on sophisticated diagnostic platforms.

Platform Combinations are useful for achieving maximal multiplexing among genetic, epigenetic, and expression assays. For example, total DNA and RNA can be amplified in a single reaction in a single tube for purposes of single-array detection of DNA and RNA viruses. Another example is the combination of methylation and gene copy number profiling on the same arrays. In principle, all three amplified libraries from a single patient samples can be combined and hybridized to a single array for simultaneous genetic, epigenetic, and expression analysis.

Derivative Rubicon Technologies are patented processes that have value independent of the amplification platforms. These include:

- Oligonucleotide compositions for whole genome amplification of bisulfite-converted DNA
- Oligonucleotide compositions to greatly reduce background of MDA WGA
- Oligonucleotide compositions for hot-start PCR with any polymerase
- Non-enzymatic random fragmentation to improve labeling for microarrays and FISH
- Non-enzymatic random fragmentation to increase reproducibility of conventional qPCR
- Enrichment procedures to reduce background on microarrays and next-gen sequencing
- Single-step preparation of amplimers for next-gen sequencing

Summary

Rubicon fills a novel and key role in nucleic acid testing. In response to the known problems in nucleic acid analysis caused by variations in DNA or RNA quantity and quality, Rubicon has developed elegant and effective pre-analytical processes for amplification, standardization and enrichment of nucleic acids for research and clinical applications. These tools are currently used in thousands of laboratories to improve genetic, epigenetic and expression research using the major analytical platforms., These pre-analytical processes will continue to greatly accelerate the discovery of new disease genes and biomarkers, as well produce the next generation of more sensitive and robust patient tests.

Contact Information

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