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Fred Russell Kramer

Personal

Birth	July 7, 1942 – New York City
Family	Married forty years, widowed, two children, four grandchildren

Education

1956 - 1959	The Bronx High School of Science
1959 - 1964	University of Michigan – B.S. with Honors in Zoology
1964 - 1969	The Rockefeller University – Ph.D. (with Vincent Allfrey)
1969 - 1972	Columbia University – Postdoctoral training (with Sol Spiegelman)

Appointments

1962 - 1964 Laboratory Technician, Cytogenetics Laboratory
Carnegie Institution of Washington, Ann Arbor, Michigan
(with Berwind Kaufmann and Helen Gay)

1969 - 1986 Department of Genetics and Development
and Institute of Cancer Research
College of Physicians and Surgeons
Columbia University

1969 - 1971	Fellow of the American Cancer Society
1971 - 1972	Research Associate
1972 - 1973	Instructor
1973 - 1980	Assistant Professor
1980 - 1983	Senior Research Associate
1983 - 1986	Research Scientist

1986 - present The Public Health Research Institute

1986 - present	Co-Director, Laboratory of Molecular Genetics
2000 - 2006	Director, Office of Technology Transfer
2006 - present	Associate Director for Business Development

1987 - 2014 Department of Microbiology
New York University School of Medicine

1987 - 2003	Research Professor
2003 - 2014	Adjunct Professor

2003 - present Professor of Microbiology, Biochemistry and Molecular Genetics
Public Health Research Institute, New Jersey Medical School

2003 - 2013	University of Medicine and Dentistry of New Jersey
2013 - present	Rutgers, The State University of New Jersey
2015 - present	Associate Member, Cancer Institute of New Jersey

Awards

2005 Jacob Heskel Gabbay Award in Biotechnology and Medicine
2023 Fellow of the National Academy of Inventors

Professional groups

American Association of University Professors
American Society for Biochemistry and Molecular Biology
American Society for Microbiology
Association for Molecular Pathology
New York Academy of Sciences
Society of the Sigma Xi

Fred Russell Kramer

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RESEARCH SYNOPSIS

For the past fifty four years, our laboratory has been exploring nucleic acid structure to understand the role that it plays in macromolecular interactions that control biological processes. The work has led to the design of novel nucleic acid molecules and the development of experimental techniques that enable the construction of extremely sensitive and specific molecular diagnostic assays. More than one hundred people have worked in the laboratory or participated as close collaborators. The following paragraphs provide sketchs of some of the significant research themes that our laboratory has pursued.

Mechanism of RNA replication

The laboratory studied the mechanism of RNA-directed RNA synthesis catalyzed by the bacteriophage polymerase, Qβ replicase. No one knew the mechanism by which the viral replicase selectively copies Qβ genomic RNA, while ignoring the vast number of other RNA molecules that are present in the bacterial host. Qβ RNA was too large to be studied with the techniques that were then available. However, we discovered a much smaller RNA (MDV-1 RNA) in Qβ-infected *Escherichia coli* that is replicated in the same manner as Qβ RNA (Kacian *et al*., 1972). Using classical enzymatic and electrophoretic techniques, we determined the complete nucleotide sequence of both complementary strands of MDV-1 RNA (Mills *et al*., 1973). This was the longest nucleic acid that had ever been sequenced. Knowledge of the sequence enabled experiments to be carried out that provided insights into the mechanism of RNA replication. We discovered that each complementary strand of MDV-1 RNA possessed extensive secondary structures (Klotz *et al*., 1980). We demonstrated that the rate of RNA synthesis was determined by pauses in polymerization that occur where secondary structures form in the nascent strand (Mills *et al.*, 1978), and we showed that structural reorganizations occur during product strand elongation (Kramer & Mills, 1981). We also developed an electrophoretic technique for separating the complementary strands that enabled the elucidation of the overall mechanism of RNA-directed RNA synthesis (Dobkin *et al*., 1979), and we utilized chemical and enzymatic nucleic acid modification methods to identify the sequences and structures that are required for the selective recognition of the RNA by the replicase, and for the initiation of product strand synthesis (Mills *et al*., 1980; Bausch *et al*., 1983; Nishihara *et al*., 1983).

Novel nucleic acid sequencing techniques

Rapid nucleic acid sequence analysis was essential to further studies of replication. We developed a chain-termination method for RNA sequence analysis (Kramer & Mills, 1978) at the same time that Fred Sanger developed a chain-termination method for DNA sequence analysis. Knowledge of the extensive secondary structure of MDV-1 RNA led us to realize that both of these sequencing techniques were compromised by the persistence of strong secondary structures during electrophoretic separation of the partially synthesized strands. We introduced a widely adopted solution to this problem, which was based on the use of modified nucleosides, such as inosine, that form weaker secondary structures (Mills & Kramer, 1979). Years later, we conceived novel techniques that enable entire genomes to be sequenced in a concerted manner by hybridization to oligonucleotide arrays (Chetverin & Kramer, 1993; 1994). These techniques were licensed exclusively to the Affymetrix Corporation (U.S. Patents 6,103,463 and 6,322,971).

In vitro evolution of replicating RNA populations

The *in vitro* replication of RNA by Qβ replicase provides a model system for studying precellular evolution. When MDV-1 RNA is replicated *in vitro*, the number of RNA molecules doubles every 20 seconds, resulting in an exponential increase in the number of RNA strands. Occasionally, errors occur during replication, producing RNA molecules with a mutated nucleotide sequence. When replication is carried out in the presence of an inhibitor of replication, mutant molecules that resist the inhibitor have a selective advantage, and if allowed to replicate for hundreds of generations, these mutants become predominant in the RNA population. Since phenotype and genotype reside in the same molecule, sequence analysis of the selected RNAs provided insights into the mechanism of Darwinian evolution.

Our laboratory carried out extensive studies on the *in vitro* evolution of replicating populations of MDV-1 RNA. Utilizing serial transfer techniques, hundreds of replicative generations could be completed in a day. By imposing different selective pressures, different variants emerged. Sequence analysis of the replicating RNA populations at different times during their evolution elucidated how the nucleotide changes that occurred conferred resistance to the particular inhibitor that was used (Kramer *et al*., 1974). Parallel molecular evolution experiments carried out in the presence of the chain elongation inhibitor, ethidium bromide, confirmed that many different genotypic pathways lead to the same phenotypic result, just as in the evolution of organisms. These experiments laid the foundation for modern *in vitro* selection techniques that are used to isolate nucleic acid molecules possessing predetermined catalytic activities.

The results of the *in vitro* evolution experiments also provided useful insights into the structural constraints that are required for an RNA to be replicatable. Though mutations occur everywhere in an RNA, the only mutations selected during the evolution of MDV-1 RNA occurred in single-stranded regions of the molecule, indicating that double-stranded structures are essential to the replicative process. When ribonuclease T1 was used as a selective agent, the mutants that arose were significantly resistant to the nuclease. The macromolecular dimensions of both the nuclease and the RNA limited cleavage to only a few sites on the exterior of the RNA molecule. The selected RNAs possessed non-cleavable nucleotide substitutions at just those exposed sites. These experiments elucidated the tertiary structure of MDV-1 RNA, enabling us to design exponentially amplifiable recombinant RNAs.

Recombinant RNAs

Many investigators wished to use the exponential amplification of RNA by Qβ replicase to synthesize large amounts of any mRNA or any genomic RNA. However, Qβ replicase is highly specific for Qβ phage RNA. We devised a scheme that enabled the replication of any heterologous RNA. Novel RNA templates were constructed by covalently inserting heterologous RNA sequences within the MDV-1 sequence at a single-stranded site that occurs on the exterior of the MDV-1 RNA molecule (Miele *et al*., 1983). The resulting recombinant RNAs possessed all of the secondary and tertiary structures that are required for replication, and the presence of the inserted sequence on the exterior of the molecule did not interfere with access to the structures required for replication. Consequently, Qβ replicase was able to catalyze the exponential synthesis of the entire recombinant RNA. Moreover, the recombinant RNAs were bifunctional, in that they retained the biological activity of the inserted sequence, as well as the replicatability of the MDV-1 RNA.

We constructed recombinant RNAs that contained the entire mRNA sequence encoding the protein chloramphenicol acetyltransferase. These recombinant molecules were amplified exponentially *in vitro* by incubation with Qβ replicase, and the replicated RNA served as template for the cell-free synthesis of enzymatically active chloramphenicol acetyltransferase (Wu *et al*., 1992). We demonstrated that these recombinant mRNAs could be continuously synthesized and that large quantities of biologically active protein could be produced in a coupled replication-translation system that contained both Qβ replicase and bacterial ribosomes (Ryabova *et al*., 1994). We also constructed amplifiable recombinant RNAs that contained entire viroid genomes (U.S. Patent 5,871,976), and the recombinant, by itself, was infectious when placed on the leaves of tomato plants.

Extremely sensitive gene detection assays

With the advent of the AIDS crisis, it became imperative that very sensitive assays be developed for the detection of pathogenic retroviruses. We realized that an attractive strategy for detecting rare targets is to link a nucleic acid probe to a replicatable reporter that can be amplified exponentially after hybridization to reveal the presence of the target (Chu *et al*., 1986). We therefore covalently linked MDV-1 RNA to an oligonucleotide probe that was complementary to a predetermined genetic target. The resulting molecules were used in assays in which the probes bind specifically to target sequences, unbound probes are washed away, and the probe-target hybrids are incubated with Qβ replicase to generate a large number of easily detected reporter molecules. Since as little as a single molecule of MDV-1 RNA can serve as template for the exponential synthesis of millions of RNA copies by Qβ replicase, these assays were extremely sensitive.

We also realized that it was simpler to perform these assays with recombinant MDV-1 RNA molecules in which a probe sequence is embedded within the MDV-1 RNA, rather than being attached to the RNA by a linker. We constructed recombinant-RNA probes and demonstrated that they were bifunctional, in that they bound specifically to their targets, and after they were bound they served as templates for their own exponential amplification (Lizardi *et al*., 1988). We demonstrated that recombinant-RNA hybridization probes could be used in sensitive gene detection assays (Lomeli *et al*., 1989; Kramer *et al*., 1992). The inclusion of intercalating fluorescent dyes, such as ethidium bromide, in the reaction mixtures to detect the reporter RNA enabled the assays to be carried out in real-time under homogeneous conditions in sealed tubes (Kramer & Lizardi, 1989; Lomeli *et al*., 1989). We also demonstrated that the time required to synthesize a given quantity of reporter RNA is inversely linearly proportional to the logarithm of the number of target molecules originally present in a sample, thus enabling quantitative determinations over an extremely wide range of target concentrations (U.S. Patent 5,503,979). This quantitative analytical technique has found wide application in real-time clinical assays that utilize polymerase chain reactions.

The sensitivity of Qβ replicase assays employing recombinant RNAs was limited by the inability to wash away every unbound probe. Persistent nonhybridized probes were amplified along with hybridized probes, generating a background signal that obscured the presence of rare targets. We investigated a number of different ways to eliminate this background (Kramer & Lizardi, 1989; Blok *et al*., 1997; U.S. Patents 5,118,801 and 5,312,728). Rather than trying to improve existing washing techniques (which were already quite efficient), we altered the design of the probes so that they could not be replicated unless they were hybridized to their target. We divided the recombinant-RNA probes into two separate molecules, neither of which could be amplified by itself, because neither contained all of the elements of sequence and structure that are required for replication by Qβ replicase. The division site was located in the middle of the embedded probe sequence. When these "binary probes" were hybridized to adjacent positions on their target sequence, they could be joined to each other by incubation with an appropriate ligase, generating a replicatable reporter RNA, which was then exponentially amplified by incubation with Qβ replicase. Nonhybridized probes, on the other hand, because they were not aligned on a target, could not be ligated, and signal generation was strictly dependent on the presence of target molecules. Because there were no background signals, the resulting assays were extraordinarily sensitive. As little as a single HIV-1 infected cell could be detected in samples containing 100,000 uninfected lymphocytes (Tyagi *et al*., 1996). This technique was licensed to Vysis (U.S. Patents 5,759,773 and 5,807,674) and was used in automated assays that detect the genes of many different infectious agents in human clinical samples.

Molecular beacons

We invented novel hybridization probes called "molecular beacons," which enable the direct detection of specific nucleic acids in living cells and in diagnostic assays (Tyagi & Kramer, 1996; Kramer *et al*., 2009). These probes are hairpin-shaped oligonucleotides with a fluorophore at one end and a nonfluorescent quencher at the other end. When they are not bound to a target nucleic acid, the fluorophore is in contact with the quencher and the probes are dark. When these probes bind to their targets, they undergo a conformational reorganization that separates the fluorophore from the quencher, resulting in a bright fluorescence signal that indicates the presence of the target. Because these probes only fluoresce when they are bound to target sequences, there is no need to isolate the probe-target hybrids to determine the amount of target present in a sample.

We showed that the mechanism of fluorescence quenching involves the transient formation of a nonfluorescent fluorophore-quencher complex, thus any desired fluorophore can be used as a label (Tyagi *et al*., 1998; Marras *et al*., 2002). When a set of molecular beacons are prepared, each specific for a different target sequence, and each labeled with a differently colored fluorophore, different nucleic acid targets can be detected simultaneously in the same assay tube or in the same cell. Moreover, by taking their thermodynamic behavior into consideration (Bonnet *et al*., 1999), molecular beacons can be designed so that they are significantly more specific than corresponding conventional linear hybridization probes. Molecular beacons can be designed in such a manner that the presence of even a single nucleotide substitution in a target sequence prevents the formation of a probe-target hybrid (Tyagi *et al*., 1998).

Our laboratory demonstrated the advantages of using molecular beacons as amplicon detector probes in quantitative, real-time, exponential amplification assays. We designed extremely sensitive, multiplex, clinical PCR assays that simultaneously detect four different pathogenic retroviruses in blood (Vet *et al*., 1999); and we designed "wavelength-shifting" molecular beacons (Tyagi *et al*., 2000) that enable many different genetic targets to be detected simultaneously in the same sample, utilizing simple instruments that possess a monochromatic light source. We also pioneered the use of molecular beacons for high-throughput "spectral genotyping" (Kostrikis *et al*., 1998); and we demonstrated the ease with which molecular beacons can distinguish single-nucleotide polymorphisms in PCR assays (Marras *et al*., 1999). We showed that molecular beacons work well in NASBA assays (Van Beuningen *et al*., 2001), as well as in PCR assays; and we demonstrated how molecular beacons can be used to monitor *in vitro* transcription in real time (Marras *et al*., 2004).

Our laboratory also designed a panel of assays that identify mutations in potential parents that cause Tay-Sachs disease and cystic fibrosis in the children of Ashkenazi Jews; and we developed a single-tube, multiplex assay that utilizes molecular beacons for the detection of bacteria that can be used as agents of bioterror (Varma-Basil *et al*., 2004a): *Yersinia pestis*, *Bacillus anthracis*, *Burkholderia mallei*, and *Francisella tularensis*. We also developed a single-tube version of a PCR assay that rapidly identifies multidrug-resistant *Mycobacterium tuberculosis* in sputum samples (El-Hajj *et al*., 2001). This assay underwent clinical trials (Varma-Basil *et al*., 2004b), was developed for commercial distribution, was endorsed by the World Health Organization, and is now the principal assay for the direct detection of tuberculosis utilized throughout the world. We have also contributed to the development of assays that detect hospital-acquired infections caused by pathogenic fungi and by methicillin-resistant and vancomycin-resistant *Staphylococcus aureus*.

Highly multiplex screening assays

Our laboratory has developed multiplex screening assays that utilize color-coded molecular beacons in single-tube gene amplification reactions that identify which infectious agent, if any, is present in a clinical sample (U.S. Patents 7,385,043 and 7,771,949). The first assay of this type is able to identify the 15 most prevalent bacterial species that are found in blood samples taken from febrile patients (Marras *et al*., 2018). Unlike classical blood cultures, which take many days to yield results, these “molecular blood cultures” require only two hours to complete. Each of the 15 species-specific molecular beacons is labeled with a unique combination of two differently colored fluorophores selected from a palette of six differently colored fluorophores. The two-color fluorescence signal that arises during the course of a PCR assay that amplifies a segment of the bacterial 16S ribosomal RNA gene uniquely identifies the species that is present. Future assays will utilize three differently colored fluorophores (selected from a palette of seven colors) to uniquely label each of 35 species-specific molecular beacons. This will enable simultaneous screening for the presence of both common species and rarely seen species, such as agents of bioterror. Widespread use of these assays will enable the rapid identification of common infectious agents, while at the same time providing an early warning system that will help contain the spread of major epidemics.

We have also designed highly multiplex screening assays based on a different principle. In these assays, only four differently colored molecular beacons are present during the amplification of a segment of the bacterial 16S ribosomal RNA gene. Unlike the assays described above, these molecular beacons contain relatively long probe sequences, enabling them to bind to amplified 16S ribosomal RNA gene

segments generated from many different bacterial species. The stability of each of the four resulting probe-target hybrids depends upon how well each of the molecular beacons matches the amplified target sequence. After amplification, the mixture of fluorescent probe-target hybrids is melted apart by raising the temperature and simultaneously determining, for each of the four differently colored probes, the temperature at which each hybrid falls apart (seen as a loss of fluorescence). The resulting set of four melting temperatures serves as a unique spectral signature that identifies which species is present (U.S. Patents 7,662,550 and 9,260,761). We demonstrated that 27 different species of *mycobacteria* can be uniquely identified with the aid of only four of these “sloppy” molecular beacons (El-Hajj *et al*., 2009); and we demonstrated that sloppy molecular beacon probes can identify 94 different species of bacteria (across 64 genera) in rapid, PCR assays designed to detect and identify bacterial species that can cause sepsis if present in the bloodstream (Chakravorty *et al*., 2010).

Self-reporting oligonucleotide arrays

We have demonstrated that molecular beacons are useful for the determination of gene expression profiles (Manganelli *et al*., 1999; Dracheva *et al*., 2001). We are exploring the use of arrays of molecular beacons for the simultaneous quantitation of hundreds of different mRNAs in a sample. Each molecular beacon is immobilized at a different location on the surface of a glass chip. Instead of enzymatically adding a fluorophore to the target mRNAs and hybridizing those targets to an array of linear probes, when an array consists of immobilized molecular beacons, the mRNAs need not be labeled and the molecular beacons become fluorescent when the targets bind to them. Hairpin-shaped probes are significantly more specific than linear probes, and the intensity of the fluorescence generated by the molecular beacons is directly proportional to the number of mRNAs that are bound.

We have also investigated distributed array formats, in which many different molecular beacon probes are used at the same time. Each type of molecular beacon probe is immobilized on a different microbead, and tens of thousands of beads are used in an assay. After hybridization to a mixture of mRNAs, the fluorescence of each bead is rapidly read by a spectral analyzer that determines the number of target mRNAs bound to each bead from the fluorescence of the molecular beacons on its surface. In order to facilitate this approach, we have developed a rapid method for telling which bead contains which probe (U.S. Patent 7,741,031). In this technique, additional hairpin-shaped nucleic acids possessing quenchers and differently colored fluorophores at each end are also immobilized on the surface of each bead. These additional hairpins do not serve as probes; instead the presence or absence of each hairpin serves as a binary element in a “serial number” that identifies the bead to which they are attached. For example, three different-length hairpins can be used, each labeled with one of five differently colored fluorophores, for a total of 15 distinctive elements that can be present or absent on the surface of the bead. The serial number of each bead in a collection of perhaps 100,000 beads is then simultaneously read by raising the temperature and noting, for each bead, which fluorescent colors appear on the surface of the bead as the temperature is raised, causing the three different-length hairpins to denature. The availability of practical gene expression profiling arrays should enable the identification of gene ensembles that control development, the discovery of new metabolic pathways, the exploration of cellular responses to viral and bacterial infection, and the development of high-throughput assays that identify new therapeutic agents.

Detection of mRNAs in living cells

One of the most exciting programs in our laboratory is the direct detection of mRNAs in living cells. Conventional *in situ* hybridization techniques require the "fixing" of cells to enable the unbound probes to be washed away. Fixing denatures and crosslinks the proteins, resulting in cell death. Thus, *in situ* hybridization provides a static view of mRNA distribution and is not effective for the investigation of dynamic processes. Because molecular beacons only become fluorescent when they bind to their target, there is no need to fix and wash the cells, and the synthesis, movement, localization, and disappearance of mRNAs can be viewed as a function of time. We have shown that molecular beacons are excellent probes for visualizing mRNAs in living cells, and we have used them in experiments with many different cell types. We found that molecular beacons can be synthesized from modified nucleotides that do not occur naturally, such as the 2'-*O*-methylribonucleotides, in order to prevent digestion of the molecular beacons by cellular nucleases and to prevent cleavage of the target mRNAs by cellular ribonuclease H. We also found that the interfering effects of autofluorescence from cellular

components can be overcome by using wavelength-shifting molecular beacons, which have large Stokes shifts that enable them to fluoresce at longer wavelengths (Tyagi *et al*., 2000). Furthermore, molecular beacons are not toxic to cells, and different mRNAs in the same cell can be visualized simultaneously with differently colored molecular beacons. And finally, we have linked molecular beacons to tRNA sequences in order to ensure that the probes are retained within the cytoplasm (Mhlanga *et al*., 2005).

The injection of molecular beacons into living cells allows the expression of particular genes to be monitored as a function of genetically programmed development, or as a response to external stimulation. With the aid of deconvolving and confocal fluorescence microscopy, we used molecular beacons to visualize the formation, transport, and localization of *oskar* mRNA in living *Drosophila* embryos (Bratu *et al*., 2003). We also used molecular beacons to follow the movement of β-actin mRNA into growing lamellipodia as lymphocytes move across surfaces. In addition, we used molecular beacons to track the movement and localization of CaMKII, Map-2, β-actin, and Arc mRNA in primary cultures of rat hippocampal neurons, in order to understand how the stimulation of presynaptic dendrites leads to mRNA localization and to the long-term potentiation of postsynaptic dendrites, which is an attractive model system for studying cellular mechanisms of memory formation (Batish *et al*., 2012). We followed the transport of specific mRNAs from the neuronal nucleus to postsynaptic dendritic sites, to determine the kinetics of mRNA movement and to elucidate the mechanism by which mRNAs are localized in stimulated dendrites.

Tracking Individual mRNA molecules

Although the fluorescence from a single molecular beacon bound to an mRNA is not sufficiently bright to be seen above the background fluorescence in a living cell, we devised a method that enables 96 molecular beacons to bind to a single mRNA molecule, which allows specific mRNAs to be seen and followed as they are synthesized, processed, and move within the nucleus and through the nuclear pores to the cytoplasm (Vargas *et al*., 2005). The technique that we developed involves the cloning of a synthetic sequence into the region of a target gene that encodes the 3'-untranslated region of the particular mRNA molecules that we wish to see and follow. The synthetic sequence contains 96 tandemly repeated molecular beacon binding sites. The presence of 96 probes on the 3' end of each mRNA does not prevent the binding of nuclear proteins. The motion of these individual mRNA-protein complexes were recorded by time-lapse photography. Analysis of their tracks demonstrated that they move freely by Brownian diffusion within the extranucleolar, interchromatin space.

Detection of somatic mutations that characterize cancer cells

Our laboratory has long been interested in developing PCR assays that use allele-discriminating gene amplification primers to detect DNA sequences containing rare somatic mutations that occur in cancer cells, without interference from abundant closely related normal DNA sequences (U.S. Patents 6,277,607 and 6,365,729). In the past few years, we have designed “SuperSelective” PCR primers that are able to detect as few as 10 mutant DNA fragments in the presence of 1,000,000 closely related wild-type fragments (Vargas *et al*., 2016; U.S. Patents 9,909,159 and 10,815,512). Moreover, we have added unique tag sequences to each SuperSelective primer that enable the resulting amplicons to be detected with differently colored molecular beacons. And most recently, we have found that the addition of selectivity enhancing agents (such as tetramethylammonium chloride or *bis*-tetramethylammonium oxalate) to the SuperSelective PCR buffer can completely suppress the amplification of normal gene sequences (Vargas *et al*., 2018).

Our current work involves the development of multiplex PCR assays that detect somatic mutations relevant to the selection of an appropriate therapy for individual cancer patients, utilizing DNA fragments that occur transiently in routine blood samples (liquid biopsies). During treatment, liquid biopsies can document the effectiveness of therapy, as the target mutations become less abundant. The beauty of this non-invasive approach is that these multiplex SuperSelective PCR assays will be so sensitive that the occurrence of a resistance mutation can be detected long before cells possessing that mutation cause a recurrence of symptoms. Moreover, the mutational profile of the patient at that time will enable therapy to be altered to take into account that patient’s particular somatic-mutational situation. These assays will be sensitive, rapid, and inexpensive, and should provide an efficient route towards achieving advances in the treatment of different cancers.

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Highly multiplex screening assays that utilize molecular beacons

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El-Hajj H, Marras SAE, Tyagi S, Kramer FR, and Alland D (2001) Detection of rifampin resistance in *Mycobacterium tuberculosis* in a single tube with molecular beacons. J Clin Microbiol 39, 4131-4137.

Varma-Basil M, El-Hajj H, Colangeli R, Hazbón MH, Kumar S, Bose M, Bobadilla del Valle M, Garcia LG, Hernandez A, Kramer FR, Osornio JS, Ponce de León A, and Alland D (2004b) Rapid detection of rifampin resistance in *Mycobacterium tuberculosis* isolates from India and Mexico by a molecular beacon assay. J Clin Microbiol 42, 5512-5516.

Highly selective nucleic acid amplification primers for multiplex PCR assays

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Wu F, Della-Latta P, Tyagi S, and Kramer FR (2010) Detection of pathogenic organisms with multicolor molecular beacons. In "Molecular Microbiology: Diagnostic Principles and Practice, 2nd Edition," Persing DH, Tenover FC, Tang Y-W, Nolte FS, Hayden RT, and van Belkum A, eds, American Society of Microbiology, Washington, DC, 245-254.

Patents and Patent Applications

AUTOCATALYTIC REPLICATION OF RECOMBINANT RNA

Kramer FR, Miele EA, and Mills DR
Columbia University
Licensed Exclusively to Gene-Trak Systems — 1988 to 1999
Licensed Exclusively to EvoGenix — 2006 to 2012

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
United States	05/25/1984	06/614,350	11/22/1988	4,786,600
United States	12/05/1994	08/349/379	04/15/1997	5,620,870
United States	06/06/1995	08/467,816	02/16/1999	5,871,976

REPLICATIVE RNA REPORTER SYSTEMS

Chu B, Kramer FR, Lizardi P, and Orgel LE
Columbia University and The Salk Institute for Biological Studies
Licensed Exclusively to Gene-Trak Systems — 1988 to 1999

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
United States	04/16/1988	06/852,692	09/18/1990	4,957,858
United States	09/22/1992	07/949,766	11/15/1994	5,364,760
Australia	04/15/1987	73068/87	06/21/1990	600942
Austria	04/15/1987	87903131.8	05/18/1994	0266399
Belgium	04/15/1987	87903131.8	05/18/1994	0266399
France	04/15/1987	87903131.8	05/18/1994	0266399
Germany	04/15/1987	87903131.8	05/18/1994	P3789849.3
Great Britain	04/15/1987	87903131.8	05/18/1994	0266399
Italy	04/15/1987	87903131.8	05/18/1994	0266399
Liechtenstein	04/15/1987	87903131.8	05/18/1994	0266399
Luxembourg	04/15/1987	87903131.8	05/18/1994	0266399
Sweden	04/15/1987	87903131.8	05/18/1994	0266399
Switzerland	04/15/1987	87903131.8	05/18/1994	0266399
The Netherlands	04/15/1987	87903131.8	05/18/1994	0266399

METHOD OF USING REPLICATABLE HYBRIDIZABLE RECOMBINANT RNA PROBES

Kramer FR and Lizardi PM
Columbia University
Licensed Exclusively to Gene-Trak Systems — 1988 to 1999

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
United States	08/26/1994	08/296,866	04/02/1996	5,503,979
Australia	04/20/1989	35407/89	08/17/1992	632047
Canada	04/17/1989	596864	08/04/1998	1339981
France	04/19/1989	89107037.7	05/31/1995	0346594
Germany	04/19/1989	89107037.7	05/31/1995	68922882.1
Great Britain	04/19/1989	89107037.7	05/31/1995	0346594
Italy	04/19/1989	89107037.7	05/31/1995	0346594
Japan	04/20/1989	1-505056	05/26/2000	3071796

REPLICATIVE RNA-BASED AMPLIFICATION/DETECTION SYSTEMS

Axelrod VD, Kramer FR, Lizardi PM, and Mills, DR
Columbia University
Licensed Exclusively to Gene-Trak Systems — 1988 to 1999

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
United States	07/02/1992	07/908,833	10/18/1994	5,356,774
United States	10/17/1994	08/323,631	04/15/1997	5,620,851
Canada	09/07/1989	610547	08/11/1998	1339991
France	09/08/1989	89910808.8	08/21/1996	0386228
Germany	09/08/1989	89910808.8	08/21/1996	68926999.4
Great Britain	09/08/1989	89910808.8	08/21/1996	0386228
Italy	09/08/1989	89910808.8	08/21/1996	0386228
Japan	09/08/1989	1-510113	05/26/2000	3071797

TARGET-DEPENDENT SYNTHESIS OF AN ARTIFICIAL GENE FOR THE SYNTHESIS OF A REPLICATABLE RNA

Kramer FR and Lizardi PM
Assigned to Gene-Trak Systems — 1989

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
United States	05/26/1989	07/358,399	05/12/1992	5,112,734
France	05/25/1990	90908800.7	04/12/1995	0473693
Germany	05/25/1990	90908800.7	04/12/1995	69018631.2
Great Britain	05/25/1990	90908800.7	04/12/1995	0473693
Italy	05/25/1990	90908800.7	04/12/1995	0473693
Japan	05/25/1990	2-508298	06/29/2001	3205555

NUCLEIC ACID PROBES CONTAINING AN IMPROVED MOLECULAR SWITCH

Lizardi PM, Kramer FR, Tyagi S, Guerra CE, and Lomeli-Buyoli HM
The Public Health Research Institute and The Salk Institute for Biological Studies
Licensed Exclusively to Gene-Trak Systems — 1988 to 1999

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
United States	09/30/1988	07/251,696	06/02/1992	5,118,801
Australia	09/29/1989	43466/89	09/29/1989	647376
Finland	09/29/1989	911536	09/29/1989	911536
France	09/29/1989	89911480.5	04/17/1996	0436644
Germany	09/29/1989	89911480.5	04/17/1996	68926302.3
Great Britain	09/29/1989	89911480.5	04/17/1996	0436644
Italy	09/29/1989	89911480.5	04/17/1996	0436644
Japan	09/29/1989	510691/89	07/24/1998	2806455
Spain	09/28/1989	8903275	11/21/1991	2023290

ASSAYS AND KITS INCORPORATING NUCLEIC ACID PROBES CONTAINING AN IMPROVED MOLECULAR SWITCH

Lizardi PM, Kramer FR Tyagi S, Guerra CE, Lomeli-Buyoli HM, Chu BC, Joyce GF, and Orgel LE
The Public Health Research Institute and The Salk Institute for Biological Studies
Licensed Exclusively to Gene-Trak Systems — 1988 to 1999

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
United States	05/04/1992	07/878,230	05/17/1994	5,312,728

REPLICATABLE HYBRIDIZABLE RECOMBINANT RNA PROBES AND METHODS OF USING SAME

Kramer FR, Lizardi PM, Miele EA, and Mills DR
Columbia University
Licensed Exclusively to Gene-Trak Systems — 1988 to 1999

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
United States	07/07/1995	08/484,992	07/16/2002	6,420,539

SENSITIVE NUCLEIC ACID SANDWICH HYBRIDIZATION ASSAYS AND KITS

Tyagi S, Kramer FR, Lizardi PM, Landegren UD, and Blok HJ
The Public Health Research Institute
Licensed Exclusively to Vysis — 1994 to 1999

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
United States	02/24/1995	08/393,888	06/02/1998	5,759,773
France	01/14/1994	94906682.3	05/22/2002	0688366
Germany	01/14/1994	94906682.3	05/22/2002	69430665.7
Great Britain	01/14/1994	94906682.3	05/22/2002	0688366
Italy	01/14/1994	27343BE/2002	05/22/2002	0688366
Japan	01/14/1994	6-516371	03/10/2006	3778925

DIAGNOSTIC ASSAYS AND KITS FOR RNA USING RNA BINARY PROBES AND A RIBOZYME LIGASE

Lizardi PM, Tyagi S, Landegren UD, Kramer FR, and Szostak JW
The Public Health Research Institute
Licensed Exclusively to Vysis — 1994 to 1999

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
United States	07/15/1996	08/683,045	07/29/1997	5,652,107

SELECTION OF RIBOZYMES THAT EFFICIENTLY CLEAVE TARGET RNA

Kramer FR, Dubnau D, Drlica KA, and Pinter A
The Public Health Research Institute

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
United States	08/18/1992	07/931,560	04/01/1997	5,616,459
Australia	07/16/1991	85175/91	11/09/1994	651380
Canada	07/16/1991	2,087,519	10/22/2002	2,087,519
Germany	07/16/1991	91916167.9	01/26/2000	69131942.1
France	07/16/1991	91916167.9	01/26/2000	0600877
Great Britain	07/16/1991	91916167.9	01/26/2000	0600877
Italy	07/16/1991	91916167.9	01/26/2000	0600877

CELL-FREE METHOD FOR SYNTHESIZING A PROTEIN

Kramer FR, Miele EA, and Mills DR
Columbia University
Licensed Exclusively to EvoGenix — 2006 to 2012

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
United States	04/29/1994	08/235,199	02/11/1997	5,602,001

COUPLED REPLICATION-TRANSLATION METHODS AND KITS FOR PROTEIN SYNTHESIS

Wu Y, Ryabova LA, Kurnasov OV, Morosov IY, Ugarov VI, Volianik EV, Chetverin AB, Zhang D, Kramer FR, and Spirin AS
The Public Health Research Institute
Co-marketing Agreement with the Institute of Protein Research, Pushchino, Russia — 1994

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
United States	08/23/1994	08/294,610	09/17/1996	5,556,769

METHOD OF SORTING A MIXTURE OF NUCLEIC ACIDS ON A BINARY ARRAY

Chetverin AB and Kramer FR
The Public Health Research Institute
Licensed Exclusively to Affymetrix — 1997 to 2012

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
United States	05/23/1994	08/247,530	08/15/2000	6,103,463

NOVEL OLIGONUCLEOTIDE ARRAYS AND THEIR USE FOR SORTING, ISOLATING, SEQUENCING, AND MANIPULATING NUCLEIC ACIDS

Chetverin AB and Kramer FR
The Public Health Research Institute
Licensed Exclusively to Affymetrix — 1997 to 2012

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
United States	09/30/1998	09/164,249	11/27/2001	6,322,971
France	02/19/1993	93906138.8	10/06/2004	0675966
Germany	02/19/1993	93906138.8	10/06/2004	0675966
Great Britain	02/19/1993	93906138.8	10/06/2004	0675966
Italy	02/19/1993	93906138.8	10/06/2004	0675966

OLIGONUCLEOTIDE-FACILITATED COALESCENCE

Kramer FR, Alsmadi OA, and Tyagi S
The Public Health Research Institute

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
United States	08/12/2003	10/398,833	10/31/2006	7,129,087
Australia	10/11/2001	2002232379	11/09/2006	2002232379
Brazil	10/11/2001	PI0114618-1	06/10/2014	PI0114618-1
Canada	10/11/2001	2,425,193	09/20/2011	2,425,193
China	10/11/2001	01820003.6	02/08/2006	ZL01820003.6
France	10/11/2001	01987791.9	01/30/2008	1332220
Germany	10/11/2001	01987791.9	01/30/2008	60132670.9
Great Britain	10/11/2001	01987791.9	01/30/2008	1332220
India	10/11/2001	544/DELNP/2003	09/03/2010	239131
Italy	10/11/2001	01987791.9	01/30/2008	48458BE2008
Japan	10/11/2001	2002-536415	07/09/2008	4115833

HYBRIDIZATION PROBES FOR NUCLEIC ACID DETECTION, UNIVERSAL STEMS, METHODS AND KITS

Tyagi S, Kramer FR, and Lizardi PM
PHRI Properties, Inc.
Licensed Non-Exclusively to 77 Companies — 1997 to 2014

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
Australia	11/14/1994	12921/95	09/09/1998	697841
Belgium	11/14/1994	95904104.7	01/02/2008	0728218
Belgium	06/21/2007	07075511.1	02/15/2012	1921169
Canada	11/14/1994	2,176,348	11/02/2004	2,176,348
Denmark	06/21/2007	07075511.1	02/15/2012	1921169
France	11/14/1994	95904104.7	01/02/2008	0728218
France	06/21/2007	07075511.1	02/15/2012	1921169
Germany	11/14/1994	95904104.7	01/02/2008	0728218
Germany	06/21/2007	07075511.1	02/15/2012	1921169
Great Britain	11/14/1994	95904104.7	01/02/2008	0728218
Great Britain	06/21/2007	07075511.1	02/15/2012	1921169
Ireland	06/21/2007	07075511.1	02/15/2012	1921169
Italy	11/14/1994	95904104.7	01/02/2008	0728218
Italy	06/21/2007	07075511.1	02/15/2012	1921169
Japan	11/14/1994	7-514079	01/25/2008	4071277
Portugal	11/14/1994	95904104.7	01/02/2008	0728218
Portugal	06/21/2007	07075511.1	02/15/2012	1921169
Spain	06/21/2007	07075511.1	02/15/2012	1921169
Sweden	11/14/1994	95904104.7	01/02/2008	0728218
Sweden	06/21/2007	07075511.1	02/15/2012	1921169
Switzerland	06/21/2007	07075511.1	02/15/2012	1921169
The Netherlands	11/14/1994	95904104.7	01/02/2008	0728218
The Netherlands	06/21/2007	07075511.1	02/15/2012	1921169

DETECTABLY LABELED DUAL CONFORMATION OLIGONUCLEOTIDE PROBES, ASSAYS AND KITS

Tyagi S, Kramer FR, and Lizardi PM
PHRI Properties, Inc.
Licensed Non-Exclusively to 77 Companies — 1997 to 2016

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
United States	05/12/1995	08/439,819	07/20/1999	5,925,517
United States	03/15/1999	09/268,402	08/15/2000	6,103,476
Australia	05/13/1996	52324/96	02/25/1999	702598
Belgium	05/10/1996	96303544.9	10/22/2008	0745690
Canada	05/10/1996	2,176,266	10/18/2011	2,176,266
Denmark	05/10/1996	96303544.9	10/22/2008	0745690
France	05/10/1996	96303544.9	10/22/2008	0745690
France	10/21/2008	08018375.9	11/05/2014	2053134
Germany	05/10/1996	96303544.9	10/22/2008	0745690
Germany	10/21/2008	08018375.9	11/05/2014	2053134
Great Britain	05/10/1996	96303544.9	10/22/2008	0745690
Great Britain	10/21/2008	08018375.9	11/05/2014	2053134
Italy	05/10/1996	96303544.9	10/22/2008	0745690
Japan	05/13/1996	8-118110	09/08/2006	3850914
Portugal	05/10/1996	96303544.9	10/22/2008	0745690
Sweden	05/10/1996	96303544.9	10/22/2008	0745690
Switzerland	10/21/2008	08018375.9	11/05/2014	2053134
The Netherlands	05/10/1996	96303544.9	10/22/2008	0745690

NON-COMPETITIVE CO-AMPLIFICATION METHODS

Kramer FR, Tyagi S, Alland D, Vet J, and Piatek A
PHRI Properties, Inc.
Licensed Non-Exclusively to 77 Companies — 1997 to 2018

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
United States	10/20/2000	09/508,343	10/08/2002	6,461,817
Australia	09/11/1998	94846/98	05/02/2002	743011
Canada	09/11/1998	2,303,414	05/20/2008	2,303,414
Japan	09/11/1998	2000-510898	05/15/2009	4306957

NUCLEIC ACID DETECTION PROBES HAVING NON-FRET FLUORESCENCE QUENCHING AND KITS AND ASSAYS INCLUDING SUCH PROBES

Tyagi S and Kramer FR
PHRI Properties, Inc.
Molecular Beacons Applications Licensed Non-Exclusively to 77 Companies — 1997 to 2017, and Non-Molecular Beacons Applications Licensed Non-Exclusively to Abbott Laboratories — 2006 to 2017, to Gen-Probe — 2011 to 2017, and Semi-Exclusively to Biosearch Technologies — 2012 to 2017

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
United States	12/12/1997	08/990,176	11/21/2000	6,150,097
Australia	04/14/1997	29224/97	10/21/1999	713667
Canada	04/14/1997	2,252,048	03/11/2008	2,252,048
France	04/14/1997	97923412.7	05/14/2008	0892808
Germany	04/14/1997	97923412.7	05/14/2008	69738687.2
Great Britain	04/14/1997	97923412.7	05/14/2008	0892808
Italy	04/14/1997	97923412.7	05/14/2008	49295BE/2008
Japan	04/14/1997	9-537293	01/05/2007	3898228

WAVELENGTH-SHIFTING PROBES AND PRIMERS

Tyagi S, Kramer FR, and Marras SAE
PHRI Properties, Inc.
Licensed Non-Exclusively to 77 Companies — 1997 to 2019

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
United States	07/28/1998	09/123,764	03/14/2000	6,037,130
Australia	07/28/1999	52402/99	10/23/2003	763106
Canada	07/28/1999	2,336,489	05/03/2011	2,336,489
France	07/28/1999	99937602.3	11/12/2008	1100971
Germany	07/28/1999	99937602.3	11/12/2008	1100971
Great Britain	07/28/1999	99937602.3	11/12/2008	1100971
Italy	07/28/1999	99937602.3	11/12/2008	1100971
Japan	07/28/1999	2000-562560	09/08/2006	3851088

ASSAYS FOR SHORT SEQUENCE VARIANTS

Tyagi A, Kramer FR, and Alland D
PHRI Properties, Inc.
Licensed Non-Exclusively to 77 Companies — 1997 to the present

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
United States	07/24/2002	10/110,907	02/16/2010	7,662,550
United States	11/09/2009	12/614,917	02/16/2016	9,260,761
Australia	10/13/2000	80240/00	06/23/2005	781945
Canada	10/13/2000	2,387,306	08/04/2009	2,387,306
France	10/13/2000	00970925.4	08/16/2006	1230387
Germany	10/13/2000	00970925.4	08/16/2006	60030145.1
Great Britain	10/13/2000	00970925.4	08/16/2006	1230387
Italy	10/13/2000	00970925.4	08/16/2006	1230387
Japan	10/13/2000	2001-533197	02/10/1012	4919568

HOMOGENEOUS MULTIPLEX SCREENING ASSAYS AND KITS

Kramer FR
PHRI Properties, Inc.
Licensed Non-Exclusively to 77 Companies — 1997 to the present

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
United States	04/30/2003	10/426,556	06/10/2008	7,385,043
United States	05/07/2008	12/116,642	08/10/2010	7,771,949
Australia	04/29/2004	2004236725	11/21/2008	236725
China	04/29/2004	200480018729.6	12/25/2009	200480018729.6
Japan	04/29/2004	2006-514227	01/07/2011	4658928
Canada	04/29/2004	2,524,080	10/22/2013	2,524,080
France	04/29/2004	04751175.3	12/31/2014	1629108
Germany	04/29/2004	04751175.3	12/31/2014	1629108
Great Britain	04/29/2004	04751175.3	12/31/2014	1629108
Italy	04/29/2004	04751175.3	12/31/2014	1629108
Switzerland	04/29/2004	04751175.3	12/31/2014	1629108

OPTICALLY DECODABLE MICROCARRIERS, ARRAYS AND METHODS

Kramer FR, Tyagi S, Marras SAE, and Trunfio HE
The Public Health Research Institute

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
United States	03/02/2004	10/791,502	06/22/2010	7,741,031
Australia	03/02/2004	2004219707	08/06/2009	2004219707
Canada	03/02/2004	2,518,147	05/01/2012	2,518,147
China	03/02/2004	200480012243.1	11/24/2010	CN1784590-B
France	03/02/2004	04716455.3	06/17/2009	1604172
Germany	03/02/2004	04716455.3	06/17/2009	602004021569.4
Great Britain	03/02/2004	04716455.3	06/17/2009	1604172
Italy	03/02/2004	04716455.3	06/17/2009	4952BE/2009
Japan	03/02/2004	2006-508969	12/17/2010	4646904

HIGH SPECIFICITY PRIMERS, AMPLIFICATION METHODS AND KITS

Tyagi S, Kramer FR, and Vartikian R
The Public Health Research Institute
Optioned to Johnson & Johnson — 2000 to 2002

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
United States	05/24/1999	09/317,350	08/21/2001	6,277,607
United States	07/12/2001	09/903,915	04/02/2002	6,365,729
Australia	05/03/2000	46939/00	05/06/2004	769219
Canada	05/03/2000	2,375,027	07/27/2010	2,375,027
Japan	05/03/2000	2000-619817	08/18/2006	3843215
France	05/03/2000	00928752.5	05/07/2008	1185546
Germany	05/03/2000	00928752.5	05/07/2008	60038796.8
Great Britain	05/03/2000	00928752.5	05/07/2008	1185546
Italy	05/03/2000	00928752.5	05/07/2008	49228BE/2008

HIGHLY SELECTIVE NUCLEIC ACID AMPLIFICATION PRIMERS

Marras SAE, Vargas-Gold DY, Tyagi S, and Kramer FR
Rutgers University
Licensed to Cepheid and Bio-Rad Laboratories — 2021 to the present

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
United States	08/06/2015	14/766,139	03/06/2018	9,909,159
United States	01/22/2018	15/877,007	10/27/2020	10,815,512
Australia	08/05/2015	2014214725	06/25/2020	2014214725
Belgium	08/03/2015	14748774.8	04/11/2018	2953958
Canada	08/04/2015	2,900,259	12/29/2020	2,900,259
China	08/08/2015	2014800199742	01/22/2019	2014800199742
France	08/03/2015	14748774.8	04/11/2018	2953958
Germany	08/03/2015	14748774.8	04/11/2018	2953958
India	08/07/2015	2606/KOLNP/2015	09/09/2022	406237
Israel	08/04/2015	240347	05/29/2019	240347
Italy	08/03/2015	14748774.8	04/11/2018	2953958
Japan	08/06/2015	2015-557131	06/28/2019	6544861
Korea	09/04/2015	10-2015-7024265	02/25/2021	10-2222546
New Zealand	08/07/2015	710851	03/02/2021	710851
Singapore	08/05/2015	11201506146T	05/29/2019	11201506146T
South Africa	08/05/2015	2015/05637	07/27/2016	2015/05637
Sweden	08/03/2015	14748774.8	04/11/2018	2953958
Switzerland	08/03/2015	14748774.8	04/11/2018	2953958
United Kingdom	08/03/2015	14748774.8	04/11/2018	2953958

Divisional Applications

United States	10/23/2020	17/078,463	09/07/2021	11,111,515
Belgium	02/07/2018	18156307.3	10/02/1019	3346016
Canada	10/15/2020	3,096,478	06/22/2021	3,096,478
France	02/07/2018	18156307.3	10/02/1019	3346016
Germany	02/07/2018	18156307.3	10/02/1019	3346016
Israel	01/30/2019	264555	02/01/2022	264555
Italy	02/07/2018	18156307.3	10/02/1019	3346016
Korea	02/23/2021	10-2021-7005395	01/22/2022	10-7005395
Sweden	02/07/2018	18156307.3	10/02/1019	3346016
Switzerland	02/07/2018	18156307.3	10/02/1019	3346016
United Kingdom	02/07/2018	18156307.3	10/02/1019	3346016
China	01/02/2019	2019100031106	Pending	

MULTIPLEX NUCLEIC ACID ASSAYS FOR CLOSELY RELATED ALLELES, AND REAGENTS THEREFOR

Marras SAE, Vargas-Gold DY, Tyagi S, and Kramer FR
Rutgers University
Licensed to Cepheid and Bio-Rad Laboratories — 2021 to the present

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
United States	10/05/2018	16/091,824	01/03/2023	11,542.547
Australia	11/02/2018	2017246663	12/15/2022	2017246663
Belgium	11/05/2018	17779722.2	02/17/2021	3440226
China	12/07/2018	2017800357947	05/31/2022	2017800357947
France	11/05/2018	17779722.2	02/17/2021	3440226
Germany	11/05/2018	17779722.2	02/17/2021	3440226
Italy	11/05/2018	17779722.2	02/17/2021	3440226
Japan	10/03/2018	2018-552172	08/24/2021	6933858
Singapore	11/03/2018	11201808740T	04/30/2021	11201808740T
South Africa	11/05/2018	2018/07405	04/28/2021	2018/07405
Sweden	11/05/2018	17779722.2	02/17/2021	3440226
Switzerland	11/05/2018	17779722.2	02/17/2021	3440226
United Kingdom	11/05/2018	17779722.2	02/17/2021	3440226
Canada	11/04/2018	3,020,165	Pending	
India	11/03/2018	201817041693	Pending	
New Zealand	11/05/2018	748011	Pending	

ASSAY METHODS AND KITS FOR DETECTING RARE SEQUENCE VARIANTS

Vargas-Gold DY and Kramer FR
Rutgers University
Licensed to Cepheid and Bio-Rad Laboratories — 2021 to the present

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
South Africa	03/15/2022	2022/03111	Allowed	
United States	03/22/2022	17/754,032	Pending	
Europe *	03/15/2022	20871738.9	Pending	
Australia	03/07/2022	2020359655	Pending	
Canada	03/10/2022	3150825	Pending	
China	03/31/2022	2020800692434	Pending	
India	03/10/2022	202217013118	Pending	
Israel	03/30/2022	291819	Pending	
Japan	04/01/2022	2022-520562	Pending	
Korea	04/29/2022	10-2022-7014741	Pending	
New Zealand	03/07/2022	785920	Pending	
Singapore	03/09/2022	11202202397Y	Pending	

* Will be pursued in Belgium, France, Germany, Italy, Sweden, Switzerland, and the United Kingdom

HIGH-THROUGHPUT METHOD FOR CHARACTERIZING IMMUNE CELL REPERTOIRES USING PCR AND MELTING PROFILE ANALYSIS WITH SLOPPY MOLECULAR BEACON PROBES

Marras SAE, Dikdan RJ, Kwang S, Kramer FR, and Tyagi S
Rutgers University

Jurisdiction	Filing Date	Application	Issue Date	Patent Number
United States	09/14/2023	63/582,589		

Expert Consultancies

1996 Consultant to Invitrogen in Promega vs. Invitrogen (W.D. Wisconsin, Case No. 95-C-0821-C). Prepared affidavits and was orally deposed.

2001 Consultant to Georgetown University in patent interference case (No. 104,670) against Applied BioSystems regarding U.S. Patent 5,538,848. Prepared two declarations and was orally deposed.

2002 Consultant to Gen-Probe in a matter against Vysis, Inc. (Case No. 99-CV-2668-H AJB). Orally deposed and testified at trial.

2004 Consultant to Gen-Probe in an arbitration involving Bayer Healthcare LLC (JAMS 1240015058). Orally deposed and testified at the arbitration hearing, providing a basic technology tutorial.

2006 Consultant to Gen-Probe in a patent litigation against Bayer Healthcare LLC (Case Nos. 04-CV-565LAB and 05-CV-1668 B). Orally deposed and submitted several declarations, including declarations relating to claims construction and a basic technology tutorial.

2007 Consultant to Stanford University in a patent litigation (Board of Trustees of the Leland Stanford Jr. Univ. v. Roche Molecular Systems, Inc., No. C-05-04158-MHP). Prepared a declaration and was orally deposed. The matter was resolved in the Supreme Court of the United States of America.

2008 Consultant to Gen-Probe in an arbitration filed by Digene Corporation against Roche and Gen-Probe (Digene Corp. v. F. Hoffman-La Roche Ltd., Case No. 50-181-T-00502-06)). Testified at the arbitration hearing, providing a basic technology tutorial, along with opinions in rebuttal to Digene's expert on matters relating to the scope of patents relating to human papillomaviruses.

2011 Prepared declarations for Troll Busters LLC in the reexaminations of Roche's U.S. Patent 5,804,375 and Applied BioSystems U.S. Patent 5,928,907.

2011 and 2012 Consultant to Gen-Probe in a patent litigation against Becton Dickinson (Case No. 09cv2319 BEN). Submitted a declaration and testified at a claims construction tutorial hearing, providing a basic technology tutorial and opinions with regard to Gen-Probe's automation patents; filed an expert report; filed a rebuttal expert report; and was deposed by Becton Dickinson.

2012 Consultant to Biosearch Technologies in a patent litigation against Life Technologies (Life Technologies Corporation et al. v. Biosearch Technologies, Inc., *et al*., Case No. 3:12-cv-00852-WHA). Submitted several expert reports and was orally deposed by Life Technologies.

2013 Consultant to Gen-Probe in a patent litigation against Beckman Coulter. Submitted declarations concerning *Inter partes* reexaminations of two Gen-Probe U.S. patents 7,482,143 and 7,524,652.

2013 Consultant to Becton Dickinson in a patent litigation brought by Enzo Life Sciences (Case No. 12-cv-275-LPS).

2015 Consultant to Illumina in a patent litigation against Roche. Submitted declaration and was orally deposed concerning *Inter partes* reexamination of Illumina's U.S. Patent 7,955,794 (Case IPR2014-01093).

2017 Consultant to Johns Hopkins University in patent litigations involving Myriad Genetics, Bio-Rad Laboratories, RainDance Technologies, and Ambry Genetics. Prepared declarations concerning *Inter partes* reexamination of Johns Hopkins' U.S. Patents 6,440,706, 7,824,889, 7,915,015, and 8,859,206 (Case Nos. IPR2017-01102, IPR2017-01105, IPR2017-01106, IPR2017-01107, and IPR2017-02096).

2018 Consultant to BioMérieux in a patent litigation involving Becton Dickinson. Prepared declarations concerning *Inter Partes* review (Case No. IPR2018-01566).

2019 Consultant to Former Security Holders and Nonholders of Ionian Technologies in an intellectual property dispute against Alere, Inc. Prepared declarations concerning the underlying inventions. (Case No. 37-2017-00016861-CU-BC-CTL).

Formative Experiences

1957 Cloud chamber

At the Bronx High School of Science, in a course called Science Techniques Laboratory, I designed and built an operating cloud chamber that utilized dry ice and alcohol to detect cosmic rays entering the atmosphere.

1958 Gaseous discharge tube

For the New York City High School Science Fair, I built an operating gaseous discharge tube, utilizing an induction coil, a six-volt car battery, and a vacuum pump, and I observed colored bands generated by ionized gases.

1959 Crystallization of inorganic salts

For the New York City High School Science Fair, I prepared 49 glass plates, each possessing a non-porous barrier, and I filled each plate with a solution containing a different chemical salt. After waiting weeks for evaporation to occur, I analyzed the crystal structures formed by each compound.

1960 Amphibian and reptile zoo

As a Nature Counselor (and Eagle Scout) at the Ten Mile River Boy Scout Camp, I maintained a zoo of live animals, including frogs, newts, turtles, and snakes that are found in New York State forests, and involved the campers in the care and appreciation of those animals.

1961 Bubble chamber simulation computer program

As a member of the Unified Science Program at the University of Michigan, I prepared a FORTRAN computer program that simulated the tracks of atomic particles that occur in the bubble chamber utilized at the synchrotron facility in the Brookhaven National Laboratory, including the generation of randomly located bubbles along parabolic tracks, and random probabilistic excursions on either side of each track. This program was then used at Brookhaven National Laboraotory as input for the development of a computer program that analyzed the tracks generated during each bubble chamber cycle, in order to decide if it was worthwhile to photograph the event.

1962 Solution to the step-plating problem in printed circuit manufacture

As a summer employee in the Quality Control Laboratory of Printed Circuits, Inc., which manufactured printed circuits that were incorporated into the guidance computers of United States naval missiles, I spent the summer exploring why those printed circuits had a defect, called step plating, that changed the ability of the circuit to carry electricity, and caused the missiles to explode. I found that step plating was caused by a contamination on the copper surface of the circuit boards, and I discovered that placement of the circuits in a boiling water bath prior to nickel and gold plating removed the contaminants. The manufacturing process was then altered to include a boiling water bath.

1962 Concept of “self” involves neurological experiences to be integrated by the brain over time

As an undergraduate philosophy student, I was challenged to explore the concept of “self”, by examining the philosophy of Martin Buber. Instead, I explored the experience of patients who underwent prefrontal lobotomies as a treatment for intractable pain. After recovering from the surgical severing of the nerves that connect the left and right areas of their brain’s prefrontal cortex, the patients were asked if they still felt the pain. The answer was always yes, but the patients seemed happy and did not suffer. I noted that the suffering was eliminated because the person suffering the pain a few moments ago was not the same person suffering the pain when asked the question. I concluded that the concept of “self” requires integration of one’s awareness over time; and that the neorological structure and operation of the brain is designed to integrate experiences over time.

1963 Identification of cytochrome oxidase as the immediate enzyme interacting with oxygen

As a zoology major at the University of Michigan, I repeated and significantly modified the classic experiment of Keilin and Hartree, utilizing the repeated motion of cilia in live clam gills as an indicator of the uptake of oxygen from the atmosphere, in order to identify which enzyme is responsible for oxygen uptake. I built a sealed chamber in which the motion of the clam cilia was inhibited by the introduction of a mixture of carbon monoxide and oxygen. I then observed and quantitated the rate of ciliary movement through a microscope as a function of the wavelength of light shined on the tissue by a monochrometer. The result was an action spectrum documenting the reversal of inhibition by the absorption of the light. The action spectrum matched the absorption spectrum of cytochrome oxidase, identifying it as the immediate enzyme that binds oxygen.

1963 Studies on Transformational Grammer

As a Research Assistant to Anatol Rapoport and William Livant at the Mental Health Research Institute of the University of Michigan, I conducted a study of students asked to carry out a series of grammatical tasks designed to see whether Noam Chomsky's theory of transformational grammer actually operated in the minds of the subjects. The project was supported by the Rome Air Development Center of the United States Air Force to gather information that could be used to develop language translation computer programs. The results did not support Chomsky's theory.

1964 Structure of DNA in lampbrush chromosomes

As a Technician in the Cytogenetics Laboratory of the Carnegie Institution of Washington, located at the University of Michigan, in the laboratory of Berwind Kaufmann and Helen Gay, I chose as my Honors Project a repeat of the classic experiment of Joseph Gall that determined the number of DNA molecules that form the loops and the spine of lampbrush chrosomes isolated from oocytes of the newt, *Triturus viridescens*. I subjected the chromosomes to mild concentrations of deoxyribonuclease, which causes a chromosomal break whenever cleavage occurs at the same position on adjacent strands of a DNA molecule. Photographs were taken at time intervals through an inverted phase microscope, and the number of breaks that occurred in the loops and the number of breaks that occurred in the spine were compared as a function of time. A mathematical model was then used to identify the different number of DNA strands forming the backbone of the chromosomal loops and spines.

1960-1964 The Michigan Daily

I was a reporter, night editor, and member of the Senior Staff of the Michigan Daily, which is the 133-year-old independent student newspaper of the University of Michigan. The paper came out each morning, was a member of the Associated Press, and in my senior year won an award from the National Press Club as the best student newspaper in the United States. I served as a Pressman's Assistant between my sophmore and junior years, running the presses that printed the paper six days a week. I served as Summer Editor between my junior and senior years, and I served as Associate Editorial Director in my senior year. I also served as an elected Member of the University's Board in Control of Student Publications.

1965 Marine embryology at the Bermuda Biological Station

I was a student in the Marine Embryology course at the Bermuda Biological Station for Research, and I carried out an independent project after the conclusion of the course that demonstrated that messenger RNAs in the oocytes of sea urchins are coated in proteins. For many years afterward, I served as a Member of the Corporation of the Bermuda Biological Station.

1966 Operant conditioning of dinoflagellates

I carried out an independent one-year-long project with a fellow graduate student, Lewis Kleinsmith, at The Rockefeller University to see whether there are changes in the RNA of the dinoflagellate, *Gonyaulax polyhedra*, when it undergoes operant conditioning (i.e., a project designed to see if RNA plays a role in memory). We learned to culture the dinoflagellates, and built an incubator whose lights shined at night so that their natural bioluminescence is strongest in the daytime (their night). These

dinoflagellates receive energy through their chloroplasts, give off blue bioluminescent light when agitated or when electrically stimulated, and swim strongly by virtue of their flagella. We established a laboratory, and we designed an elaborate electronic training apparatus that enabled us: a) to periodically cause the dinoflagellates to emit bioluminescent light in response to mechanical agitation or to electrical stimulation; and b) to periodically reward the dinoflagellates by shining a red light on the test tube to feed them through their chloroplasts. Instruments recorded the luminescent response. The idea was to train them so that a reward would engender a bioluminescent response without an electrical or mechanical stimulation. Then we would analyze their RNA to detect any changes that occurred. The training failed because the dinoflagellates used their flagella to swim away from the electro-mechanical paddles by which we stimulated bioluminescence in the test tube.

1966 Messenger RNA synthesis during the lampbrush chromosome stage of *Xenopus* oogenesis

As a graduate student, working with Alfred Mirsky and Eric Davidson at The Rockefeller University, I studied the synthesis of RNA in the lampbrush chromosome stage oocytes of the frog, *Xenopus laevis*. We found that messenger RNAs, representing only about 2% of the total synthesized RNA in the cell, are actively synthesized from the chromosomal loops of these oocytes, and those maternal mRNA molecules are stored in the oocyte for use as templates for protein synthesis in the embryo after fertilization occurs (Davidson *et al*., 1966). It is now understood that these mRNAs become coated in proteins that protect them from degradation in the oocyte, and they only become active once proteases stored in the head of the sperm digest away the protein coats after fertilization occurs. My main role in this project was to develop an *in vitro* protein synthesis assay that was used to estimate the amount of mRNA present in the oocytes.

1967 Collection of live frogs in Panama and Trinidad

I carried out a six-week long expedition, with Eric Davidson, to the Smithsonian Tropical Research Institute on Barro Colorado Island in Gatun Lake in the middle of the Panama Canal, and to The New York Zoological Society Tropical Research Station (Simla) in the Arima valley of Trinidad. The purpose of the expedition was to capture approximately 500 live frogs, *Engystomops pustulosus*, and transport them back to The Rockefeller University in New York City, where a breeding colony was established. We chose this species to collect because they lay their eggs in rain puddles and their subsequent development is consequently very rapid. We also established a breeding colony in Trinidad, in association with the University of the West Indies. The frogs were collected at night and the females were located by the peeps of the mating males. Dangers included the presence of caymens in the ponds, chiggers in the bush, and poisenous caterpillars in the trees.

1968 Chemical synthesis of ribonuclease A

For my biochemistry qualifying examination at The Rockefeller University, working in collaboration with Bruce Merrifield, I devised a protocol for the chemical synthesis of the 124-amino acid-long protein bovine pancreatic ribonuclease A.

1969 Evolutionary selection chooses both protein sequence and the rate of translation

Working in the laboratory of Vincent Allfrey, my thesis advisor at The Rockefeller University, and with colleagues that included James Schwartz at New York University School of Medicine, and Har Gohbind Khorana and Robert Wells at the University of Wisconsin, I studied the interaction between the genetic code and the mechanism of protein synthesis. Utilizing artificial template RNAs, I found that the rate of addition of an amino acid to a growing protein chain is dependent on the identity of which synonym codon is used to specify the addition of each amino acid. A synonym codon that is translated by a readily available transfer RNA is rapidly added to the growing protein chain. On the other hand, a synonym codon for the same amino acid that is translated by a rare transfer RNA is only added slowly, as it takes more time for that transfer RNA to be added to the ribosome. The most significant conclusion of my dissertation was that evolutionary selection of a genetic sequence not only determines the amino sequence of its encoded protein, but through the evolutionary choice of which synonym codon is selected for encoding each amino acid, evolution also influences the rate of synthesis (and consequent amount of synthesis) of the encoded protein.