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요약

본 발명은, 진단 매개변수를 한정하고 매개변수가 최적화되도록 매개변수간 관계식을 설정하며 진단 적용에 대해 최적인 마커 그룹을 선택함을 포함하여, 진단 적용에 사용하기 위한 마커의 포트폴리오 선택방법에 관한 것이다. 진단 매개변수는 유전자의 상대적 발현도의 척도 및 유전자의 발현도의 측정시의 변화량 척도를 포함하며, 진단 및 판별 매개변수간 관계식은 평균 변화량 관계식일 수 있다.

본 발명의 추가 양태는 본 방법을 실행하도록 프로그램된 기기 및 이의 작동을 위한 지시사항을 포함하는 제품이다.

색인어

마커, 포트폴리오, 매개변수, 유전자 발현, 진단

명세서

발명의 상세한 설명

발명의 목적

발명이 속하는 기술 및 그 분야의 종래기술

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본 발명은 진단 마커의 포트폴리오 선택에 관한 것이다.

her-2-neu와 같은 몇몇 단일 유전자 진단 마커가 현재 사용되고 있다. 그러나, 일반적으로 한개의 특정 유전자에 대한 분자적 진단으로는 질환을 용이하게 진단하지 못한다. 빈번하게 다중 마커가 요구되며, 상이한 유전자 변형을 기본으로 하는 검정에 포함될 수 있는 이러한 마커의 수는 심지어 수백개의 유전자로 확대될 수도 있다. 마커를 포트폴리오로 분류하여, 결과를 수득하는데 필요한 가장 적은 수의 마커의 사용하여 가장 신뢰성있는 결과를 수득하는 것이 바람직하다. 이는 핵산 증폭 단계와 같은 다수의 단계를 포함하는 검정에서 특히 필요하다.

발명이 이루고자 하는 기술적 과제

본 발명은, 진단 매개변수를 한정하고 매개변수가 최적화되도록 매개변수간 관계식을 설정하며 이러한 관계식을 사용하여 진단 적용에 대해 최적인 마커 그룹을 선택하는, 진단 적용에 사용하기 위한 마커의 포트폴리오 선택방법에 관한 것이다.

본 발명의 또다른 양태에서, 진단 매개변수는 유전자의 상대적 발현도의 척도 및 유전자 발현도 측정시의 변화량 척도를 포함하며, 진단 및 판별 매개변수간 관계식은 평균-변화량 관계식이다.

본 발명의 추가 양태는, 본 발명의 방법을 수행하도록 프로그램된 기기 및 이의 작동을 위한 지시사항을 포함한 제품이다.

발명의 구성 및 작용

본 발명의 방법은, 유전자 발현 측정방법을 기준으로 하여, 단백질뿐만 아니라 관련 세포의 유전자 발현 패턴을 측정하는 어떠한 방법과도 연관시켜 사용할 수 있다. 유전자 발현 프로필을 설정하기에 바람직한 방법에는 단백질 또는 펩타이드를 암호화할 수 있는 유전자에 의해 생성된 RNA의 양을 측정하는 것이 포함된다. 이는 역전사효소 PCR(RT-PCR), 경쟁적 RT-PCR, 실시간 RT-PCR, 차동 RT-PCR, 노던 블롯 분석 및 기타 관련 시험에 의해 수행된다. 개별적인 PCR 반응을 사용하는 이러한 기술을 사용할 수도 있지만, mRNA로부터 생성된 복사 DNA(cDNA) 또는 복사 RNA(cRNA)를 증폭시킨 후 미세정렬을 통해 분석하는 것이 가장 좋다. 다수의 상이한 정렬 형태 및 이의 제조방법은 당분야의 숙련가에게 공지되어 있으며, 본원에서 참고문헌으로 인용된 미국 특허 제5,445,934호, 제5,532,128호, 제5,556,752호, 제5,242,974호, 제5,384,261호, 제5,405,783호, 제5,412,087호, 제5,424,186호, 제5,429,807호, 제5,436,327호, 제5,472,672호, 제5,527,681호, 제5,529,756호, 제5,545,531호, 제5,554,501호, 제5,561,071호, 제5,571,639호, 제5,593,839호, 제5,599,695호, 제5,624,711호, 제5,658,734호 및 제5,700,637호에 기술되어 있다.

미세정렬 기술은, 수천개의 유전자의 정상 상태 mRNA 수준을 동시에 측정하여 비조절된 세포 증식의 개시, 정지 및 조절과 같은 효과를 동정하는 강력한 수단을 제공할 수 있다. 2가지의 미세정렬 기술이 현재 널리 사용되고 있다. 첫번째는 cDNA 정렬이고 두번째는 올리고뉴클레오타이드 정렬이다. 이러한 칩의 구조에는 차이가 있더라도 모든 하부 데이타 분석 및 출력은 본질적으로 동일하다. 이러한 분석의 결과는 전형적으로 미세정렬에서 공지된 위치에 있는 핵산 서열과 하이브리드된 샘플의 cDNA 서열을 검출하는데 사용되는 표지된 프로브로부터 수용된 시그날의 강도 측정치이다. 전형적으로, 시그날의 강도는 cDNA 양에 비례하므로 샘플 세포에서 발현되는 mRNA 양에 비례한다. 이러한 다수의 기술은 입수가능하며 유용하다. 유전자 발현을 측정하기 위한 바람직한 방법은, 본원에서 참고문헌으로 인용된 미국 특허 제6,271,002호(Linsley 등), 제6,218,122호(Friend 등), 제6,218,114호(Peck 등) 및 제6,004,755호(Wang 등)에서 찾아볼 수 있다.

발현 수준의 분석은 상기한 강도를 비교하여 수행한다. 시험 샘플중 유전자의 발현 강도 대 대조 샘플중 유전자의 발현 강도의 비율 매트릭스를 산출하여 수행하는 것이 가장 좋다. 예를 들어, 질환이 있는 조직의 유전자 발현 강도를, 동일 유형의 정상 조직으로부터 생성된 발현 강도와 비교할 수 있다(예: 질환이 있는 결장 조직 샘플과 정상 결장 조직 샘플). 이러한 발현 강도의 비율은 시험 샘플과 대조 샘플의 유전자 발현의 배수 변화(fold change)로 나타난다.

비정상 세포에는 상향 조절 또는 하향 조절과 같이 특이적으로 발현되는 조절된 유전자가 있다. 상향 조절 및 하향 조절은 검출가능한 차이가 동일한 기준에 비해 유전자의 발현량으로 (이를 측정하는데 사용되는 시스템의 잡음 기여도를 능가하여) 발견되는 것을 의미하는 상대적인 용어이다. 이러한 경우에, 기준은 정상 세포의 유전자 발현 측정치이다. 따라서, 비정상 세포의 문제의 유전자는 동일한 측정 방법을 사용한 기준 수준에 비해 상향 또는 하향 조절된다.

바람직하게는 상향 및 하향 조절 수준은, 하이브리드된 미세정렬 프로브의 강도 측정치의 배수 변화를 기준으로 하여 구별한다. 예를 들어, 이러한 구별에 1.5배 이상의 차이가 사용되는 경우, 질환이 있는 세포는 정상 세포에 비해 1.5배 이상 또는 1.5배 미만의 강도가 나타난다는 것을 알 수 있다.

이를 구별하는데는 또다른 방법이 유용하다. 예를 들어, 통계적 시험법을 사용하여 다양한 샘플 그룹간에 가장 유의

적으로 상이한 유전자를 찾을 수 있다. 예를 들어, 스튜던트 t-시험(Student t-test)는 두 그룹간 중요한 차이를 발견하는데 사용할 수 있는 확고한 통계법이다. p 값이 작을수록 그 유전자가 상이한 그룹간에서 차이가 난다는 보다 강력한 증거가 된다. 그럼에도 불구하고 미세정렬은 동시에 하나 이상의 유전자를 측정하기 때문에, 수만개의 통계적 시험법이 한번에 요구될 수도 있다. 이러한 이유로, 무작위/치환 실험뿐만 아니라 시닥 보정법(Sidak correction)을 사용하여 변화시키고 조정하여 작은 p 값을 수득하는 것이 가능하다.

t-시험에 의해 p 값이 0.05 미만인 경우, 유전자가 유의적으로 차이가 난다는 증거가 된다. 보다 강력한 증거는 시닥 보정을 실시한 후의 p 값이 0.05 미만인 것이다. 각 그룹에서 샘플 수가 보다 많은 경우, 무작위/치환 시험 후 p 값이 0.05 미만인 것이 유의차 나타내는 가장 강력한 증거가 된다.

그룹중의 유전자 세트에 대해 수득된 정보를 진단, 예후 또는 치료 선택과 같이 임상적으로 관련된 판단을 하기 위한 합당한 기준으로 제공하기 위해서, 유전자를 그룹으로 분류할 수 있다. 이러한 유전자 세트는 본 발명의 포트폴리오를 구성한다. 가장 좋은 진단 마커의 경우, 의학적 판단을 보정하기에 충분한, 가장 적은 수의 마커를 사용하는 것이 종종 바람직하다. 이는 시간 및 자원의 부적절한 사용을 방지할 뿐만 아니라 미해결된 추가 분석 처리가 지연되는 것을 방지한다. 바람직한 최적의 포트폴리오는, 판단에 최소한의 마커를 사용하지만 실제 판단이 정확할 가능성을 극대화하는 조건에 부합하는 포트폴리오이다. 이러한 조건에는 일반적으로 민감성 및 특이성 요구치가 포함된다. 검출 방법을 기본으로 하는 미세정렬에서, 포트폴리오의 민감성은 정상 상태에 비해 질환이 있거나 이상 상태에 있는 유전자 발현으로 나타나는 배수 차이에 반영될 수 있다. 다른 상태에 있는 유전자의 발현에 비해 상당한 배수 변화가 나타난다면 유전자의 특이적 발현 검출은 민감한 것이다. 민감성의 또다른 측면은 잡음으로부터 시그날을 구별하는 능력이다. 예를 들어, 유전자 세트의 발현이 질환 상태로 정의된 적절한 민감성을 나타낼 수 있지만 하나(예를 들어, 미세정렬에서의 강도 측정치)에 의해 생성된 시그 날이 소정의 설정(즉, 임상 실험실)시의 잡음으로부터 용이하게 구별되는 수준 이하라면, 이 유전자는 최적의 포트폴리오로부터 배제시켜야한다. 최적의 포트폴리오를 한정하는 상기한 조건을 설정하는 과정은 본 발명의 방법에 포함될 수 있다.

특이성은 관심있는 조건을 지닌 유전자 발현의 시그날과 상관된 통계적 수치에 반영될 수 있다. 유전자 세트의 특이적 발현이 관심있는 조건 이외에 다수의 상태(예를 들어, 다수의 질환 상태)에서 배수 변화가 큰 것으로 관찰된다면, 유전자 세트에 대한 유전자 발현 특징은 비특이적이다. 데이타의 상관성 또는 데이타의 일치도(예: 표준편차)의 통계학적 수치, 상관계수 등을 이러한 척도로서 사용할 수 있다. 포트폴리오에 포함시키기 위해 고려되는 유전자 그룹에서, 발현 수치에서 표준편차가 작다는 것은 특이성이 크다는 것과 상관성이 있다. 유사한 발현 패턴을 나타내는 유전자는 동일 인자에 의해 조절되어 동일한 방향으로 유전자를 진행시킬 수 있다. 샘플을 분류하는데 있어 이러한 인자가 충분하지만 필수적이지는 않다면, 마커가 이러한 단일 인자와 모두 연관되어 있을 경우에는 이러한 유전자로 샘플을 정확히 동정하는데는 실패할 것이다. 다각화는 가능한한 적은 수의 마커를 선택하게 하지만, 데이타 세트에 포함된 다수의 상이한 최적의 발현 패턴을 포함한다.

본 발명의 방법에서는, 유전자 마커 그룹을 선택하여 진단 적용에 사용한다. 이러한 마커 그룹이 '포트폴리오'이다. 진단 적용에는 피험자의 질환 상태 또는 이상의 검출 또는 동정, 피험자가 소정의 질환 또는 질병이 있을 가능성 측정, 질환 또는 질병이 있는 피험자가 치료에 반응할 가능성 측정, 질환 또는 질병이 있는 피험자의 예후 측정(또는 이의 진행 또는 퇴행), 및 질환 또는 이상이 있는 피험자의 치료 효과 측정을 포함한다. 예를 들어, 본 방법은 피험자가 결장암에 걸릴 가능성 또는 피험자의 결장암 존재여부, 또는 세포독성 약물에 대해 피험자가 호의적으로 반응할 가능성을 검출하기 위한 포트폴리오를 설정하는데 사용할 수 있다.

본 발명의 방법에 의해 선택된 포트폴리오는 정확하고 정밀한 결과를 확보하는 다수의 여러 유형 마커를 포함하며, 포트폴리오를 포함하는 유전자 수의 측면에서 경제적이다. 본 발명의 방법은 다수의 유전자 발현이 수반되는 임의의 질환, 질병 또는 상태에 대해 최적의 유전자 발현 포트폴리오를 설정하는데 사용할 수 있다. 본 발명에서 최적의 포트폴리오는 다음 매개변수중 둘 이상의 예정된 표준에 따라 (실시되는 분석 조건을 기준으로 하여) 피험자의 상태를 평가하는 유전자 발현 프로필을 의미한다: 정확도, 정밀도 및 포트폴리오를 포함한 유전자의 수.

보다 바람직하게는, 포트폴리오에 사용되는 마커는 mRNA를 발현하는 핵산 서열('유전자')이다. 마커는 건강한 개체에서는 정상적으로 발현될 수 있지만, 진단 적용할 필요가 있는 사건이 발생되는 경우에는 보다 많이 발현되거나 보다 적게 발현된다. 또한, 진단 적용할 필요가 있는 사건이 일어난 경우를 제외하고는 발현이 일어나지 않을 수도 있다.

마커는, 본 발명에서 사용되는 진단 매개변수인, 진단 평가를 실시하기 위해 비교되는 특징, 징후 또는 측정치의 원인이 된다. 유전자 발현 수준 지표는 가장 바람직한 진단 매개변수이다. 이러한 지표에는 상기한 바와 같은 미세정렬로부터 판독된 강도의 수치가 포함된다. 마커의 상대적인 메틸화도의 지표와 같이 다른 진단 매개변수도 가능하다.

서로 연관되어 있는 수학적/통계적 수치를 사용하여 진단 매개변수를 판별할 수 있다. 바람직한 판별은 유전자 발현을 나타내는 평균 시그날 판독치 및 이러한 판독의 변화량 수치이다. 가장 바람직한 판별은 상이한 그룹 판독치 사이의 평균 시그날 비율(예를 들어, 미세정렬 강도 수치) 및 시그날 비율 척도의 표준편차를 사용하는 것이다. 이러한 수

학적/통계적 수치의 다수는 소정의 백분위수로 환산되어 사용될 수 있다.

진단 매개변수 판별의 관계식을 사용하여 진단 적용에 유용한 마커 선택을 최적화한다. 전형적으로는 선형 또는 2차 프로그램된 알고리즘을 사용하여 실시한다. 그러나, 입력 데이타 선택 또는 데이타 출력 보충에 발견적 접근법을 적용하거나 사용할 수도 있다. 가장 바람직한 관계식은, 본원에서 참고문헌으로 인용된 다음 문헌에 기술된 바와 같은 평균-변화량 관계식이다[참고문헌: Mean-Variance Analysis in portfolio Choice and Capital Markers by Harry M. Markowitz(Frank J. Fabozzi Associates, New Hope, PA: 2000, ISBN:1-883249-75-9)]. 재정적 투자 포트폴리오를 위한 주식 선택의 측면에서 관계식을 가장 잘 이해할 수 있다. 이로써 관계식이 전개되고 명확해진다.

주식의 포트폴리오를 최적화하기를 희망하는 투자자는, 수입 및 위험 인자의 사실적 비율이 있는 다수의 가능한 주식으로부터 선택을 할 수 있다. 평균 변화량 방법은, 기대되는 소정의 수입 수준에 대해 (변화량 또는 표준편차에 의해 측정된) 위험을 최소화하고 소정의 위험 수준에 대해 기대되는 수입을 최대화하는 모든 가 능한 포트폴리오를 동정하기 위해 선형 프로그래밍 또는 2차 프로그래밍의 임계선 알고리즘을 사용한다. 표준편차를 기대되는 수입에 대해 플롯화할 경우, 효율 영역이 생성된다. 효율 영역을 따라 주식을 선택하면 수입 및 위험의 측면에서 최적화된 다양한 주식 포트폴리오를 수득할 수 있다.

평균 변화량 관계식을 본 발명의 방법에 사용하는 경우, 재정 포트폴리오 선택에 사용된 수입 및 위험 인자 값이 미세정렬 시그날 강도 및 표준편차와 같은 진단 매개변수로 대체된다. 평균 변화량 관계식을 적용하는 경우, 상업용 컴퓨터 소프트웨어 적용(예: ' Wagner Associates Mean-Variance Optimization Application', 본 명세서에서는 '바그너 소프트웨어'로 지칭)이 가장 바람직하다. 이 소프트웨어는 마코비츠 센스(Markowitz sense)에서 효율 영역 및 최적의 포트폴리오를 결정하기 위해 '바그너 어소시에이트 평균-변화량 최적화 라이브러리'(Wagner Associates Mean-Variance Optimization Library)의 기능을 사용한다. 재정 적용의 경우에도 이러한 방법이 사용되므로, 소프트웨어에 의해 요구되는 사항을 동정할 수 있도록 입력 데이타를 예비 처리할 필요가 있다. 예를 들어, 바그너 소프트웨어를 미세정렬 강도 측정치와 함께 사용한 다음, 후속되는 데이타 전환 방법을 사용한다.

각각의 유전자 기준 및 실험 값 사이의 관계식이 먼저 설정되어야 한다. 바람직한 방법은 다음과 같이 수행한다. 기준 부류를 선택한다. 전형적으로는 이는 문제의 상태를 지니지 않은 집단의 유전자를 포함한다. 예를 들어 유방암을 진단하기 위해서 유전자의 포트폴리오를 선택하는 경우, 유방암이 없는 환자의 샘플을 사용하여 기준 부류를 정할 수 있다. 기준 부류가 선택되면, 기준 부류 샘플에서 각 유전자의 유전자 발현 지표에 대한 산술평균 및 표준편차를 계산한다. 이러한 지표는 전형적으로는 미세정렬 판독의 형광도이다. 컴퓨터로 처리한 통계적 데이타를 사용하여 각 유전자의 기준값(X * 표준편차+평균)을 계산한다. 이는 비교되는 모든 다른 샘플의 유전자에 대한 기준 판독값이다. X는 포트폴리오를 설정하는 사람에 의해 선택된 가변 엄격도이다. X 값이 보다 높은 것이 낮은 값보다 엄격하다. X는 바람직하게는 0.5 내지 3, 보다 바람직하게는 2 내지 3, 가장 바람직하게는 3이다.

그런 다음, 기준 판독값에 대해 (문제의 상태를 나타내는) 각 실험 샘플간의 비율은 계산한다. 비율은 데이타를 용이하게 처리하기 위해 소프트웨어에 의해 상용로그 값으로 전환시킨다. 이는 하향 조절된 유전자가, 바그너 소프트웨어를 사용하는 마크맨 평균-변화량 알고리즘에 따른 최적화에 필요한 마이너스 값을 나타낼 수 있도록 한다.

이러한 전환된 비율을 포함하는 예비 처리 데이타는, 재정 분석 목적에 사용되는 바그너 소프트웨어에서 일반적으로 사용되는 자산 수입 수치 대신에 입력값으로 사용된다.

효율 영역을 설정하는 경우, 영역의 한 지점에 상응하는 소정의 입력 수준(수입) 또는 변화량에 대해 최적화된 포트폴리오를 선택한다. 이러한 입력치 또는 변화량은 포트폴리오를 설정하는 사람에 의해 예비 측정된 표준이다. 달리 언급하 면, 최적의 포트폴리오를 추구하는 사람은 허용될 수 있는 입력 수준(민감성 지표) 또는 소정의 변화량 수준(특이성 지표)를 측정하고, 입력 수준 또는 변화량에 상응하는 효율 영역에 있는 유전자를 선택한다. 바그너 소프트웨어는, 입력 수준 또는 변화량을 선택하는 경우에 이러한 유전자를 선택할 수 있다. 주식 포트폴리오의 주식과 같이, 포트폴리오에서 각각의 유전자에 대한 중요성을 할당할 수도 있다.

포트폴리오가 진단용인 경우에 샘플이 질병이 있는지를 측정하는 것은, 포트폴리오를 설정하는데 사용된 특이적 발현 유전자의 계산 값을 지닌 환자 샘플과 포트폴리오의 유전자 발현을 비교하여 수행할 수 있다. 바람직하게는 포트폴리오 값은 포트폴리오 선택 과정에서 유전자에게 할당된 중요성에 따라 포트폴리오에 있는 각 유전자의 강도 수치를 합하여 먼저 산출한다. 기준 그룹에 대한 포트폴리오 값의 Y * 표준편차+평균에 의해 경계 값을 계산하는데, 여기서 Y는 상기한 X와 같이 동일한 의미를 지니는 엄격도 값이다. 기준 부류의 경계 값을 초과하는 포트폴리오 값을 지닌 샘플은 질병이 있는 것으로 분류한다. 경우에 따라, 이러한 방법은 신뢰 수준을 향상시키기 위한 공지된 통계적 방법에 따라 반복적으로 수행할 수 있다.

임의로 가장 정확한 예측값을 수득할 때까지 이 과정을 반복할 수 있다.

포트폴리오 선택 과정 및 공지되지 않은 특징은 다음과 같이 요약할 수 있다:

1. 기준 부류를 선택한다.

2. 기준 부류 샘플에 대한 각 유전자의 평균 및 표준편차를 계산한다.

3. 각 유전자에 대해 X * 표준편차+평균를 계산한다. 이는 비교할 다른 모든 샘플의 판독 결과를 기준으로 한다. X는 가변 엄격도로 X값이 높은 것은 낮은 것보다 더 엄격하다.

4. 단계 3에서 계산된 기준 판독치에 대한 각 실험 샘플간의 비율을 계산한다.

5. 1 미만의 비율은 마이너스로 전환시킨다(예를 들어, 상용로그를 사용하는데, 하향 조절된 유전자는 이제 정확하게 MV 최적화에 필요한 마이너스 값을 갖는다).

6. 상기한 전환 비율은 소프트웨어 적용시 일반적으로 사용되는 수입 평가 대신에 입력치로서 사용한다.

7. 소프트웨어는 효율 영역을 플롯화하고 효율 영역에 따른 임의의 지점에서 최적화된 포트폴리오로 반환시킨다.

8. 효율 영역에서 바람직한 반환 또는 변화량을 선택한다.

9. 포트폴리오 선택 알고리즘에 의해 산출된 중요성에 따라 각 유전자의 강도 수치를 합하여 각각의 샘플의 포트폴리오 값을 계산한다.

10. 다수의 Y에 기준 그룹의 평균 포트폴리오 값 및 기준 포트폴리오 값의 표준편차를 더하여 경계 값을 계산한다. 이 경계를 초과하는 값은 실험 부류로서 분류하여야 한다.

11. 임의로 가장 정확한 예측값을 수득할 때까지 이 과정을 반복할 수 있다.

기준 및 실험 계산치를 역전시켜 임의로 제2 포트폴리오를 만들 수 있다. 이로써 원래의 기준 부류에서 상향 조절된 유전자의 새로운 포트폴리오가 제조된다. 이러한 제2 포트폴리오 값은 제1 포트폴리오로부터 빼서, 다수의 포트폴리오를 기준으로 한 새로운 분류 값을 만들 수 있다.

유전자 발현 데이타로부터 유전자를 예비 선택하여 포트폴리오를 선택하는 방법에 대한 입력치로서 사용할 수 있는 또다른 유용한 방법은 다음의 역치를 기준으로 한다.

수학식 1

$$1 \le \left| \frac{(\mu_t - \mu_n)}{(\sigma_t + \sigma_n)} \right|$$

상기식에서,

μ_t 은 질환 또는 이상이 있는 공지된 서브세트의 평균이고,

μ_n 은 정상 샘플 서브세트의 평균이며,

$\sigma_t + \sigma_n$ 은 표준편차 합이다.

$0.5 \le \left| \frac{(\mu_t - MAX_n)}{(\sigma_t + \sigma_n)} \right|$ 와 같은 관계식에 따라 데이타를 예비 선택함으로써 잡음 컷 오프에 대한 시그날을 사용할 수도 있다. 이는 특이적 변형을 기준으로 하여 예비 선택된 유전자가 임상적으로 중요한 방식으로 구별되도록 한다. 이는 진단 매개변수를 측정하는 과정에 비례하여 기기의 잡음 수준을 능가한다. 이러한 범주에 따 라 예비 선택된 각각의 마커의 경우, 칼럼이 샘플을 나타내고 열(row)이 마커를 나타내며 각각의 원소가 하기 수학식에 따라 마커 발현에 대해 강도 수치가 정상화되도록 매트릭스를 설정한다.

수학식 2

$$\left|\frac{(\mu_t - I)}{\mu_t}\right|$$

상기식에서,

I는 강도 측정치이다.

재정 포트폴리오 소프트웨어에 있어 입력치를 생성하는 이러한 과정을 사용하면, 소프트웨어로 최적의 포트폴리오를 한정하는 추가의 경계 조건을 설정할 수 있다. 예를 들어 포트폴리오 크기는 고정 범위 또는 마커의 수로 제한할 수 있다. 이는 보다 엄격한 데이타 예비 선택 범주를 만들거나(예: $0.5 \le \left|\frac{(\mu_t - MAX_n)}{(\sigma_t + \sigma_n)}\right|$ 대신에 $0.8 \le \left|\frac{(\mu_t - MAX_n)}{(\sigma_t + \sigma_n)}\right|$) 포트폴리오 크기를 제한하는 것과 같은 프로그래밍 형태를 사용하여 실시할 수 있다. 예를 들어, 최적의 10개의 유전자내에서만 효율 영역이 선택되도록 경계 조건을 설정할 수 있다. 효율 영역을 결정하기 위해 예비 선택된 유전자 모두를 사용한 다음, 선택된 유전자의 수를 제한할 수도 있다(예를 들어, 10개 이하).

포트폴리오를 선택하는 과정에는 발견적 규칙이 적용될 수 있다. 바람직하게는, 이러한 규칙은 임상적 결과를 수득하는데 사용되는 기술의 이해 및 생물학을 기본으로 하여 설정된다. 보다 바람직하게는 이는 최적화 방법의 출력치에 적용된다. 예를 들어, 포트폴리오 선택의 평균 변화량 방법은, 유방암이 있는 피험자에서 특이적으로 발현되는 다수의 유전자의 경우 미세정렬 데이타에 적용할 수 있다. 방법의 출력치는 질환이 있는 유방 조직뿐만 아니라 말초 혈액에서 발현되는 몇몇 유전자를 포함할 수 있는 유전자 세트를 최적화한다. 시험 방법에 사용된 샘플이 말초 혈액으로부터 수득되고 유방암의 경우에 특이적으로 발현되는 특정 유전자가 말초 혈액에서도 특이적으로 발현될 수 있다면, 말초 혈액에서 특이적으로 발현되는 유전자를 제외한 효율 영역으로부터 선택된 포트폴리오에 발견적 규칙을 적용할 수 있다. 물론 규칙은, 예를 들어 데이타 예비 선택 동안에 규칙을 적용함으로써 효율 영역 형성 이전에 적용할 수도 있다.

해당 생물학과 본질적으로 관련이 없는 다른 발견적 규칙을 적용할 수도 있다. 예를 들어, 포트폴리오의 백분률이 특정 유전자 또는 유전자들에 의해 나타나는 규칙을 적용할 수 있다. 바그너 소프트웨어와 같이 시판되는 소프트웨어는 이러한 유형의 발견을 용이하게 수용한다. 이는, 예를 들어 정확도 및 정밀도 이외의 인자(예를 들어, 예상된 허가 비용)가 하나 이상의 유전자를 포함하는 바람직함에 영향을 미치는 경우에 유용할 수 있다.

정확도 및 정밀도 검정과 같이 예정된 특징에 따라 포트폴리오를 최적화하는 한, 평균-변화량 관계식 외에 다른 관계식을 본 발명의 방법에 사용할 수 있다. 두가지 예로 마틴 동시 방정식법[참고문헌: Elton, Edwin J. and Martin J. Gruber (1987), Mordern portfolio Theory Investment Analysis. Third Edition, John Wiley, New York, 1987] 및 유전적 알고리즘[참고문헌: Davis, L., (1989), Adapting Operator Probabilities in Genetic Algorithms, in Proceedings of the Third international Conference on Genetic Algorithms, Morgan Kaufmann: San Mateo, pp.61-69]이 있다. 공지된 경향을 나타내는 마커 검출 기술에서와 같이 불균일한 데이타를 처리하는 평균-변화량 관계식을 채택한 다수의 방식이 있다. 여기에는, 예를 들어 참고 시그날의 평균 제곱(마이너스) 변화량의 제곱근이 참고 시그날 이하의 시그날 값만을 포함하는 반-변화량 방법이 있다.

본 발명의 제품에는 치료, 진단, 예후 및 또다른 질환 평가에 유용한 포트폴리오를 구성하는, 유전자 발현 특징의 설명이 포함된다. 이러한 설명은 컴퓨터 판독가능한 매체(자기, 광학 등)과 같은 기기에 의해 자동적으로 판독될 수 있는 매체에 적합하다. 제품에는 이러한 매체에서 유전자 발현 프로필을 평가하기 위한 지시사항을 포함할 수 있다. 예를 들어, 제품은 또한 상기한 유전자 포트폴리오의 유전자 발현 특징을 비교하기 위한 컴퓨터 지시사항이 있는 CD-ROM이 포함될 수 있다. 제품은 또한 환자 샘플로부터의 유전자 발현 데이타를 비교할 수 있도록 디지털 기록된 유전자 발현 프로필을 포함한다. 다르게는, 프로필은 상이한 제시 형식으로 기록될 수 있다. 그래프 기록이 이러한 형식중 하나이다.

본 발명에 따라 제조한 제품의 상이한 유형에는 유전자 발현 프로필을 나타내는데 사용되는 형식화된 검정 또는 매체가 있다. 이들은, 예를 들어 서열 상보체 또는 프로브가 매트릭스에 고정되어 있어 관심있는 유전자의 서열 표시가 이의 존재여부에 대한 판독가능한 측정치와 일치하는 미세정렬을 포함할 수 있다. 이러 한 미세정렬이 최적화된 포트폴리오를 포함하는 경우, 기질에 적용되고 샘플과 반응하며 분석기에 의해 판독되고 결과를 처리하며 (때론) 검증해야만하는 cDNA 또는 올리고뉴클레오타이드의 수를 최소화함으로써 시간, 처리 단계 및 자원을 절약할 수 있다.

본 발명에 따른 다른 제품은, 하이브리드화, 증폭 및 본 발명의 방법을 통해 설정된 포트폴리오에서 유전자의 발현 수준을 나타내는 시그날 생성을 수행하기 위한 시약 키트로 제조할 수 있다. 본 발명에 따라 제조한 키트에는 유전자 발현 프로필을 측정하기 위해 형식화시킨 검정이 포함된다. 이는 시약 및 지시사항과 같이 검정을 수행하는데 필요한 물질 몇몇 또는 전부를 포함할 수 있다.

실시예

실시예 1: 최적화된 포트폴리오 생성

유전자 발현 데이타는 11가지 상이한 유형의 암을 나타내는 조직 샘플로부터 최근 수득하였다. 데이타는 다음 문헌[참고문헌: Cancer Research 61: 7388-7393, 2001 and http://carrier.gnf.org/welsh/epican, Andrew I. Su et al., 'Molecular Classification of Human Carcinomas by Use of Gene Expression Signatures]에 공개되어 있다. 데이타는 'U95' 올리고뉴클레오타이드 미세정렬(시판원: Affymetrix, Inc.)을 사용하여 수득한 강도 수치를 포함한다.

공개된 데이타로부터의 유전자 발현의 측정치를 사용하여, 순환 세포가 유방암, 전립선암, 난소암, 결장직장암 또는 폐암의 존재여부를 나타내는지를 측정하기 위한 마커의 패널에 대해 최적인 유전자 발현 포트폴리오를 선택한다. 이러한 순환 세포는 상피 세포가 바람직하다.

본 연구의 데이타는 24개 선암, 12개 침윤 관 유방 선암, 21개 결장직장 선암, 23개 난소 선암 및 25개 폐 선암 샘플로부터 수집하였고, 19개 전립선 선암, 12개 유방암, 12개 결장암, 13개 난소암, 13개 난소암 및 89개 폐암의 추가 샘플로부터 데이타를 수집하였다.

기준 부류로서 정상 샘플의 수집물로부터 판독한 강도를 이용하여 각 유전자의 산술평균 및 표준편차를 계산하고, 각 유전자의 수치(X * 표준편차+평균)를 계산하였다. 가변 엄격도 X는 본 경우에서 3의 값이다. 그런 다음, 기준 값 계산치에 대해 본 연구에서 기술한 실험 샘플 사이의 비율을 계산하였다. 비율은 상용로그로 전환시켰다. 그런 다음, 이 값을 바그너 소프트웨어에 대한 입력치로서 사용하였다.

이러한 과정으로, (잡음 비율 지점에 대한 최상의 시그날에서 선택된) 선택된 차이 수준에서 변화량이 가장 적은, 각 종양 유형에 대한 마커의 최소 세트에 따른 효율 영역을 선택하였다. 소프트웨어로 최적화하여, 전립선 암의 경우 2개, 유방암의 경우 5개, 결장암의 경우 6개, 난소암의 경우 2개 및 폐암의 경우 9개의 마커를 포함하여 24개의 유전자의 포트폴리오 선택하였다(표 1).

[표 1]

암 유형	번호	명칭	설명	서열 번호
PR	NM_001648	KLK3	칼리크레인 3, (전립선 특이적 항원)	서열 1
PR	NM_005551	KLK2	칼리크레인 2, 전립선	서열 2
BR	NM_004064	CDKN1B	사이클린-의존적 키나제 억제제 1B (p27, Kip1)	서열 34
BR	NM_002411	MGB1	마마글로빈 1	서열 3
BR	NM_005264	GFRA1	GDNF 패밀리 수용체 알파 1	서열 4
BR	None	C18ORF1	염색체 18 개방 판독 프레임 1	서열 98
BR	NM_000095	COMP	연골 올리고머 매트릭스 단백질	서열 67
CO	NM_001804	CDX1	꼬리형 호메오 박스 전사 인자 1	서열 8
CO	NM_001046	SLC12A2	용질 전달 패밀리 12 (나트륨/칼륨/클로라이드 운반체), 구성원 2	서열 9
CO	NM_001285	CLCA1	클로라이드 채널, 칼슘 활성화됨, 패밀리 구성원 1	서열 11
CO	NM_007052	NOX1	NADPH 옥시다제 1	서열 13
CO	NM_002457	MUC2	뮤신 2, 장/기관지	서열 14
CO	NM_004063	CDH17	카데린 17, LI 카데린	서열 15
LU_A	NM_021950	MS4A2	막-스패닝 4-도메인, 서브패밀리 A, 구성원 2	서열 17

LU_A	NM_000964	ASAHL	N-아실스핑고신 아미도하이드롤라제(산 세라미다제)형	서열 18
LU_A	NM_006495	EVI2B	동종숙주 바이러스 삽입 부위 2B	서열 20
LU_A	NM_006864	LILRB3	백혈구 면역글로불린형, 서브패밀리 B	서열 21
LU_A	X67301	none	IgM 경쇄 불변 영역에 대한 호모 사피엔스 mRNA(Ab63)	서열 22
LU_A	NM_002123	HLA-DQB1	주조직적합 복합체, 부류 II, 베타 1	서열23
LU_S	NM_000673	ADH7	알콜 데하이드로게나제 7 (부류 IV), 뮤 또는 시그마 폴리펩타이드	서열 24
LU_S	NM_003722	TP63	p53에 강한 상동성을 갖는 종양 단백질 63kDa	서열 26
LU_S	None	SOX2	SRY (성 결정 영역 Y)-박스 2	서열 32
OV	NM_000906	NPR1	나트륨이뇨 촉진 펩타이드 수용체 A/구아닐레이트 사이클라제 A	서열 28
OV	NM_000378	WT1	윌림스 종양 1	서열 30

실시예 2: 발견적 단계

실시예 1에서 수득한 포트폴리오에 발견적 규칙을 추가로 적용시켰다. 동정된 유전자/마커가 말초 혈액에서 발현되거나 익히 공지된 조직 마커(예를 들어, PSA, 마마글로빈 등)인 경우, 이러한 유전자/마커의 포트폴리오로부터 제거하는 규칙이다. 규칙의 적용은, 상피 세포와 같이 말초 혈액에서 발견되는 성분을 검정하여 환자 샘플을 수득하는 선별 적용에 사용하기 위해 최적화된 유전자/마커 포트폴리오의 설정을 가능하게 한다. 선택된 포트폴리오의 결과는 표 2에 나타낸 바와 같이 31개의 유전자를 포함한다.

[표 2a]

암 유형	**기탁번호**	**명칭**	**설명**	서열
PR	Hs.12784	KIAA0293	KIAA0293 단백질	서열 67
PR	NM_006562	LBX1	D. 멜라노가스터 호메오도메인 단백질과 유사한 전사 인자	서열 33
PR	NM_016026	LOC51109	CGI-82 단백질	서열 34
PR	HG2261-HT2352	없음	항원	서열 99
PR	NM_012449	STEAP	전립선의 6개의 트랜스막 상피 항원	서열 35
PR	NM_001634	AMD1	S-아데노실메티오닌 데카복실라제 1	서열 36
PR	HG2261-HT2351	없음	항원	서열 100
PR	NM_006457	LIM	LIM 단백질 (랫트 단백질 키나제 C-결합 에니그마와 유사)	서열 37
BR	NM_005853	IRX5	이러쿼이 호메오박스 도메인 5	서열 38
BR	NM_005264	GFRA1	GDNF 패밀리 수용체 알파 1	서열 39
BR	none	C18ORF1	염색체 18 개방 판독 프레임 1	서열 98
BR	NM_000095	COMP	연골 올로고머 매트릭스 도메인 (가성연골무형성, 골단 형성이상 1, 다중)	서열 41

CO	NM_001265	CDX2	꼬리형 호메오박스 전사 인자 2	서열 43
CO	NM_001046	SLC12A2	용질 전달 패밀리 12 (나트륨/칼륨/클로라이드 운반체), 구성원 2	서열 44
CO	NM_001285	CLCA1	클로라이드 채널, 칼슘 활성화, 패밀리 구성원 1	서열 46
CO	NM_004063	CDH17	카데린 17, LI 카데린 (간-장)	서열48
OV	NM_000906	NPR1	나트륨이뇨 펩타이드 수용체 A/구아닐레이트 사이클라제 A(심방나트륨이뇨 펩타이드 수용체 A)	서열 50
OV	NM_005504	BCAT1	측쇄 아미노트랜스퍼라제 1, 세포질	서열 52
OV	NM_002398	MEIS1	메이스 1 (마우스) 상동체	서열 53

[표 2b]

OV	없음	SPON1	스폰딘 1, (f-스폰딘) 세포외 매트릭스 단백질	서열 69
OV	NM_001692	없음	M25809:사람 막내 단백질 펌프 서브유닛 mRNA |GenBank==M25809	서열 54
OV	NM_002774	KLK6	칼리크레인 6 (뉴로신, 자임)	서열 55
LU_A	NM_000964	ASAHL	N-아실스핑고신 아미도하이드롤라제 (산 세라미다제)형	서열 56
LU_A	NM_002838	PTPRC	단백질 티로신 포스파타제, 수용체 유형 C	서열 58
LU_A	NM_015364	MD-2	MD-2 단백질	서열 59
LU_A	NM_006875	PIM2	pim-2 종양유전자	서열 60
LU_S	NM_005554	KRT6A	케라틴 6A	서열 61
LU_S	NM_000673	ADH7	알콜 데하이드로게나제 7 (부류 IV), 뮤 또는 시그마 펩타이드	서열 62
LU_S	NM_003722	TP63	p53에 강한 상동성을 갖는 종양 단백질 63 kDa	서열 64
LU_S	없음	SOX2	SRY (성 결정 영역 Y)-박스 2	서열 32
LU_S	NM_005688	ABCC5	ATP-결합 카세트, 서브 패밀리 C (CFTR/MRP), 구성원 5	서열 66

실시예 3: 예후 포트폴리오

임상적 결과가 공지된 환자 샘플 세트를 사용하여 본 발명의 포트폴리오 선택방법을 시험하였다. 샘플은 본원에서 참고문헌으로 인용된 다음 문헌에서와 같이 설정하였다[참고문헌: van't Veer, L. J et al. Gene Expression Profiling Predicts Clinical Outcome of Breast Cancer, Nature, 415, 530-536, (2002)]. 이 연구에서 유방 조직 샘플은 산발성 유방 종양이 있는 78명의 환자로부터 수득하였다. 환자는 모두 55세 미만으로 5㎝ 미만의 종양을 나타내었다. 모든 림프절은 음성이었다. 34명의 환자의 경우 5년내에 원거리 전이가 나타났지만 44명은 동일한 기간내에 원거리 전이가 나타나지 않았다.

본 연구의 데이타는 본 발명의 방법에 따라 처리하였다. 상실된 수치의 백분율이 높아서 54개의 샘플수를 추가 분석으로부터 삭제하였다. 각 유전자에 대한 강도 수치의 평균 및 표준편차는 기준으로서 비전이 샘플을 사용하여 계산하였다. 그런 다음, 판별 값 X * (표준편차+평균)를 각 기준 유전자에 대해 계산하였다(X는 3의 수치를 갖는다). 이 수치는 수득한 포트폴리오의 엄격성을 보장한다. 그런 다음, 각 전이 샘플에서 기준 값에 대한 판별 값의 비율을 계산하였다. 비율은 상용로그로 전환시켰다. 그런 다음, 이 데이타를 바그너 소프트웨어에 입력시켜 효율 영역을 산출하고, 이로부터 16개 유전자의 포트폴리오를 선택하였다. 기준 값 및 실험 값을 역전시키고, 비전이 경우에서 상향 조절된 유전자를 나타내는 12개 마커의 제2 포트폴리오를 산출하였다. 제2 포트폴리오 값을 제1 포트폴리오 값으로부터 빼서 모두 28개 유전자로부터 합한 포트폴리오 값을 수득하였다. 이러한 최종 포트폴리오는 서열 70 내지 97의 유전자를 포함한다. 포트폴리오 유전자는 다음과 같이 동정되었다(서열 70, 서열 72, 서열 73 내지 77, 서열 79, 서열 80, 서열 85, 서열 87, 서열 91 내지 93, 서열 95 및 서열 97).

28개 유전자 목록(제2 포트폴리오)

전이 환자(포트폴리오 1)에서의 상향

콘티그53226_RC	서열 89
NM_012214	서열 82
NM_020386	서열 86
NM_004504	서열 81
AA555029_RC	서열 70
AL080059	서열74
AF055033	서열 73
NM_016448	서열 85
콘티그40831_RC	서열 95
콘티그63649_RC	서열 91
콘티그24252_RC	서열 93
NM_000436	서열 75
NM_002019	서열 77
콘티그55313_RC	서열 90
콘티그25991	서열 97
NM_000788	서열 76
비전이 환자에서 상향 (포트폴리오 2) AB033007	서열 71
콘티그42421_RC	서열 96
NM_003748	서열 78
NM_013262	서열 83
NM_003862	서열 79
NM_003882	서열 80
콘티그48328_RC	서열 87
NM_015416	서열 84
AB037863	서열 72
콘티그27312_RC	서열 88
콘티그32125_RC	서열 92
콘티그49670_RC	서열 94

17개 중복부

전체 명칭	
NM_003862	서열 79
NM_003882	서열 80
콘티그48328_RC	서열 87
AA555029_RC	서열 70
AL080059	서열 74
AF055033	서열73

AF055033	서열 73
NM_016448	서열 85
AB037863	서열 72
콘티그40831_RC	서열 95
콘티그63649_RC	서열 91
콘티그24252_RC	서열 93
NM_000436	서열 75
NM_002019	서열 77
콘티그32125_RC	서열 92
콘티그25991	서열 97
NM_000788	서열 76

참고문헌에 기술되어 있는 분류 정확도를 시험하기 위해, 본 발명에 따른 미세정렬 데이타로부터의 유전자 발현 특징을 비교함으로써 78개의 원래의 샘플의 예후를 결정하는데 2개의 포트폴리오를 사용하였다. 70개의 유전자 포트폴리오의 경우, 최적화된 역치에 따라 샘플의 81%가 불충분한 예후의 지표로서 모호한 특징을 포함하는 것으로 나타났다(절대 역치의 경우 85%). 이 포트폴리오는 최적화된 역치를 사용하여 예후가 좋지 못한 3명의 환자를 예후가 좋은 것으로 잘못 분류하였다(절대 역치의 경우, 5명). 예후가 좋은 12명의 환자는, 최적 역치를 사용하여 예후가 나빠진 경우에 예후가 좋은 것으로 잘못 분류하였다(절대 역치의 경우 8명).

28개의 유전자 포트폴리오의 경우, 최적화된 역치에 따라 샘플의 94%가 불충분한 예후의 지표로서 모호한 특징을 포함하는 것으로 나타났다(절대 역치의 경우 93%). 이 포트폴리오는 최적화된 역치를 사용하여 예후가 좋지 못한 3명의 환자를 예후가 좋은 것으로 잘못 분류하였다(절대 역치의 경우, 5명). 예후가 좋은 3명의 환자는, 최적 역치를 사용하여 예후가 나빠진 경우에 예후가 좋은 것으로 잘못 분류하였다(절대 역치의 경우 2명).

2개의 프로필을 비교하면, 본 발명의 방법에 따라 선택한 프로필이 훨씬 더 경제적이고 비교할만한 프로필의 결과에서보다 정확하고 신뢰할만한 결과를 생성한다는 것이 명백하다.

발명의 효과

본 발명에 의해 진단 매개변수를 한정하고 매개변수가 최적화되도록 매개변수간 관계식을 설정하며 이러한 관계식을 사용하여 진단 적용에 대해 최적인 마커 그룹을 선택하는, 진단 적용에 사용하기 위한 마커의 포트폴리오 선택방법이 제공된다.

(57) 청구의 범위

청구항 1.

a) 진단 매개변수를 한정하는 단계;

b) 마커의 최적화된 포트폴리오를 동정하는 진단 매개변수 사이의 관계식을 설정하는 단계; 및

c) 당해 포트폴리오를 선택하는 단계를 포함하는, 진단 적용에 사용하기 위한 마커의 포트폴리오 선택방법.

청구항 2.

제1항에 있어서, 마커가 유전자를 포함하는 방법.

청구항 3.

제2항에 있어서, 관계식이 유전자 발현을 기본으로 하는 방법.

청구항 4.

제2항에 있어서, 관계식이 유전자의 발현 변화량을 기본으로 하는 방법.

청구항 5.
제4항에 있어서, 진단 매개변수가 유전자의 상대적 발현도의 척도 및 유전자 발현도 측정시의 변화량의 척도를 포함하며 관계식이 평균 변화량 관계식인 방법.

청구항 6.
제1항에 있어서, 발견적 규칙의 적용을 추가로 포함하는 방법.

청구항 7.
제1항에 있어서, 진단 매개변수가 민감성 및 특이성을 나타내는 방법.

청구항 8.
진단 매개변수 사이의 관계식을 사용하여 마커를 최적화하는 지시사항을 포함하는, 마커의 포트폴리오를 동정하기 위해 프로그램된 다목적 컴퓨터를 포함하는 기기.

청구항 9.
제8항에 있어서, 마커가 유전자를 포함하는 기기.

청구항 10.
제9항에 있어서, 관계식이 유전자 발현을 기본으로 하는 기기.

청구항 11.
제9항에 있어서, 관계식이 유전자 발현의 변화량을 기본으로 하는 기기.

청구항 12.
제9항에 있어서, 지시사항이 유전자의 상대적 발현도의 척도 및 유전자 발현도 측정시의 변화량의 척도를 포함하며 관계식이 평균 변화량 관계식인 기기.

청구항 13.
제8항에 있어서, 발견적 규칙을 실행하는 지시사항을 추가로 포함하는 기기.

청구항 14.
제8항에 있어서, 진단 매개변수가 민감성 및 특이성을 나타내는 기기.

청구항 15.
a) 진단 매개변수를 알고리즘에 입력하기 위한 지시사항;

b) 마커의 최적화 포트폴리오를 동정할 수 있도록 진단 매개변수와 연관된 상기한 알고리즘을 실행하기 위한 지시사항; 및

c) 상기한 포트폴리오를 선택하기 위한 지시사항을 포함하여, 진단 적용에 사용하기 위한 마커의 포트폴리오를 선택하기 위한 지시사항을 포함하는 제품.

청구항 16.
제15항에 있어서, 마커가 유전자를 포함하는 제품.

청구항 17.
제16항에 있어서, 관계식이 유전자 발현을 기본으로 하는 제품.

청구항 18.
제17항에 있어서, 관계식이 유전자 발현 변화량을 기본으로 하는 제품.

청구항 19.
제17항에 있어서, 진단 매개변수가 유전자의 상대적 발현도의 척도 및 유전자 발현도 측정시의 변화량 척도를 포함하며 관계식이 평균 변화량 관계식인 제품.

청구항 20.
제16항에 있어서, 발견적 규칙의 적용을 위한 지시사항을 추가로 포함하는 제품.

청구항 21.
제16항에 있어서, 진단 매개변수가 민감성 및 특이성을 나타내는 제품.

청구항 22.
제16항에 있어서, 지시사항이 기기 판독가능한 제품.

청구항 23.
제22항에 있어서, 지시사항이 컴퓨터 판독가능한 제품.

청구항 24.
제1항에 따른 방법에 따라 선택한 진단 포트폴리오.

청구항 25.
제24항에 있어서, 유전자를 포함하는 포트폴리오.

<110> Ortho-Clinical Diagnostics, Inc

<120> Selection of markers

<130> 5-1999-029538-1

<150> 60/368,790

<151> 2002-03-29

<160> 100

<170> Kopatentin 1.71

<210> 1

<211> 1466

<212> DNA

<213> human

<400> 1

agccccaagc ttaccacctg cacccggaga gctgtgtgtc accatgtggg tcccggttgt 60

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<400> 87

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<211> 400

<212> DNA

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<213> human

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<211> 1017

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专利名称(译)	选择标记		
公开(公告)号	KR1020030078803A	公开(公告)日	2003-10-08
申请号	KR1020030019839	申请日	2003-03-29
[标]申请(专利权)人(译)	奥索临床诊断有限公司		
申请(专利权)人(译)	妥临床钻石刀论学，细胞器的鼻子		
当前申请(专利权)人(译)	妥临床钻石刀论学，细胞器的鼻子		
[标]发明人	JATKOE TIM		
发明人	JATKOE,TIM		
IPC分类号	G06F19/20 G06F9/00 C12Q1/68 G06F17/18 G01N33/48 G01N33/50 G01N33/53 G06F19/18 G06F17/00 A61B5/00 G01N37/00		
CPC分类号	G06F19/18 G06F19/20 G16B20/00 G16B25/00		
代理人(译)	李，何炳 李昌勋		
优先权	60/368790 2002-03-29 US		
外部链接	Espacenet		

摘要(译)

本发明涉及一种标记组合选择方法，它意味着为诊断应用选择最佳标记组，限制诊断参数，并在参数之间建立相关公式，使参数最优化，并用于诊断应用。诊断参数包括在测量基因和基因的相对表达图的绝对温标的表达程度时的绝对温度变化量。并且诊断和辨别参数之间的相对公式可以是平均符号翻转相对公式。本发明的附加方面所看到的方法可以参考包括为了执行而编程的仪器的指令及其操作的产品。标记，组合，参数，基因表达，诊断。

[표 1]

암 유형	번호	명칭	설명	서열 번호
PR	NM_001648	KLK3	칼리크레인 3, (전립선 특이적 항원)	서열 1
PR	NM_005551	KLK2	칼리크레인 2, 전립선	서열 2
BR	NM_004064	CDKN1B	사이클린- 의존적 키나제 억제제 1B (p27, Kip1)	서열 34
BR	NM_002411	MGB1	마마글로빈 1	서열 3
BR	NM_005264	GFRA1	GDNF 패밀리 수용체 알파 1	서열 4
BR	None	C18ORF1	염색체 18 개방 판독 프레임 1	서열 98
BR	NM_000095	COMP	연골 올리고머 매트릭스 단백질	서열 67
CO	NM_001804	CDX1	꼬리형 호메오 박스 전사 인자 1	서열 8
CO	NM_001046	SLC12A2	용질 전달 패밀리 12 (나트륨/칼륨/클로라이드 운반체), 구성원 2	서열 9
CO	NM_001285	CLCA1	클로라이드 채널, 칼슘 활성화됨, 패밀리 구성원 1	서열 11
CO	NM_007052	NOX1	NADPH 옥시다제 1	서열 13
CO	NM_002457	MUC2	뮤신 2, 장/기관지	서열 14
CO	NM_004063	CDH17	카데린 17, LI 카데린	서열 15
LU_A	NM_021950	MS4A2	막- 스패닝 4- 도메인, 서브패밀리 A, 구성원 2	서열 17