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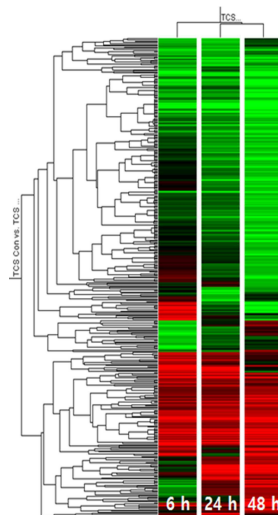
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(54) 발명의 명칭 트리클로산 노출에 대응하는 히드라 유전자 및 이를 이용한 수생태계 환경오염 진단 방법

(57) 요약

본 발명은 트리클로산(Triclosan, TCS) 노출에 대응하는 히드라(*Hydra magnipapillata*) 유전자 및 이를 이용하여 수생태계 환경오염을 진단하는 방법에 관한 것으로, 구체적으로 히드라를 배양하여 트리클로산을 7.35 µg/L 농도로 6 h, 24 h 및 48 h 동안 노출시킨 후, 이로부터 RNA를 분리하고, cDNA를 합성한 뒤, Cy3 및 Cy5로 표지하여 혼성화하였고, oligo-마이크로어레이를 제작하여 이를 분석한 결과, 트리클로산 6시간 노출군에서는 32종의 유전자(증가 20종, 감소 12종); 24시간 노출군에서는 10종의 유전자(증가 9종, 감소 1종); 48시간 노출군에서는 114종의 유전자(증가 22종, 감소 92종)의 유전자들이 트리클로산 노출에 의해 발현량이 변화되는 것을 확인함으로써, 상기 156종의 유전자들은 트리클로산 노출을 확인할 수 있는 바이오마커로 유용하게 활용될 수 있다.

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명세서

청구범위

청구항 1

서열번호 1 내지 156으로 기재되는 모든 유전자 각각의 핵산 서열의 올리고뉴클레오티드 전부 또는 그의 상보가닥 분자가 집적된, 시료내 트리클로산(triclosan, TCS) 노출 여부 검출용 마이크로어레이 칩(microarray chip).

청구항 2

제 1항에 있어서, 상기 유전자는 히드라 (*Hydra magnipapillata*)로부터 유래된 것을 특징으로 하는 트리클로산 노출 여부 검출용 마이크로어레이 칩.

청구항 3

- 1) 피검 시료에 노출된 실험군의 히드라와, 정상 대조군의 히드라에서 각각 RNA를 분리하는 단계;
- 2) 단계 1)의 실험군 및 대조군의 RNA로부터 cDNA를 합성하면서 실험군과 대조군을 각기 다른 형광물질로 표지하는 단계;
- 3) 단계 2)의 각기 다른 형광물질로 표지된 cDNA를 제 1항의 마이크로어레이 칩과 혼성화시키는 단계;
- 4) 반응한 마이크로어레이 칩을 분석하는 단계; 및
- 5) 분석한 데이터에서 제 1항의 마이크로어레이 칩에 집적된 유전자 발현 정도를 대조군과 비교하여 확인하는 단계를 포함하는, 시료내 트리클로산 노출 여부 검출 방법.

청구항 4

제 3항에 있어서, 상기 시료는 생체시료, 식품시료, 화학시료, 공업시료, 임상시료 및 환경시료로 구성된 군으로부터 선택되는 어느 하나인 것을 특징으로 하는 트리클로산 노출 여부 검출 방법.

청구항 5

제 3항에 있어서, 상기 단계 2)의 형광물질은 Cy3, Cy5, FITC(poly L-lysine-fluorescein isothiocyanate), RITC(rhodamine-B-isothiocyanate) 및 로다민(rhodamine)으로 이루어진 군으로부터 선택되는 어느 하나인 것을 특징으로 하는 트리클로산 노출 여부 검출 방법.

청구항 6

- 1) 피검시료에 노출된 실험군의 히드라와, 정상 대조군의 히드라에서 각각 RNA를 분리하는 단계;
- 2) 단계 1)의 RNA를, 서열번호 1 내지 156으로 기재되는 각각의 유전자에 상보적이고 유전자를 증폭할 수 있는 프라이머 쌍을 모두 사용하여 정량 실시간 RT-PCR(Quantitative real-time reverse transcript polymerase chain reaction, qRT-PCR)을 수행하는 단계; 및
- 3) 단계 2)의 유전자 산물을 대조군과 비교하여 발현 정도를 확인하는 단계를 포함하는, 시료내 트리클로산 노출 여부 검출 방법.

청구항 7

제 6항에 있어서, 상기 시료는 생체시료, 식품시료, 화학시료, 공업시료, 임상시료 및 환경시료로 구성된 군으로부터 선택되는 어느 하나인 것을 특징으로 하는 트리클로산 노출 여부 검출 방법.

청구항 8

제 1항의 마이크로어레이 칩을 포함하는 트리클로산 노출 여부 검출용 키트.

청구항 9

제 8항에 있어서, 스트렙타비딘-알칼리 탈인화효소 접합물질(streptavidin-like phosphatase conjugate), 화학형광물질(chemifluorescence) 및 화학발광물질(chemiluminescent)로 이루어진 형광물질군으로부터 선택되는 어느 하나를 추가적으로 포함하는 것을 특징으로 하는 트리클로산 노출 여부 검출용 키트.

청구항 10

제 8항에 있어서, 혼성화에 사용되는 완충용액, RNA로부터 cDNA(complementary DNA)를 합성하기 위한 역전사효소, dNTPs(deoxynucleotide triphosphates) 및 rNTPs(ribonucleotide triphosphates, 사전 혼합형 또는 분리 공급형), 표식시약, 및 세척 완충용액으로 이루어진 반응 시약군으로부터 선택되는 어느 하나를 추가적으로 포함하는 것을 특징으로 하는 트리클로산 노출 여부 검출용 키트.

청구항 11

서열번호 1 내지 156으로 기재되는 각각의 유전자에 상보적이고 유전자를 증폭할 수 있는 프라이머 쌍을 모두 포함하는 트리클로산 노출 여부 검출용 키트.

발명의 설명

기술 분야

[0001] 발명은 트리클로산(Triclosan, TCS) 노출에 대응하는 히드라 (*Hydra magnipapillata*) 유전자 및 이를 이용하여 수생태계 환경오염을 진단하는 방법에 관한 것이다.

배경 기술

[0003] 트리클로산(Triclosan, TCS)은 항균 및 항진균 활성을 갖는 화학물질로, 치약, 방취제, 비누, 세제 등과 같은 개인 청결용품에 사용된다. 미국 식품의약국(US Food and Drug Administration)에 의하면 트리클로산이 인간에게 위험하다고 알려진 것은 아직 없다고 하지만, 그 위해성에 대해서는 계속 논란이 진행 중이다. 즉, 트리클로산이 독성을 일으키고 면역력을 저하시키며 체내의 호르몬 조절, 특히 에스트로겐 관련 반응과 갑상선 호르몬 관련 반응에 문제를 일으킬 수도 있는 것으로 알려져 있다. 미국의 비영리 환경단체인 EWG 2008년 보고서에 의하면 트리클로산은 수돗물의 염소와 반응하여 발암물질인 염화 다이옥신을 생성하고 그 자체만으로도 갑상선 기능과 갑상선 호르몬을 방해하는 물질로 의심하고 있다.

[0004] 생물들은 환경오염, 기후변화 및 병원성 미생물의 감염 등과 같은 외부 환경변화에 대응하여, 항상성을 유지하기 위해 생체방어기작을 진화시켜왔다. 이 생체방어기작의 대부분은 특정 유전자의 발현량을 능동적으로 변화시켜, 특정 단백질의 양을 조절하는 것으로 나타난다. 따라서 특정 변화에 대한 특이적인 유전자들을 발굴하여 이들의 발현량 변화를 모니터링한다면, 특정지역의 환경변화에 관한 정보뿐만 아니라, 이러한 환경변화가 생명 현상에 미치는 영향 및 생태계의 건강에 관한 정보를 얻을 수 있다.

[0005] 히드라(*Hydra magnipapillata*)는 신경세포를 갖고 있는 최초의 다세포 동물로서, 생물 진화의 연구에도 매우 중요한 위치를 차지하고 있다. 이배엽성 동물로서 번식은 무성 및 유성생식에 의하며, 뛰어난 재생능력을 갖고 있다. 형태형성과 관련된 신호전달계(Hobmayer 등, 2000, Nature 407: 186-189; Arvizu 등, 2006, Differentiation 74: 305-312; Augustin 등, 2006, Dev. Biol. 296: 62-70; Kaesbauer 등, 2007, Dev. Biol. 303: 376-390), 재생과 관련된 신호 및 신호전달계(Bode, 2003, Dev. Dyn. 226: 225-236; Fujisawa, 2003, Dev. Dyn. 226: 182-189; Holstein 등, 2003, Dev. Dyn. 226: 257-267), 세포분화의 신호전달과 관련된 연구(Thomsen 등, 2004, Mech. Dev. 121: 195-204; Philipp 등, 2005, Gene Expr. Patterns, 5: 397-402) 및 조직의 fate 결정 신호(Bielen 등, 2007, Development, 134: 4187-4197)등이 알려져 있다. 이러한 신호전달계는 진화과정을 통해 모든 동물에 공통적으로 존재하므로, 히드라에서의 신호전달계 이상을 다른 동물에 외삽할 수 있다. 대부분의 동물에서 초기발생과정에만 발현하는 다양한 유전자들이 Hydra에서는 성체에서도 그 발현이 유지된다. 따라서 시기적인 제한을 받지않고 이와 관련된 신호전달계의 연구가 가능하다. 각 세포형에 대한 분자마커들이 알려져 있어, 세포분화에 대한 연구도 가능하다. 유전자 및 단백질 발현의 localization을 위한 in situ hybridization(ISH) 및 immunohistochemistry(IHC)법도 정립되어 있다. 현재 160,000 클론의 ESTs 정보 및 genome data(Chapman 등, 2010, Nature 464: 592-596)가 DB화되어 있어, 다양한 신호전달계에 속하는 유전자들에 대한 정보 검색이 가능하다.

[0006] 이에 본 발명자들은 트리클로산 노출에 대한 특이 유전자후보의 확보 및 검출을 위해, DDBJ/EMBL/NCBI 유전자 데이터베이스에 축적되어 있는 유전자 정보로부터 17,000여개의 singleton을 추출하였다. 추출된 singleton들에 대한 oligo-probe를 디자인하고, 이들을 탑재한 17K Hydra Express Gene Microarray(HEGEM)을 완성하였다. 이 17K HEGEM을 이용하여 TCS 6시간 노출군에서는 32종의 유전자(증가 20종, 감소12종); 24시간 노출군에서는 10종의 유전자(증가 9종, 감소 1종); 48시간 노출군에서는 114종의 유전자(증가 22종, 감소 92종)들이 트리클로산 노출에 의해 발현량이 변화되는 것으로 파악되며, 노출에 의해 발현량이 변화되는 유전자를 발굴, 트리클로산 노출 여부를 확인할 수 있는 바이오마커 및 이를 이용한 노출 여부를 확인하는 방법을 확립하여 본 발명을 완성하였다.

발명의 내용

해결하려는 과제

[0008] 본 발명의 목적은 트리클로산(Triclosan, TCS) 노출에 대응하는 히드라 (*Hydra magnipapillata*) 유전자 및 이를 이용한 트리클로산 노출 여부를 확인하는 방법을 제공하는 것이다.

과제의 해결 수단

[0010] 상기 목적을 달성하기 위하여, 본 발명은 서열번호 1 내지 156으로 기재되는 모든 유전자 각각의 핵산 서열의 올리고뉴클레오티드 전부 또는 그의 상보가닥 분자가 집적된, 시료내 트리클로산(triclosan, TCS) 노출 여부 검출용 마이크로어레이 칩(microarray chip)을 제공한다.

[0011] 또한, 본 발명은

- [0012] 1) 피검 시료에 노출된 실험군의 히드라와, 정상 대조군의 히드라에서 각각 RNA를 분리하는 단계;
- [0013] 2) 단계 1)의 실험군 및 대조군의 RNA로부터 cDNA를 합성하면서 실험군과 대조군을 각기 다른 형광물질로 표지하는 단계;
- [0014] 3) 단계 2)의 각기 다른 형광물질로 표지된 cDNA를 상기 마이크로어레이 칩과 혼성화시키는 단계;
- [0015] 4) 반응한 마이크로어레이 칩을 분석하는 단계; 및
- [0016] 5) 분석한 데이터에서 상기 마이크로어레이 칩에 집적된 유전자 발현 정도를 대조군과 비교하여 확인하는 단계를 포함하는, 시료내 트리클로산 노출 여부 검출 방법을 제공한다.

[0017] 또한, 본 발명은

- [0018] 1) 피검시료에 노출된 실험군의 히드라와, 정상 대조군의 히드라에서 각각 RNA를 분리하는 단계;
- [0019] 2) 단계 1)의 RNA를, 서열번호 1 내지 156으로 기재되는 각각의 유전자에 상보적이고 유전자를 증폭할 수 있는 프라이머 쌍을 모두 사용하여 정량 실시간 RT-PCR(Quantitative real-time reverse transcript polymerase

chain reaction, qRT-PCR)을 수행하는 단계; 및

[0020] 3) 단계 2)의 유전자 산물을 대조군과 비교하여 발현 정도를 확인하는 단계를 포함하는, 시료내 트리클로산 노출 여부 검출 방법을 제공한다.

[0021] 또한, 본 발명은 상기 마이크로어레이 칩을 포함하는 트리클로산 노출여부 검출용 키트를 제공한다.

[0022] 아울러, 본 발명은 서열번호 1 내지 156으로 기재되는 각각의 유전자에 상보적이고 유전자를 증폭할 수 있는 프라이머 쌍을 모두 포함하는 트리클로산 노출 여부 검출용 키트를 제공한다.

발명의 효과

[0024] 본 발명에서 히드라(*Hydra magnipapillata*) 유전자 oligo-마이크로어레이를 활용한, 마이크로어레이 실험분석을 통해, 트리클로산 6시간 노출군에서는 32종의 유전자(증가 20종, 감소 12종); 24시간 노출군에서는 10종의 유전자(증가 9종, 감소 1종); 48시간 노출군에서는 114종의 유전자(증가 22종, 감소 92종)의 유전자들이 트리클로산 노출에 의해 발현량이 변화되는 것을 확인함으로써, 상기 156종의 유전자들은 트리클로산 노출을 확인할 수 있는 바이오마커로 유용하게 활용될 수 있다.

도면의 간단한 설명

[0026] 도 1은 트리클로산(Triclosan, TCS)에 노출한 히드라(*Hydra magnipapillata*)의 차등 발현 유전자 프로파일링 Hierachial clustering 결과를 나타낸 도이다.

발명을 실시하기 위한 구체적인 내용

[0027] 이하, 본 발명을 상세히 설명한다.

[0028] 본 발명은 트리클로산(triclosan; TCS) 노출에 대응하여 발현이 변화하는 히드라(*Hydra magnipapillata*) 유래의 유전자를 발굴하여, 상기 트리클로산 노출에 대하여 발현량이 변화하는 히드라 유래 유전자를 집적한 마이크로어레이 칩을 트리클로산 노출 여부 검출 및 수생태계 오염 상태를 진단하는데 이용할 수 있다.

[0030] 본 발명은 서열번호 1 내지 156으로 기재되는 각각의 핵산 서열의 올리고뉴클레오티드 전부 또는 그의 상보가닥 분자가 집적된, 시료내 트리클로산(triclosan, TCS) 노출 여부 검출용 마이크로어레이 칩(microarray chip)을 제공한다.

[0031] 상기 유전자는 히드라 (*Hydra magnipapillata*)로부터 유래된 것이 바람직하다.

[0032] 상기 트리클로산 노출 여부 검출용 마이크로어레이 칩은 당업자에게 알려진 방법으로 제작할 수 있다. 상기 마이크로어레이 칩을 제작하는 방법은 하기와 같다. 상기 탐색된 유전자를 프로브로 이용하여 마이크로어레이 칩의 기관상에 고정화시키기 위해 파이프조일렉트릭(piezoelectric) 방식을 이용한 마이크로피펫팅(micropipetting)법 또는 핀(pin) 형태의 스폿터(spotter)를 이용한 방법 등을 사용하는 것이 바람직하나 이에 한정되지 않는다. 상기 마이크로어레이 칩의 기관은 아미노-실란(amino-silane), 폴리-L-라이신(poly-Llysine) 및 알데히드(aldehyde)로 이루어진 군에서 선택되는 하나의 활성기가 코팅된 것이 바람직하나 이에 한정되지 않는다. 또한, 상기 기관은 슬라이드 글라스, 플라스틱, 금속, 실리콘, 나일론 막, 및 니트로셀룰로스 막으로 이루어진 군에서 선택될 수 있으나 이에 한정되지 않는다.

[0034] 또한, 본 발명은

[0035] 1) 피검 시료에 노출된 실험군의 히드라와, 정상 대조군의 히드라에서 각각 RNA를 분리하는 단계;

[0036] 2) 단계 1)의 실험군 및 대조군의 RNA로부터 cDNA를 합성하면서 실험군과 대조군을 각기 다른 형광물질로 표지하는 단계;

[0037] 3) 단계 2)의 각기 다른 형광물질로 표지된 cDNA를 상기 마이크로어레이 칩과 혼성화시키는 단계;

[0038] 4) 반응한 마이크로어레이 칩을 분석하는 단계; 및

[0039] 5) 분석한 데이터에서 상기 마이크로어레이 칩에 집적된 유전자 발현 정도를 대조군과 비교하여 확인하는 단계를 포함하는, 시료내 트리클로산(triclosan, TCS) 노출 여부 검출 방법을 제공한다.

[0040] 상기 시료는 생체시료, 식품시료, 화학시료, 공업시료, 임상시료 및 환경시료로 구성된 군으로부터 선택되는 어

는 하나인 것이 바람직하다.

- [0041] 또한, 상기 단계 2)의 형광물질은 Cy3, Cy5, FITC(poly L-lysine-fluorescein isothiocyanate), RITC(rhodamine-B-isothiocyanate) 및 로다민(rhodamine)으로 이루어진 군으로부터 선택되는 어느 하나인 것이 바람직하다.
- [0043] 또한, 본 발명은
- [0044] 1) 피검시료에 노출된 실험군의 히드라와, 정상 대조군의 히드라에서 각각 RNA를 분리하는 단계;
- [0045] 2) 단계 1)의 RNA를, 서열번호 1 내지 156으로 기재되는 각각의 유전자에 상보적이고 유전자를 증폭할 수 있는 프라이머 쌍을 모두 사용하여 정량 실시간 RT-PCR(Quantitative real-time reverse transcript polymerase chain reaction, qRT-PCR)을 수행하는 단계; 및
- [0046] 3) 단계 2)의 유전자 산물을 대조군과 비교하여 발현 정도를 확인하는 단계를 포함하는, 시료내 트리클로산 노출 여부 검출 방법을 제공한다.
- [0047] 상기 시료는 생체시료, 식품시료, 화학시료, 공업시료, 임상시료 및 환경시료로 구성된 군으로부터 선택되는 어느 하나인 것이 바람직하다.
- [0048] 또한, 본 발명은 상기 마이크로어레이 칩을 포함하는 트리클로산 노출 여부 검출용 키트를 제공한다.
- [0049] 상기 키트는 스트렙타비딘-알칼리 탈인화효소 접합물질(streptavidin -like phosphatase conjugate), 화학형광 물질(chemifluorescence) 및 화학발광물질(chemiluminescent)로 이루어진 형광물질군으로부터 선택되는 어느 하나를 추가적으로 포함하는 것이 바람직하다.
- [0050] 또한, 상기 키트는 혼성화에 사용되는 완충용액, RNA로부터 cDNA(complementary DNA)를 합성하기 위한 역전사효소, dNTPs(deoxynucleotide triphosphates) 및 rNTPs(ribonucleotide triphosphates, 사전 혼합형 또는 분리 공급형), 표식시약, 및 세척 완충용액으로 이루어진 반응 시약군으로부터 선택되는 어느 하나를 추가적으로 포함하는 것이 바람직하다.
- [0051] 아울러, 본 발명은 서열번호 1 내지 156으로 기재되는 각각의 유전자에 상보적이고 유전자를 증폭할 수 있는 프라이머 쌍을 모두 포함하는 트리클로산 노출 여부 검출용 키트를 제공한다.
- [0054] 이하, 본 발명을 실시예에 의해서 상세히 설명한다.
- [0055] 단, 하기 실시예는 본 발명을 예시하기 위한 것일 뿐, 본 발명이 하기 실시예에 의해서 한정되는 것은 아니다.
- [0056] <실시예 1> 히드라의 배양 및 트리클로산 노출
- [0057] <1-1> 히드라의 배양
- [0058] 히드라(*Hydra magnipapillata*)의 야생종(wild strain) 105는 1 mM NaCl, 1 mM CaCl₂, 0.1 mM KCl, 0.1 mM MgSO₄, 1 mM Tris (hydroxymethyl) aminoethane (pH7.6)에 배양하였다. 수온은 20℃로 고정하였으며, 이틀에 한번 갓 부화한 아르테미아(*Artemia*) 유생을 먹이로 공급하였다. 먹이 공급 후, 수 시간 이후에 배양액을 교환하였다.
- [0060] <1-2> 트리클로산 노출
- [0061] 히드라를 대상으로 트리클로산 노출 조건을 확립하기 위해, 트리클로산 반수치사농도(Lethal concentration 50, LC₅₀)를 결정하였다. 그 결과, 48 시간 노출 반수치사농도는 367.72 µg/L로 확인되었다. 차등발현 유전자 프로파일링을 위한 노출 농도는 48 h LC₅₀ (367.72 µg/L)의 약 1/50에 해당하는 7.35 µg/L에 히드라 20 개체를 각각 6 h, 24 h, 48 h 동안 노출하였다.
- [0063] <실시예 2> 트리클로산 노출에 의한 유전자 발현량 변화 측정
- [0064] <2-1> RNA 추출 및 cDNA 합성
- [0065] 노출 시험군(6, 24, 48시간) 및 노출하지 않은 대조군의 히드라조직에 Tri-Reagent 용액(Molecular Research Center Inc, Cincinnati, Ohio, USA) 1 ml을 넣고 유리 균질기(glass homogenizer)를 이용하여 균질화하고, 실온에 분간 방치하였다. 클로로포름 200 µl를 첨가하여 잘 섞은 후, 실온에서 10분간 방치하고, 15분간 원심

분리(12,000×g, 4℃)하였다. 그런 다음, 상층액을 수득하여, 아이소프로판올(isopropanol) 500 μl를 넣고, 상온에 5분간 방치하였다. 약 20 분간 원심분리(12,000×g, 4℃)한 후, 용액을 제거하여 침전물만을 취합하였고, 상기 침전물에 70% 에탄올 용액 50 μl를 넣어 5분간 원심분리한 뒤, 에탄올 용액을 제거하고 침전된 RNA를 건조시켰다. 건조 후, 적당량의 에틸피로카보네이트(diethylpyrocarbonate, DEPC)로 처리한 멸균수에 용해하였다

[0067] <2-2> Cy3 및 Cy5 표지

[0068] 정제된 노출군 및 대조군의 전체 RNA는 Agilent's Low RNA Input Linear Amplification Kit Plus (Agilent Technologies, USA)를 사용하여 제조사의 프로토콜을 따라 다음과 같이 Cy3 및 Cy5로 표지하였다.

[0069] 구체적으로, RNA 1 μg을 dT-프로모터 프라이머(dT-promoter primer)와 MMLV-역전사효소(MMLV-Reverse transcriptase)와 혼합하여 40℃에서 2시간 동안 역전사반응을 수행하였다. 그런 다음, T7 중합효소(T7 polymerase)를 첨가하여 40℃에서 2시간 동안 선형 증폭(linear amplification)을 수행하였다. 이와 같은 증폭과정을 통해 실험군 및 대조군의 시료를 각각 Cy3-CTP와 Cy5-CTP로 표지하였다.

[0071] <2-3> 혼성화(Hybridization) 및 스캐닝(scanning)

[0072] 형광물질이 라벨링된 cRNA 시료를 Qiagen PCR purification kit을 사용하여 정제하고, 증류수로 용출하였다. 정제된 형광표지-cRNA 시료를 혼성화 완충액(hybridization buffer)(3×SSC, 0.3% SDS, 50% 포름아미드(formamide), 20 μg Cot-1 DNA, 20 μg 효모균(yeast) tRNA)에 첨가한 후, microcon YM-30으로 농축하여 혼성화 혼합물을 만들었다. 혼성화 혼합물을 95℃로 3분 동안 가열하여 변성시키고 12,000×g에서 30초간 원심분리하며 온도를 낮추었다. 제조된 히드라 oligo-마이크로어레이에 커버슬립(coverlip)을 덮고, 변성시킨 혼성화 혼합물을 파이펫팅(pipetting)하였다. 그 후, 상기 마이크로어레이를 GT-Hyb 챔버(Chamber)에 넣고 65℃에서 16시간 동안 반응시켜 혼성화를 시켰고, 혼성화가 끝난 후, 챔버에서 마이크로어레이를 꺼내어 세척과정을 수행하고, 마이크로어레이를 회전하여 건조한 후 스캐닝 할 때까지 암실에서 보관하였다. 실험이 완료된 히드라 oligo-마이크로어레이를 Axon GenePix 4000B scanner(Axon Instrument, USA)를 사용하여 스캔하였으며, GenePix Pro 6.0 program을 이용하여, 스캔 이미지로부터 각 점을 그리딩 파일(griding file)을 이용하여 그리딩하고, 정량화하여 각 점의 Cy5/Cy3 강도 및 비율등의 분석값이 포함된 GPR 파일(GPR file)을 얻었다.

[0074] <실시예 3> 마이크로어레이 자료 분석

[0075] GenePix Pro Program에서 얻어진 GPR 파일로부터, 분석 프로그램인 GeneSpring 7.3.1(Agilent Technologies, USA)를 이용하여 아래와 같이 분석을 수행하였다:

[0076] 표준화(Normalization)는 LOWESS(locally weighted regression scatterplot smoothing)를 이용하여 수행하였고, 신뢰할 수 있는 유전자(Reliable gene)는 중앙값의 합이 배경(background) 보다 낮거나, 각 화소(pixel) 값의 표준편차가 유의하지 않은 점을 플래그 아웃(flag-out)함으로써 유의한 유전자를 얻었다. 유의한 유전자(Significant genes)는 평균화된 비율(normalized ratio) 값이 10배 이상 차이를 보이는 점을 선별하였다.

[0078] <3-1> 특이유전자후보군의 확인

[0079] 1차적으로 트리클로산 노출 시간별 유전자 발현량이 1.5배이상 변화되는 유전자들을 선별하였다. 그 결과, 6시간 노출군에서는 32종의 유전자(증가 20종(표 1), 감소12종(표 2)); 24시간 노출군에서는 10종의 유전자(증가 9종(표 3), 감소 1종(표 4)); 48시간 노출군에서는 114종의 유전자(증가 22종(표 5), 감소 92종(표 6))의 유전자들이 트리클로산 노출에 의해 발현량이 변화되는 것으로 분석되었다. 즉, 표 7 및 표 8에 나타난 바와 같이, 트리클로산 노출에 의해 총 51종의 유전자가 발현이 증가하고, 105종의 유전자 발현이 감소하는 것을 확인함으로써, 상기 156종의 유전자들은 트리클로산 노출을 확인할 수 있는 바이오마커로 활용 가능할 것으로 생각된다.

표 1

[0081] 트리클로산 6 시간 노출군에서 발현량이 1.5배이상 증가되는 유전자 목록(20종)

유전자	발현량
Glucosamine--fructose-6-phosphate aminotransferase [isomerizing] 2, partial mRNA	1.50
nematocilin B (NemB), mRNA	1.51
ferritin, partial mRNA	1.55
AHNAK nucleoprotein, partial mRNA	1.58

microtubule-actin crosslinking factor 1, partial mRNA	1.59
SNF1-like kinase 2, mRNA	1.60
Glucosamine--fructose-6-phosphate aminotransferase 1, partial	1.61
Radixin, partial mRNA	1.61
harbinger transposase derived 1, partial mRNA	1.64
oxidative stress protein, mRNA	1.68
mitochondrial RNA ligase 2, mRNA	1.76
heat shock protein 90, alpha (cytosolic), class A member 1, mRNA	1.96
5-azacytidine induced 1, mRNA	1.97
testis expressed gene 264, mRNA	2.15
Tumor necrosis factor, alpha-induced protein 8-like protein, mRNA	2.18
DEHA2D14190p, mRNA	2.37
mucin 1, cell surface associated, mRNA	3.14
Sox10, mRNA	5.79
zinc finger with UFMI-specific peptidase domain, partial mRNA	8.04
ERYTHROCYTE MEMBRANE PROTEIN PFEMP3, mRNA	8.57

표 2

[0083]

트리클로산 6 시간 노출군에서 발현량이 1.5배이상 감소되는 유전자 목록(12종)

유전자	발현량
zinc finger protein Ssu-Zic, partial mRNA	-1.50
ribonucleoside-diphosphate reductase small chain, mRNA	-1.50
glutamine rich 2, mRNA	-1.50
c-K-ras2 protein, mRNA	-1.51
phospholipase A2, mRNA	-1.55
homeobox Hx, mRNA	-1.63
homeobox protein Otx, mRNA	-1.65
GLI pathogenesis-related 2, mRNA	-1.65
potassium channel homolog, mRNA	-1.69
coenzyme Q5 homolog, methyltransferase, mRNA	-1.76
acyl-Coenzyme A dehydrogenase, long chain, mRNA	-1.82
aldehyde dehydrogenase 8A1, mRNA	-1.99

표 3

[0085]

트리클로산 24 시간 노출군에서 발현량이 1.5배이상 증가되는 유전자 목록(9종)

유전자	발현량
TANK-binding kinase 1, mRNA	1.52
variable membrane protein, partial mRNA	1.52
complement component C3-like protein, partial mRNA	1.52
WntX2, mRNA	1.60
translationally controlled tumor protein, mRNA	1.60
polyprotein, mRNA	1.72
antistasin, mRNA	1.75
Glycoprotein 3-alpha-L-fucosyltransferase A, mRNA	1.81
Probable voltage-dependent N-type calcium channel subunit alpha-1B, partial mRNA	1.91

표 4

[0087]

트리클로산 24 시간 노출군에서 발현량이 1.5배이상 감소되는 유전자 목록(1종)

유전자	발현량
Ornithine decarboxylase, partial mRNA	-1.60

표 5

[0089]

트리클로산 48 시간 노출군에서 발현량이 1.5배이상 증가되는 유전자 목록(22종)

유전자	발현량
PMP1 protein, mRNA	1.51
tfii4 large subunit, mRNA	1.52
pregnancy zone protein, partial mRNA	1.55
conserved hypothetical protein, mRNA	1.56
alpha macroglobulin, partial mRNA	1.59
selenium binding protein 1, mRNA	1.59
Protein C20orf11 homolog, mRNA	1.60
LSM14 homolog B, mRNA	1.60
C3 and PZP-like, alpha-2-macroglobulin domain containing 8, partial mRNA	1.62
HyTSR1 protein, partial mRNA	1.65
golgi reassembly stacking protein 2, mRNA	1.66
protein disulfide isomerase, mRNA	1.67
G-protein coupled receptor 112, mRNA	1.69
acheron, partial mRNA	1.76
Hexosaminidase domain-containing protein, mRNA	1.77
brachyury, mRNA	1.79
DnaJ (Hsp40) homolog, subfamily C, member 3, mRNA	1.84
Na,K-ATPase alpha subunit, mRNA	1.91
Brain-specific angiogenesis inhibitor 3, mRNA	1.98
chondroitin 4-sulfotransferase, mRNA	4.05
hydra Na channel 3, mRNA	6.25
ADP-ribosylation factor-like 11, mRNA	129.42

표 6

[0091]

트리클로산 48 시간 노출군에서 발현량이 1.5배이상 감소되는 유전자 목록(92종)

유전자	발현량
protein phosphatase 1, catalytic subunit, alpha, partial mRNA	-1.50
Latent-transforming growth factor beta-binding protein, isoform	-1.50
Late histone H2A.2.2, mRNA	-1.50
von Willebrand factor D and EGF domains, partial	-1.51
myosin V, mRNA	-1.52
poly-gamma-glutamate synthesis protein, mRNA	-1.52
PIF1p DNA helicase (yeast) homolog family member (pif-1), partial mRNA	-1.52
S-adenosylmethionine decarboxylase; SAMDC, partial mRNA	-1.53
adult brain protein 239-like protein, partial mRNA	-1.53
tyrosine kinase receptor, mRNA	-1.54
doublesex-Mab related 99B, mRNA	-1.54
hemicentin 1, partial mRNA	-1.55
Zinc transporter ZIP12, mRNA	-1.56
beta-tubulin, transcript variant 1, mRNA	-1.56
mini-collagen, transcript variant 1, mRNA	-1.56
hydra Na channel 2 (hynac2), mRNA	-1.57
beta-tubulin, transcript variant 2, mRNA	-1.57
dickkopf-3 related protein, mRNA	-1.58
voltage-dependent calcium channel alpha-2 delta subunit, partial mRNA	-1.58
sulfotransferase family 1B, member 1, mRNA	-1.58
hydra Na channel 3, partial mRNA	-1.58
General transcription factor II-I repeat domain-containing protein 2A, mRNA	-1.59

NAD ⁺ -dependent 15-hydroxyprostaglandin dehydrogenase, mRNA	-1.59
receptor for egg jelly 6, partial mRNA	-1.59
adenylate kinase 1, mRNA	-1.60
apextrin, mRNA	-1.60
Serine/threonine-protein phosphatase beta isoform, partial mRNA	-1.61
Ift46 protein, mRNA	-1.61
KSI, mRNA	-1.61
FLJ42117 protein, mRNA	-1.61
zinc finger, MYM domain containing 1, mRNA	-1.61
flagellar calcium-binding protein TB-44A, mRNA	-1.61
Cation transport regulator-like protein 2, mRNAactivin B, mRNA	-1.62
activin B, mRNA	-1.62
prdl-b protein, mRNA	-1.62
nematocyst outer wall antigen, mRNA	-1.62
HyTSR1 protein, mRNA	-1.63
Kinesin-like protein KIF19, mRNA	-1.64
calmodulin, partial mRNA	-1.64
ATP-binding cassette sub-family F member 3, mRNA	-1.64
carbonic anhydrase 7, mRNA	-1.65
Na,K-ATPase alpha subunit, partial mRNA	-1.67
advillin, mRNA	-1.69
voltage-gated sodium channel alpha subunit protein, partial mRNA	-1.70
Neuropilin-1a precursor (znrp1), mRNA	-1.71
mitogen-activated protein kinase 6, transcript variant 2, mRNA	-1.71
carbonic anhydrase 2, mRNA	-1.74
neuropeptide, mRNA	-1.75
mitogen-activated protein kinase 6, transcript variant 1, mRNA	-1.76
FERM domain-containing protein 5, mRNA	-1.76
calmodulin, mRNA	-1.78
glutathione synthetase, mRNA	-1.80
BTB (POZ) domain containing 7, mRNA	-1.82
nudix-type motif 3, mRNA	-1.83
Rho GTPase activating protein 6, mRNA	-1.84
Prion-like-(Q/N-rich)-domain-bearing protein family member (pqn-13), mRNA	-1.84
Calmodulin CG8472-PA, transcript variant 3, mRNA	-1.86
calmodulin-like 3, mRNA	-1.86
nematoblast specific protein (nb035), partial mRNA	-1.87
ferritin, mRNA	-1.88
myopalladin, mRNA	-1.89
collagen, partial mRNA	-1.90
secreted protein, mRNA	-1.90
carbonic anhydrase 7, transcript variant 1, mRNA	-1.91
epitheliopeptide HYM-301, mRNA	-1.93
Ece1 protein, partial mRNA	-1.93
Metabotropic glutamate receptor 3, partial mRNA	-1.95
SCO-spondin, partial mRNA	-1.96
Sox17 alpha, mRNA	-1.96
ventropin, partial mRNA	-1.96
Ras-like protein family member 11B, mRNA	-1.98
PXN-FBPL, mRNA	-1.98
hydra Na channel 4 (hynac4), partial mRNA	-2.00
WD repeat domain 86, mRNA	-2.04
viral A-type inclusion protein, mRNA	-2.04
deleted in colorectal carcinoma, mRNA	-2.05
minicollagen-15, mRNA	-2.08
Polycystic kidney disease 2-like 2 protein, part	-2.08
carbonic anhydrase 7, transcript variant 2, mRNA	-2.09

Cysteine and glycine-rich protein 2, mRNA	-2.16
calcium transporter 2, partial mRNA	-2.19
transglutaminase, mRNA	-2.32
TiTiN family member (ttn-1), mRNA	-2.33
Rh-associated glycoprotein, partial mRNA	-2.40
probable zinc metalloproteinase, mRNA	-2.41
gamma-glutamyl hydrolase, mRNA	-2.45
mini-collagen, transcript variant 4, mRNA	-2.61
mini-collagen, transcript variant 3, mRNA	-2.62
bcl-2-like 3, mRNA	-2.82
mini-collagen, mRNA	-2.86
Calmodulin CG8472-PA, partial mRNA	-3.02
equinatoxin V, mRNA	-4.22

표 7

트리클로산 노출에 의해 히드라에서 발현이 증가하는 유전자 목록(51종)

[0093]

서열번호	유전자
1	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing] 2, partial mRNA
2	nematocilin B (NemB), mRNA
3	ferritin, partial mRNA
4	AHNAK nucleoprotein, partial mRNA
5	microtubule-actin crosslinking factor 1, partial mRNA
6	SNF1-like kinase 2, mRNA
7	Glucosamine--fructose-6-phosphate aminotransferase 1, partial
8	Radixin, partial mRNA
9	harbinger transposase derived 1, partial mRNA
10	oxidative stress protein, mRNA
11	mitochondrial RNA ligase 2, mRNA
12	heat shock protein 90, alpha (cytosolic), class A member 1, mRNA
13	5-azacytidine induced 1, mRNA
14	testis expressed gene 264, mRNA
15	Tumor necrosis factor, alpha-induced protein 8-like protein, mRNA
16	DEHA2D14190p, mRNA
17	mucin 1, cell surface associated, mRNA
18	Sox10, mRNA
19	zinc finger with UFM1-specific peptidase domain, partial mRNA
20	ERYTHROCYTE MEMBRANE PROTEIN PFEMP3, mRNA
21	TANK-binding kinase 1, mRNA
22	variable membrane protein, partial mRNA
23	complement component C3-like protein, partial mRNA
24	WntX2, mRNA
25	translationally controlled tumor protein, mRNA
26	polyprotein, mRNA
27	antistasin, mRNA
28	Glycoprotein 3-alpha-L-fucosyltransferase A, mRNA
29	Probable voltage-dependent N-type calcium channel subunit alpha-1B, partial mRNA
30	PMP1 protein, mRNA
31	tfiia large subunit, mRNA
32	pregnancy zone protein, partial mRNA
33	conserved hypothetical protein, mRNA
34	alpha macroglobulin, partial mRNA
35	selenium binding protein 1, mRNA
36	Protein C2Oorf11 homolog, mRNA
37	LSM14 homolog B, mRNA
38	C3 and PZP-like, alpha-2-macroglobulin domain containing 8, partial mRNA
39	HyTSR1 protein, partial mRNA

40	golgi reassembly stacking protein 2, mRNA
41	protein disulfide isomerase, mRNA
42	G-protein coupled receptor 112, mRNA
43	acheron, partial mRNA
44	Hexosaminidase domain-containing protein, mRNA
45	brachyury, mRNA
46	DnaJ (Hsp40) homolog, subfamily C, member 3, mRNA
47	Na,K-ATPase alpha subunit, mRNA
48	Brain-specific angiogenesis inhibitor 3, mRNA
49	chondroitin 4-sulfotransferase, mRNA
50	hydra Na channel 3, mRNA
51	ADP-ribosylation factor-like 11, mRNA

표 8

트리클로산 노출에 의해 히드라에서 발현이 감소하는 유전자 목록(105종)

[0095]

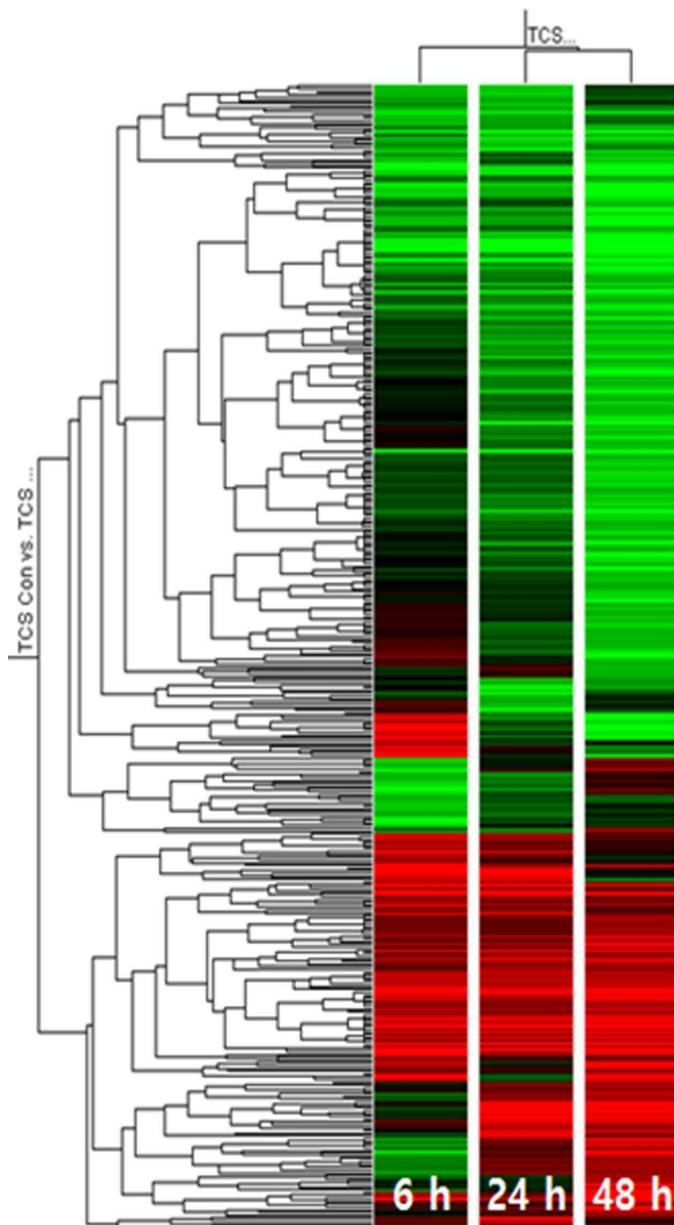
서열번호	유전자
52	zinc finger protein Ssu-Zic, partial mRNA
53	ribonucleoside-diphosphate reductase small chain, mRNA
54	glutamine rich 2, mRNA
55	c-K-ras2 protein, mRNA
56	phospholipase A2, mRNA
57	homeobox Hx, mRNA
58	homeobox protein Otx, mRNA
59	GLI pathogenesis-related 2, mRNA
60	potassium channel homolog, mRNA
61	coenzyme Q5 homolog, methyltransferase, mRNA
62	acyl-Coenzyme A dehydrogenase, long chain, mRNA
63	aldehyde dehydrogenase 8A1, mRNA
64	Ornithine decarboxylase, partial mRNA
65	protein phosphatase 1, catalytic subunit, alpha, partial mRNA
66	Latent-transforming growth factor beta-binding protein, isoform
67	Late histone H2A.2.2, mRNA
68	von Willebrand factor D and EGF domains, partial
69	myosin V, mRNA
70	poly-gamma-glutamate synthesis protein, mRNA
71	PIF1p DNA helicase (yeast) homolog family member (pif-1), partial mRNA
72	S-adenosylmethionine decarboxylase; SAMDC, partial mRNA
73	adult brain protein 239-like protein, partial mRNA
74	tyrosine kinase receptor, mRNA
75	doublesex-Mab related 99B, mRNA
76	hemicientin 1, partial mRNA
77	Zinc transporter ZIP12, mRNA
78	beta-tubulin, transcript variant 1, mRNA
79	mini-collagen, transcript variant 1, mRNA
80	hydra Na channel 2 (hynac2), mRNA
81	beta-tubulin, transcript variant 2, mRNA
82	dickkopf-3 related protein, mRNA
83	voltage-dependent calcium channel alpha-2 delta subunit, partial mRNA
84	sulfotransferase family 1B, member 1, mRNA
85	hydra Na channel 3, partial mRNA
86	General transcription factor II-I repeat domain-containing protein 2A, mRNA
87	NAD+-dependent 15-hydroxyprostaglandin dehydrogenase, mRNA
88	receptor for egg jelly 6, partial mRNA
89	adenylate kinase 1, mRNA
90	apextrin, mRNA
91	Serine/threonine-protein phosphatase beta isoform, partial mRNA

92	Ift46 protein, mRNA
93	KSI, mRNA
94	FLJ42117 protein, mRNA
95	zinc finger, MYM domain containing 1, mRNA
96	flagellar calcium-binding protein TB-44A, mRNA
97	Cation transport regulator-like protein 2, mRNA
98	activin B, mRNA
99	prdl-b protein, mRNA
100	nematocyst outer wall antigen, mRNA
101	HyTSRI protein, mRNA
102	Kinesin-like protein KIF19, mRNA
103	calmodulin, partial mRNA
104	ATP-binding cassette sub-family F member 3, mRNA
105	carbonic anhydrase 7, mRNA
106	Na,K-ATPase alpha subunit, partial mRNA
107	advillin, mRNA
108	voltage-gated sodium channel alpha subunit protein, partial mRNA
109	Neuropilin-1a precursor (znrp1), mRNA
110	mitogen-activated protein kinase 6, transcript variant 2, mRNA
111	carbonic anhydrase 2, mRNA
112	neuropeptide, mRNA
113	mitogen-activated protein kinase 6, transcript variant 1, mRNA
114	FERM domain-containing protein 5, mRNA
115	calmodulin, mRNA
116	glutathione synthetase, mRNA
117	BTB (POZ) domain containing 7, mRNA
118	Prion-like-(Q/N-rich)-domain-bearing protein family member (pqn-13), mRNA
119	Rho GTPase activating protein 6, mRNA
120	nudix-type motif 3, mRNA
121	Calmodulin CG8472-PA, transcript variant 3, mRNA
122	calmodulin-like 3, mRNA
123	nematoblast specific protein (nb035), partial mRNA
124	ferritin, mRNA
125	myopalladin, mRNA
126	collagen, partial mRNA
127	secreted protein, mRNA
128	carbonic anhydrase 7, transcript variant 1, mRNA
129	epitheliopeptide HYM-301, mRNA
130	Ecel protein, partial mRNA
131	Metabotropic glutamate receptor 3, partial mRNA
132	SCO-spondin, partial mRNA
133	Sox17 alpha, mRNA
134	ventropin, partial mRNA
135	Ras-like protein family member 11B, mRNA
136	PXN-FBPL, mRNA
137	hydra Na channel 4 (hynac4), partial mRNA
138	WD repeat domain 86, mRNA
139	viral A-type inclusion protein, mRNA
140	deleted in colorectal carcinoma, mRNA
141	minicollagen-15, mRNA
142	Polycystic kidney disease 2-like 2 protein, part
143	carbonic anhydrase 7, transcript variant 2, mRNA
144	Cysteine and glycine-rich protein 2, mRNA
145	calcium transporter 2, partial mRNA
146	transglutaminase, mRNA
147	TiTiN family member (ttn-1), mRNA
148	Rh-associated glycoprotein, partial mRNA
149	probable zinc metalloproteinase, mRNA

150	gamma-glutamyl hydrolase, mRNA
151	mini-collagen, transcript variant 4, mRNA
152	mini-collagen, transcript variant 3, mRNA
153	bcl-2-like 3, mRNA
154	mini-collagen, mRNA
155	Calmodulin CG8472-PA, partial mRNA
156	equinatoxin V, mRNA

도면

도면1



서열목록

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for diagnosing aquatic environment pollution using the same

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- <211> 563
- <212> DNA
- <213> Hydra magnipapillata
- <400> 7

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- <211> 1585
- <212> DNA
- <213> Hydra magnipapillata
- <400> 8

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<211> 748

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<400> 11

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<210> 12

<211> 2436

<212> DNA

<213> Hydra magnipapillata

<400> 12

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 <212> DNA
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 <400> 17

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- <210> 18
- <211> 1067
- <212> DNA
- <213> Hydra magnipapillata
- <400> 18

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- <211> 222
- <212> DNA

<213> Hydra magnipapillata
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 <211> 784
 <212> DNA
 <213> Hydra magnipapillata
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 gaacgtata tgttacaag tcacatcgat aaacttaac aatcattcaa ttgaaataaa 240
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 <210> 21
 <211> 1092
 <212> DNA

 <213> Hydra magnipapillata
 <400> 21
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 <212> DNA

 <213> Hydra magnipapillata
 <400> 22

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atTTTgaaag aagaaccagt ggtagtTgtt agcactagtG aagtgtttac agaaagctac 600
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 aatattTTTaa aagaagaaca agtggtagct gctagcacta gtgaggTgtt tacagaatgc 780

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gttgataaaa gtgtTaaat aactactaca gaagaaggac acgatgaacg taaacataca 1260
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- <210> 23
- <211> 285
- <212> DNA
- <213> Hydra magnipapillata
- <400> 23

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- <210> 24
- <211> 1415
- <212> DNA
- <213> Hydra magnipapillata
- <400> 24

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<210> 25

<211> 936

<212> DNA

<213> Hydra magnipapillata

<400> 25

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 <212> DNA
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<211> 945

<212> DNA

<213> Hydra magnipapillata

<400> 27

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ccggatggta cagaagctaa tcatttcaaa cgcaaaacct tttttgaaaa tggataaaaa 780

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aaaaattctt ttatactttt tataaaacaa atttattaat attgacttaa aagataaaaa 900

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<400> 28

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<212> DNA

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<210> 42

<211> 1834

<212> DNA

<213> Hydra magnipapillata

<400> 42

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aatgtttgtg atttagattt agatcgacat agaaatgatg caaatgattg taaaaatagt 180

tgtcacctg atgctatttg taataatagt atttgcgttt gtaaaaaagg ttttgttgg 240

gatggtgtaa ataaatgttc agactgttgt attgaacaga ctgttggaga agacagttac 300

actggaatat ataattttcc agtgacttta agagatgatg ttcattgttc tctctgtgta 360

tataatgcaa gtattcaatt tcaaagacga tgtttgtttg ttagtaattt tgctattcct 420

gagtgggaac ctgctattct ttcgaattgt ccttcaaaat ctgaaatata ccagaaactc 480

atcaacttgg aaaacattaa tgtcacaaca tcaaacattt taaatgttac atattactta 540

aatgacatag ttcaaaatgg taacctatca agcatatag atgttcaaat gatttcaagt 600

attataaaac agattataaa tgtgaacttc tcatcagaaa aggttaccca tggatatttg 660

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tttaatttgt ctgctgtttt tttatcatta attgatgttt tggggaaaca acaaaccaca 780

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gataatttca agctacatc agatataaaa gactacataa ttcttcttc ttcattattt 960

attgaacata atgaaactca aatttactct tttatatata gaacaaaagc atttttcagg 1020

gaacaagtag gagttataga tagtgtgata ctgtcagtct cagtaaatgg ttaccattc 1080

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 accgtgtaaa tagaataat cgatcaatga aaaa 1834

<210> 43
 <211> 1353
 <212> DNA

<213> Hydra magnipapillata

<400> 43

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 ttttaaacga agaatgcatt agcattaatg aactacataa aaaaatcata gctcaagctg 240
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 ggaataagga aggttatgta aatattaagc taatagcttc cttcaaccgt atgcgatctt 360

taacaaaaga ttttaataa attgttgacg ctcttagaac ttcgtcaaga cttgcagtag 420
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 gcaagagttt tcccaatgat ttacaatcac attactcaa acatccagaa ctaaaagaaa 660
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<210> 44
 <211> 1946
 <212> DNA
 <213> Hydra magnipapillata
 <400> 44

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 tctaagtcta atcattggta tgagttggt gatatcagca ttactaaatt tacggagccc 180

ccaacaacac aacatTTTTc aattagcaa atccaatag cgattgacaa taatgttaaa 240
 cctgaaatcc ccgactttcc atctactcc cagagtgttg agcgagcgg tttcacacc 300
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 ttgaaacaa taiggaataa tttaaagcga atgttcatt ttgattgaa ggggtcacct 420
 ccaaacatt catatTTTt taaactTTTg cctttactta agtctcttg tgcttcaggt 480
 ttattaatag aatatgaaga catgtttcca tactctggac ttctgcacca aatatcatcc 540
 ccttttgcat acagcaaagt tgagattcaa gaactTTTg atacagctaa aaaattgaac 600

atggaggtca taccacttgt tcaaacattt ggccattttg agtttgttct taaacatgag 660
 caatttcgat atttaagaga aactctgag tatgcaaac ttccatgccc gettcataat 720
 gaaacattaa gtatggtatt gaagatggt gatcaagtgt tatctgtgca tccagatatt 780
 acattaatac acctaggagg tgatgaggtt ttcaacttga aagattgcgc cagatgtaaa 840
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 aacatttgac tttattatta ttagatattt atttgttatt taatttctca aaagaaattc 1860

ttaagaaaag tttttcatta taagtatttt tactttaata attatTTTTT ttttataaaa 1920
 gttttttatt taatgttaat agttgt 1946

<210> 45
 <211> 1383
 <212> DNA
 <213> Hydra magnipapillata
 <400> 45

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 gatTTTgaaa gataacgaaa gtaaaaaaca aaaggtagag aagaaattaa ctaaagattt 120
 gatgctaaga gtaaaacttg aggataaaaa tctatgggca agctttcadc gaatgaccaa 180
 tgaaatgatt gtcacaaaa atggcagacg tatgtttccg gttttaaagt catctgtcga 240

tgggttagat cctcaatcca tgtatactat tatgctagac tttttaccag tagatgaaaa 300
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 gagtaaaata tatgtccatc ctgattcacc gaactttgga tgtcactgga tgaaaaatcc 420
 tattgtattt tccaaggtaa aattaaccaa caaggaaagt accaatgggc aagtgtcat 480
 gctgaactcg ttgcataagt acattcctcg catccacatt gtcaaggtct gcaacggtga 540
 aaaaattacg tctacacata ctttcgttga aacggaattc atcgtgttta ctgcttatea 600
 aaacgaagag ataacgaact taaaaattcg atacaaccg ttcgcaaaag cttttctgga 660

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taa 1383

- <210> 46
- <211> 1821
- <212> DNA

<213> Hydra magnipapillata

<400> 46

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tattaattaa gttgttaaga aatatttttag taatgtggac cgaaaaatca agttgttata 180
gaattttttc atgtctctct gtccttttta tgacttttga tacgaatggt tttaaagggtg 240
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cagctggtca attaacagaa gctttatctc attaccattc agctattgag ggtgatccaa 360

aaaattatat gacatacttt aagcgtgcag cagttctttt ggctttggga agatcaaaat 420
cagcccttcc tgatttgac aaaacacttg aattgagagc agattttttg agtgctcgct 480
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ttcatgtgag tagacaagat tcttctaatg cagaggetac tcagggtcta tetattattg 600
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tggttataag tcatctcgaa aatcctattg aggtatgccc ttgggatggt catttaagag 720
aaatgcgagc gaaaagtat gaacaaatag gagattactt aaaagctatt caagattatc 780

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<210> 47
<211> 3111
<212> DNA
<213> Hydra magnipapillata
<400> 47

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tccaaaaaa agaatatgga tgatctcaa aaagagcttg aaatggatga acataaaatt 180
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<211> 1048
<212> DNA
<213> Hydra magnipapillata
<400> 48

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aatagataga ataacaagtt acattgttaa tggacagaat gtatcttata acttctttgc 960

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aaagagccaa tgtgaaagta atgattaa 1048

<210> 49
<211> 1254
<212> DNA

<213> Hydra magnipapillata
<400> 49

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cgctcgatt tgaanaatgc gtcttcaatt ggtcgtgtaa agcgaatct tgaatcgata 240
aaccttttcg atcagcaaga aaaaagacaa acagtaatgc ttaaattttg ttcaactct 300
aacttttcaa ataaaactat ggatgaacac atgatttaca ctgacaaaga aagtcttctt 360

tactgetata tacctaaagc tgcttgatca acttgggaagc gaatgtttca gatatttgac 420
ggaaaaatgg atttaaatca agtgatggct gttgaaaaaa atgcagtcca taagctgcat 480
tataacaatt ttacaactct tgacgctgca caaaaagttt tccgtgagaa aaattattat 540
tcatttttag taagtcgcca tccatttgaa agattgttgt cagcgtatag aaataaattt 600
ttggaccctt acacaacgca ttatcaaaaa aagtatgggtg cagaaatcct tcgtttatat 660
cggaacgata ttacagaaga acagtattta aaaggagaag gggttacatt tcgagaattt 720
atcaagtatg ttatatcagg aaaacctttt gataaacatt ggcgtcctat gacgcaacta 780

tgctctccgt gtcgttttaa gtacagatac ttgggtaaaa tggaacatt atttgaagac 840
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agagatcgtt ataagccaat ttcaacaatt gacatgaaaa gtcaatatat gagtttgaaa 960
gcttcagaaa ttagaaagct atactacatg tataaagatg attttctcgc ttttggctat 1020
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atataatggt ttatcaacaa aatagttgta ttactagttg tttttcattt ctagcatttt 1140
atattgtttt aaatttttat ttatgatttt ataaaaatac ataataatgag aactgttttt 1200

ttttttttt gcttacgatt attaaaattg taacaaggta ataataaaga taat 1254

<210> 50
<211> 1362
<212> DNA

<213> Hydra magnipapillata

<400> 50

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 gacaaaagaa acaattattt tcgaagagct atatggtttt taattacggt tggcgcgttt 180
 atctatgctg ttgaaaaagt ttatgaaagt actgttaatt acttttctta tccattcaaa 240
 accgctcgca tgaaaattta tgtcaatgaa ttaaattttc cggctgtatc attctgtaat 300

ttgaatgatt tttgttttag caaactaaat ggcaccaaac ttgatgaatc aatttgttac 360
 cctgatgadc ctgaaaaaaaa caatgtctca gaaatagaaa ttagcaacat tacttctgat 420
 gccacaattc gattagatca aatgttagtt gattgtgagt ttgaaggcaa aaaatgcact 480
 catgaaaact ttacagattt ctggactatg cagggcgaac tttgttttac atttaactct 540
 ggaaaaaatt ctcatcatt attaaaggta agtggagttg ggggtttaag aagcttaaaa 600
 ctgacaatta atgtccaaca ttatgaatat tatcgagatg aaatggctgg tgggattcat 660
 ttaatgattc atggacagga tgaggaacca gtaaaaatgc aaggacaaat agtatctcca 720

ggatattctt tttatgttaa agttgaaaaa aaaactataa tgaacttaga aaaaccttac 780
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 cctggaaca ttccatattg cagtttgccct gaattacaaa actgtacatg gatagaatgg 960
 gcaaagtta acaaggataa aatgtataag tgcccgttgcc catgtaaaat tgatttgtat 1020
 ggagtgagct taictcgagc attgtttccg actaccaat attctagcat acttgcgtaa 1080
 cagtttcgca aacagcctca tgttttaagt attgtacaca atattactga tgagctctta 1140

tttatgagag ataatttact tcgttttatt atttattatg atgatttgtc gtacgaggta 1200
 cttgaacaga aaccaagtta tgaaacatta gtctggttag gagatattgg tggacagata 1260
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 gtgatataca cacggttttt tcagaagttt acttccat ga 1362

<210> 51

<211> 819

<212> DNA

<213> Hydra magnipapillata

<400> 51

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cattctttta ctggtttag atgcagctgg aaaaacaaca atactttata agttaagct 180

caacgaaaac gtcacaacca ttctacaat cgggtttaat gtggaagagg tgactccagt 240

aaaaaacgtt acitttacca tgtgggatgt tggaggtcag gaaaaaattc gacctttatg 300

gagacactat tatcaaggtg ccgaaggact agtttttgtt gttgatgctt gtgacgtttt 360

aagaattcaa gaagcgcgtg aagagttttt ttctgtttta aaagatgaag gcattgaaaa 420

aggaattcct gcagtgattc ttgcaaaaa acaagatctc ccaaatgcat taaaatcgtg 480

ggaactagt gataaaaatc ggttaaaaga attaagcggg aatccttggc acgttcaaga 540

aatgtgcgct ttaacagggg atggtttata tgaaggtatt caaagacttg cagatatggt 600

aaaaacatat caaagagaaa acaaagtttag atggaacag catgaaacta aagtaaaaaa 660

tgtataatca tcaacaatat tattcctatc acgatgataa taaataaagt actattttat 720

cagtgaatat ttttttaaat ttttagcttg taaactatth aacttacttg aaaatatctt 780

tataaaatcc gaattttttg attaataaca aggaaaaaa 819

<210> 52

<211> 1257

<212> DNA

<213> Hydra magnipapillata

<400> 52

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tacttagatc acatgagtc atcaacatct aggtatgccg atcacaatgt ttcagatcac 120

gttgcttcac ctctgggat ttttaatgca cattctgcca tgatgagtc atctcaggct 180

gttaggaagt taggtacgta cgcttcacgt tcatctagtc aaagtgaata ccaacattct 240

tctggagtcg gaaattctga ttttatgaat ggatataaaa caatacacga ggaaccacca 300

atattaactg cagcacagcc aactcattca ataccaacta tgatgaatga cacaaatagc 360

catgctggga gaacacattg tatgtttgct agtgggtcac tgcatcataa tactgaata 420

aactcacgat ttactctaa tttgaatcaa aatcacggat attcttctaa cccaagtlacc 480

ccttcgtctt tttatcctct atcccacac gaattatcgc acaacagcca ctatcctgga 540

aaatacccaa agcacgatta tgcaagtgc gtttaagccag catacaacta tcagccacta 600

aattcatctt acgaatacta cggaagccca cagtacggat actcaaatct tcaacctcat 660

aacagttttc ggcaacctct taatcaagtt atatcatgca tgtggcaaga tcaaaccaca 720

aaaggaaaac cgtgcggaaa acagttttt gttatgatgg acatagtgc gcatttagct 780

gaagatcacg tgggcttaaa tgactcgaca gaacatataat gttattggaa agattgccca 840
cgagcaggac ttgctttcaa agctaataat aaacttatta atcacctaag agtacacaca 900

ggtgaaaaac cttttccatg tccattccca ggatgtggta aactatttgc aagatcgaa 960
aatftaaaga tacataagcg aacgcacacc ggtgaaagac cgtttgtgtg tgaatttaca 1020
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tcccttcgca aacatatgaa attgcatggg aagcctgata ccgtaaaaga tataaaactt 1200
tcaaaaataa caaactcaga ttcaaaaata agaacttctg atcaagattt acattcc 1257

<210> 53
<211> 1092
<212> DNA

<213> Hydra magnipapillata

<400> 53

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atcttaata aagacctctc acgcttgggt gttaaacca tcaattcca acacaaggta 180
atftgggac tttataaaaa acaaaaagac gcatattgga ccgctgaaga aattgatttt 240
agtaatgac gtcattgatt tttaaagtt gataaaaata tccaacattt tattaagaga 300
atfttagcat tftttgcagg agccgattca attgttaaca ttaatttta gaaaaatta 360

tcaaaaataa ctgtcaaga agctgaaatt gcttatggat tccaacaaat gatggaagat 420
acgcatagtg aagtttatgc agacatgta atcaaaaata ttacagatcc ggttgaacgc 480
gacgaactta ttaatgcgtt taaaacagtt gcatctatta agagaatgat tcaatgggct 540
caacgatgga ttaattctaa acgcagaatt gggtactcga ttgtcgtatt ttgtatttt 600
gagggattaa tgttttcggg ggctfttgcc ggaatatatt ggctcaaaa gattcttggc 660
gaagatataa tgaagggtt aatacaatca aataattta ttgccaaaga tgaaggcatg 720
cacacaattt tcggatgtgt catttacacc ttattattt atagactgtc tfttgaggag 780

atfttgaat taatgaaaga ggctgttga atftgcaat cftttacaaa agacgcaatt 840
tgtgttgaca tgattggat gaacgtcga cttatgtata attacattga gtatgttgc 900
gacaggctta tggatatact cggttatgaa aagatcttta tgacaaaaat tctgatcag 960
tftcagttta tggacacaat cggftttctt aataaggaca acttctttga acgtcgtgca 1020
accgaatctc aaaaagctta taatgaccga aataggctg actggaactt taaactctc 1080

cctatttact aa 1092

<210> 54

<211> 666

<212> DNA

<213> Hydra magnipapillata

<400> 54

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gactggttca ctgataagga gtttatattt cgacaagata atgatacatg tcatatggac 120

aacgggtgta aagatttggga ggaactttct ttccaaacta accgtgcatg cggaggcgct 180

catacattga ttgagcagca taaaagaaac ccaagtaaaa accaaacata tttcaaagac 240

gaaggagatc aatttcagaa agaagataaa gtaatagacg ttcttggctg cgacggacac 300

atttataaag gaaatttgc aacaccacta ccagctatac ctagatcacc acttacatta 360

gaaaccgata cagttgaact aaccgatgag aacaacgaga gcgttctatc acaattatct 420

aatcattgga aagtaaactt gaatgcaaca gcttaatata aaactgttta tttaaatfff 480

aacattttat attttttga tattttactt gttaacgaat tgaataaag tatattttca 540

aaaaacaaaa actacactaa tgtaaaatta atatatattg tttcaactac tttataatfc 600

atataaaaca actccaaatt ataaacgatg atacacacag ccatacaatc acgcattgaa 660

agatct 666

<210> 55

<211> 1200

<212> DNA

<213> Hydra magnipapillata

<400> 55

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gaagatggcg ttggaaaatc tgctttaata aaccaaatag ttgaaaaaaaa atttattgaa 120

aagtataatc cgacagtffc aatcacctt acgcacgttg ttgagtttga aggtcatctt 180

tgcgtgtgtt tgttagttga tactgtctgg gtggctgact ttccggctat gagaaagtta 240

gccatatcaa aaggaaacgc atttgtagtt gtatatgctg tggataaccg aaaatcattt 300

gaaacggcaa aaaatftaat acgagaaata aaacttctaa aaggtacagc gaaagaaact 360

cgcgttgtac ttgtaggaaa caaaaaggat ttacctcgta ctgtcactta taaagaaggt 420

ttagattatt caaaaatgat tcaagaagac aactgcgtga gtacatttat tgaagcaagc 480

gcaaaagaac gagaaagtcc aacagaaata ttgtataaaa tattaatat gttttgcct 540
 cccattaaac gactagaaga atacaataat ctaaagaaaa agccttcatt atattcgtcg 600
 acattaagca accgacgac ttccaagtca ctaataagac gtaaacaag ttgcggttca 660
 aaaaatgaaa cgactcaaaa agatagggta attggtgata tagaaggtag tgttttgaa 720
 acaaaatcctt caccaaccgg aagatttaga agtcgttcaa aaagccacc tattatttcg 780

gttaaaggta tattagaaac attatctcca aagacttcta gaagaaagt ttcagccaat 840
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 aaaacaaacc gtctgtgta ctttacacat tcgacaagcc taaacagttc cagtttagaa 960
 ttttcaata gttaacctc gatgagtagc catgacagcg gtcttggtga cacaatcgc 1020
 cagcagctg gtcttagtga tgtttcaaat aaaaagactt tgagtgaat tcatatcaga 1080
 cttacaaat cactttcaga acccctcgt tcggaaagaa ggacgatgaa acaataata 1140
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1200

- <210> 56
- <211> 730
- <212> DNA
- <213> Hydra magnipapillata
- <400> 56

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 aagcatttct tgaagatcaa aaaaaacaag gattttcaga aagagaagta ttgaaaaaa 180
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caaaatcaga taaggatcgc ggattctttg atcaactcga tgaatgctgt ttgaaacacg 360
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 agaatactta tgagcctaca ccggatggtt cccccaacc atactttcgt gtcctaaac 660
 attataagta atataaata tctttgtaa tattattaaa aatataatta ttttaaagct 720

ccttattaaa 730

<210> 57
 <211> 541
 <212> DNA
 <213> Hydra magnipapillata
 <400> 57

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 atacgaacca ctttttctag tgatcaagtg ttcttacttg aaatgcaatt taataaacia 180
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acccaaagca atatatactc ccaaaaaatt caaaattggt tacgacctaa tatatttatg 360
 agctcagaga aaacgcgcaa agaatttttt tcagtttctt ctgtattata tcctcaacac 420
 gaacatgtat ttatgatgaa aagagagcat gaaaaccgca caaacattta ttaaagccgg 480
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<210> 58
 <211> 1452
 <212> DNA
 <213> Hydra magnipapillata
 <400> 58

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ttttcttgaa aaagaaaaag atttatcttg tgcgcatga ccatgtccga tagccccttg 120
 gatagtcctat taacatgaa ttctggagca ataaagtcag agactccacc taaaagacgg 180
 cgtgaaagga cacttttac aaaagcacia cttgacgtgc tggaagatat gtttgaaaa 240
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 ctcatcgaa ctttctctaa aagtagtggg ggcactccac actcttacc aaatacaagc 480

aattatcctc cgctatggaa atatccaggt gcagaccaca gtccaccata tgcaggaaat 540
 aatataattg aagaaccgtt ttatagacia tcaaacctt acgctccat ctacacctat 600
 gaatataatt ttctagtgagg gagaccacca tactttactc agccacctta ttatagtcgg 660
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 ttaccagtga aaatgtcggf ttagcaaaat ttcaagcctt tatgaaaagg aaaatggctg 1260
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 tagcagcgat caaaaatgga aaaatatatg ttattgaaac aactttttaa ttttctattt 1380
 atcaattaac tgatttaacg tataaacctg ttttagattt ttttgtaaa tatattatta 1440
 ctgtaataga aa 1452

 <210> 59
 <211> 1056
 <212> DNA
 <213> Hydra magnipapillata
 <400> 59

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 gcaaaaaagt gcttaaagcg tttgttattt gattacgacg aacaaactca ggaaggggag 180

 aacattgctg taatgaaaga tattgaagtt tctggaacca cagtggtaga ctactggtat 240
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 tcttctttgc gaagagcttc gagcatgggt tccagtttac caaaagcttc tggaccata 600

 caatctgatg gtgtttttcg tgatgaatta cttacgtcac ataactata cagaagtcgg 660
 cataatgcta agccactttc attaagcagt ggattgacac taaaagctca gcaggttgcc 720
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gcttgacta aaggaattac aggaaatgaa gcttcctcca tttggtataa cgaagagaat 840
gatttcaatt ataataacaa gagtttctca atgaaaacag gaagctttac tcaaatgata 900
tgaaagaaa ctaaagaaat tggcgtaggt cgagcaatag atgacagagg ttgcactcat 960
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ttacctaag gtccacctcc aaaatattt tattga 1056

<210> 60
<211> 1437
<212> DNA
<213> Hydra magnipapillata
<400> 60

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gttgctggac aactatttca gacgtaccga actacacttg aacgatatcc ggatacttta 180
cttgagatc caaaaaagcg acaacatttt aaaaatccaa aaacaaaaga actatTTTTT 240
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gacagtaaat taatagaaaa aatgcaactt gataatggtt taaaagaaat agatacgaaa 420
gaaaatttac cattatttaa accgtttcgc attttatggg aattttttga ataccagag 480
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aattcaaaa tgacgatacc aagtgtcaat gcagatgga caaaatattc taatacggct 660
gttattgtct ggtttacaat tgaatTTTTA atccggtga tttcttgtcc taacaaacta 720

atgttttttc taaatgggtg taacatcatt gattttttat caatactacc attctatttg 780
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gctttgccag tcctataat tgtttccaat tttgagtttt attataaaaa agatcaaaac 1200
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 agaagatatt caatgtctca aaacctgat ggtttgtcaa ctaaacttga cgagtcacca 1380
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 <211> 732
 <212> DNA
 <213> Hydra magnipapillata
 <400> 61
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 ggctgcacc gcgctggaa ggcctacacc gtgctggtgg ccaacctca agagggcgac 180
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 ggcaagtcgg gtcaagtcgt gcataccgat atcaaccaag ccatgctctc caccgcccgc 300
 aaccgtttgc tggatgcggg catcgtgttg cccacgctgg tgtgcatgc ggagaagttg 360
 ccttccccg acaactactt caatgtggc agcgtggcct tcggtttgcg caacatgacc 420
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<400> 65

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 <213> Hydra magnipapillata
 <400> 66

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 <400> 67

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 <400> 68

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<210> 69
 <211> 585
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<213> Hydra magnipapillata

<400> 69

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<210> 70

<211> 1233

<212> DNA

<213> Hydra magnipapillata

<400> 70

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- <211> 502
- <212> DNA
- <213> Hydra magnipapillata
- <400> 72

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- <211> 788
- <212> DNA
- <213> Hydra magnipapillata
- <400> 73

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gatgcaatat aa 3132

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- <211> 1110
- <212> DNA
- <213> Hydra magnipapillata
- <400> 75

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agaagacaac agatgcaaga ttcaaaacag agattgactc cacttccacg gggagatata 240

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cacacgcaaa gttatgtacc aataccaatg agatattttg gcaaatcggc aagtttaaaa 480

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tacaacggaa aaatataccc agacgatcgc tgccacaaaa gtagtggat gcacatata 660

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<211> 732

<212> DNA

<213> Hydra magnipapillata

<400> 76

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<210> 77

<211> 576

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<400> 77

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<210> 78
<211> 2325
<212> DNA
<213> Hydra magnipapillata
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- <211> 654
- <212> DNA
- <213> Hydra magnipapillata
- <400> 79

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<210> 80

<211> 1902

<212> DNA

<213> Hydra magnipapillata

<400> 80

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gaaactggag ttaacatggg gtttaacta gaactagatt tgaatcaaa agattctctt 720

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 <211> 1873
 <212> DNA
 <213> Hydra magnipapillata
 <400> 81

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<210> 82
<211> 1045
<212> DNA
<213> Hydra magnipapillata
<400> 82

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 <211> 2158
 <212> DNA
 <213> Hydra magnipapillata
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 <210> 84
 <211> 970
 <212> DNA
 <213> Hydra magnipapillata
 <400> 84

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gaatttataa tgaaaaagat cattaacaat tcctctttta aggaaatgag agagaaagaa 720
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- <210> 85
- <211> 885
- <212> DNA
- <213> Hydra magnipapillata
- <400> 85

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 aaaaagttta tgaaggtaca atgaactact tttcgtaccc attttact gttcgcata 240
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 aatagaaaa aaagacgatt ataaacttag aagcgcata caaaacaaaa tgtggttcag 780

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- <210> 86
- <211> 1398
- <212> DNA
- <213> Hydra magnipapillata
- <400> 86

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 <211> 891
 <212> DNA

 <213> Hydra magnipapillata
 <400> 87
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<210> 88
<211> 3859
<212> DNA
<213> Hydra magnipapillata
<400> 88

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 <211> 957
 <212> DNA
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 <400> 89

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<210> 90
 <211> 1961
 <212> DNA
 <213> Hydra magnipapillata
 <400> 90

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- <210> 91
- <211> 745
- <212> DNA
- <213> Hydra magnipapillata
- <400> 91

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- <210> 92
- <211> 1590
- <212> DNA
- <213> Hydra magnipapillata
- <400> 92

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- <210> 93
- <211> 781
- <212> DNA
- <213> Hydra magnipapillata
- <400> 93

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- <210> 94
- <211> 2307
- <212> DNA
- <213> Hydra magnipapillata
- <400> 94

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 <212> DNA

 <213> Hydra magnipapillata
 <400> 95

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 <210> 96
 <211> 955
 <212> DNA
 <213> Hydra magnipapillata
 <400> 96

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 agaagagaaa gaacaacgac aagcgctgtt caaacaatit gataatggta atggattitit 180
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- <210> 97
- <211> 759
- <212> DNA
- <213> Hydra magnipapillata
- <400> 97

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ttaaataagc tcgtaaaaaa tataactagc gattaaatgt atgatgacaa tctgatgaag 660
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<210> 98

<211> 1134

<212> DNA

<213> Hydra magnipapillata

<400> 98

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<210> 99

<211> 957

<212> DNA

<213> Hydra magnipapillata

<400> 99

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<210> 100

<211> 2375

<212> DNA

<213> Hydra magnipapillata

<400> 100

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 <400> 101
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<400> 103

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- <211> 423
- <212> DNA

<213> Hydra magnipapillata

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- <211> 2079

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 <211> 1846
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 <400> 110

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- <211> 900
- <212> DNA
- <213> Hydra magnipapillata
- <400> 111

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gaatttcattg cagttcattg gaataccgat ttatatcaga cagaaaatga agctatcgcg 480

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gaatgggaac aacacaactg ttataagttc caacgtcaat ctgattcctc gacgattaca 840

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<210> 112

<211> 1322

<212> DNA

<213> Hydra magnipapillata

<400> 112

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 <210> 113
 <211> 1759
 <212> DNA

 <213> Hydra magnipapillata
 <400> 113

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 <211> 2156
 <212> DNA
 <213> Hydra magnipapillata
 <400> 114

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 tatgatataa tcaaactgtg tgagaatgat tctgggcaca aaagaatgca aagaagtggT 180

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 <211> 614
 <212> DNA
 <213> Hydra magnipapillata
 <400> 115

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 atgtTTTTTT gttc 614
 <210> 116
 <211> 288
 <212> DNA
 <213> Hydra magnipapillata
 <400> 116
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gaaaaatttg gtttactcaa cacttttagag aatgatatgc aaaagctttt caattcaaag 660

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<210> 118

<211> 1751

<212> DNA

<213> Hydra magnipapillata

<400> 118

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aggggtgcta a 1751

<210> 119
<211> 1725
<212> DNA

<213> Hydra magnipapillata

<400> 119

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gataaatgtg tcaatcttag caataacgaa tcacctcaa tgtctccgtt taaacaaat 180

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cagcctccag actgtgcaat cgaaatggag aattgtgtat taaacaaaga acaaagtffc 480

tcaattaaaa ccgaagatgt tccaaggagg aaaaagcttt caagtgttat taatgcact 540

aaaaaaactc gatcaagaat acataacgaa ggtgcttctt tatggacacc aatggaaat 600

agtcgttggga agtctggaga aaaagtgtt gaaactaaaag atatttcttt gcaccaatta 660

actgaatcag agcgtgaaaa attgaacag tacggattag aaagattacg agaaaaaat 720

tttgattgtc atttaataat ccccaaagaa ttattgtttg catcaggttg cagtttgcac 780

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tctcctagge cttataacce tgtgttttcg gtggatctaa accaagtat tgagaatgat 900

aaacttattg aaaaagcgca aaaagaggca atgcaaaaac ttgataaaaa tgaagagctt 960

ggggaaaact ttgaaacgaa tgaaaaagat caatcaaaca aaaacagtat aactagaact 1020

aaggtctgtg aagaggcgtt tacaaccgag gatcatgaag attacaactt gaattatfff 1080

aggacaatat gtacgtgca agaagaagac gaggatgaat ggagcaccaa taaaagaaaa 1140

tcaaggttaa ttgaagetct tgcactttct tcaacatctg cttcttcttt gtgcatactt 1200

gataaagaaa tagagtttaa acaacttctt cttcaaatc caaaagtcatt ttgcaaaca 1260

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gaagaaagta atccaaacga tgttgacgca cttttaaag agtttttaag atgtttacct 1440

gatccattgt tgactagaga actttaccaa gttttttat ctttagcaaa aaaagaaaca 1500

caaacaaaag aagaaaaagt tgaaattatt cagcagctta tctggttgct acctgtggct 1560

aatcgagata ctttagaatg tctcctaaat tgcttgcgaa tggtagctga ccattcccaa 1620

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gccactttaa tggctccaaa tattctacac agatgcaaag tatga 1725

<210> 120

<211> 671

<212> DNA

<213> Hydra magnipapillata

<400> 120

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atggaattaa ttttgggtt gagtcttaga tcatcagtat aatctttaa aatgaaagca 120

caagttccaa gaacctatga caaagaagc tataaacttc gagcaggatg tttatgttat 180

aaggatgctg caaaaaaga aatattactg gtatccagct caagcaatga ttctctttgg 240

gttgttctg ctggtggcat agacctgga gaaaatccaa ttcaagctgc tattagagaa 300

gcatatgaag aggctggagt aataggtgtg gttggtgatt gtgttggagt gtttcaatta 360

ttgcgctgaa aagaatacgt gaaaagtgat ttaaatagtt ccaggaatat atcaactta 420

catacatatt taaaagatta attaaaaaga acaaaagcaa tttaaaatta tacatgacga 480

atacttaaaa gttatataag ggattaaaat tgtacatgac aaatattcaa gtcattaaaa 540

gatgataaaa attgtaccaa gaaaaacca aactattagt tgatataaag catagataaa 600

agctaataaa aatatttatt tatagaatta ttaaaactaa aaataagtgc ttattacatg 660

atacttaaaa t 671

<210> 121

<211> 1044

<212> DNA

<213> Hydra magnipapillata

<400> 121

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agatagcaat caataatta tcgaataatt ttttttaca agagaacaat gaaaataaaa 180

agaggaaagt gtaaaattgc aaatattgtc agagaagagc aggaatggc tcaaaccgtt 240

acgcctgaaa gcatgacggc cttaaaagaa gcatttcagg catttgacaa aatgatgac 300

ggatttatct ctaaagaaga gctaactcaa gttatgttta gtctcgaca cgtcatgtca 360

accgcagaaa tagatcagat gataagctta gtagatcgg atggaatgg tttgatagac 420

ttcaaagaat ttttgagttt aatgaacact accagtcaag aagaataaa tgataggaa 480

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 gttatggta ttaaaagaaa atttttattg tcgatagtta atgacaaaac aaaaaagca 840
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 aaagtcatct tgtactagta aaaagttaca ctctcaaga cgaactttgt gtacattaat 960
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 <210> 122
 <211> 1080
 <212> DNA
 <213> Hydra magnipapillata
 <400> 122

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 gacgagccga ctctccagt tacgtctcct ggagcaataa gcaatgaaga agattcttca 120
 aaaagcactg ataagaaaa taacgaaaca gacaatatta aggaagaacg tttcgataat 180
 agtttgctac aatcaattat ttcggaattc cgtcaccacc cagattgctt actctcaaac 240
 caaaacttgc aactaatTTT agaaggTTTg ggTgaaccta cagattcaaa tacaatacaa 300
 gagtggatca gtttttatga caaaataat aaaggaggga ttgatttagt tggattttta 360
 aggatggtga tggatcaaat tcttccaatc gaagattcag aggaggaagt tgaagattct 420
 ttcaaagtat ttgatacaga aaattccgga caaattagct gcaatgcttt agaatacgtt 480

 ctaaaaacca gaggaaatcc tttacaaca gaagaatTTT caatccttat aaataaaaac 540
 aaccctaaaa aagaaaagct atTTaattgg aaagaatTTT gtcacaagtt taccaaagaa 600
 gtttatgatg ctagtaatga ccctcTataa ctggatgaca aatggTgatc ctgtgctaaa 660
 tgtttgaagt taaacttgat ttatttatta caaaatatgc acacaaaaaa aaacaactat 720
 agttttaca taattaataa accaacactt ttaataaatt tatcaataat agttttatTa 780
 tctttatTTa acaacttgtt tgtttttaaa atgtatttag agttgtattt aaagttgtaa 840
 attaaaatta ttatgtattt aaaaatggaa tTaaaattgt aaatatgtaa gttttaaaaa 900

 ttgtattTaa atgtttaaaa aaacaagcac cttataaaaa aacataaaag tattattgat 960

gtaaacttaa tattttttt acctgtgtca atgtgattta ctttttaaaa aaatgcgaaa 1020
 attaaataac ttcgataaag caatttgtac agcaattttt ttagaagata gattattaca 1080
 1080

<210> 123
 <211> 568
 <212> DNA
 <213> Hydra magnipapillata
 <400> 123

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tcaatgaact ttcagcgatc attgtttcca aacaatccat ttgttgaagc aaatcattac 180
 ttctatgatg tagaaaaaaaa taatttagac gactcaacag aacgagacgt taaatcgaca 240
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 gaaattccta ggccagagaa ttttgatac taacatgcta caaaatagga aggagattca 360
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 tgcatttggc gtcaaaaata gatgtcatac agagttttta cctaagagga tgcagaaaaa 480
 acaaaacatt ttttttagga tttaaataaa aatgttaaaa ttagcgatat taatttcttt 540

ttctagaaga acaaatcta cgtgtata 568

<210> 124
 <211> 679
 <212> DNA
 <213> Hydra magnipapillata
 <400> 124

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 gaagatgcta ttaacaacca aataaacatg gagttgtatg caagttacca atatctttct 180
 atggcctatt attttgatca agatgatgtt gcattagacg gataactcaa gtttttcaag 240
 catcaatcag atgaagaacg tgaacatgct caggaactaa tggactacca aaataaacgt 300

ggtgggcgtg tagtttaca ggaattcaa gctcccaaat ttcaactgga tacgccagtt 360
 tcagctttag aagcagcgt taatttgag aagaaagtta acgagtcgtt gttaaatgtt 420
 catgccatcg ctgaaaaaaaa cagtgatcct cttttgtcgc acttcttaga atcagagttc 480
 ttgaacgaac aagttgagtc aatcaatgag attgccaagt tgattactaa cgctaagaga 540

tgcggcgatg gtttgggtgt ctatcaattt gataagttga gcatgtcaag ttaaagtca 600
 atgttttttt atttcattgt tttgccttga ggactataca gtgtaaaca tgtttttgt 660
 taaagaaaaa taaatatta 679

<210> 125

<211> 935

<212> DNA

<213> Hydra magnipapillata

<400> 125

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 aaaacataat tatttagaat cgatttactt atatttgatg tttacagtga ctagaagtaa 120
 tttatactaa cctcaattaa agatgataaa attaatgact gatagtcgtg aacttactag 180
 ctatcttgca tctactatg cttaaacc aa agtgaggcat tacaccgata ataactcgca 240
 aaaacaaca gctagaaaaa atacatctaa atggaaacgg gagcatttga atccgagaat 300
 agttgaaaat gaagttgagg atgatgatga agcagaggag gattttagtt atttttctaa 360

agcttattta caaccaagat ttttaactca gttaccacgt aatttatcaa aaacattagg 420
 tgaaaagctt gaagtggttt gcttaacaag cgttttgttt ccgactgaga ttgattggta 480
 tttaaatggc atagtacttt ctaactcggc agacggtaga atagttagaga agaacaacgc 540
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 gcgcgaataa aatgttaact gatagggtac tatcaaattt cgtaccgttg tttcgatc 780

agaaaattaa gcattttccg actgcaagta aaaatgtttt acttgttttg acaaaaaacat 840
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 aaattatatt actcataaat atttatgaaa aaaaa 935

<210> 126

<211> 1453

<212> DNA

<213> Hydra magnipapillata

<400> 126

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 ttttacgaag atgatgaaca tagccgtata atgcctgtca ttttttgc tgaagaatcc 120

tggaaagtta aagcaaagct ttcagatcag ttcacttctg aaattaaagg ccatgattta 180

tttttacc aaacacaaagag tgatttgctt aaagaacatc aggcagaaga aaaaaccag 240

agcttgtaca catttggagt aatagaagaa ggtgacataa aagtcacaga attgtttcat 300

gaactaaaag ctgagaaaa aacaaat tggagccaat caagttatca ttgggatcgt 360

acagcagttc cttacaaaa taattctgat attaatacaa acttgaaaa tccaagtgt 420

gctggaattg ttgatgtagg ctttataatc gactcatctt ggagtttaag agaccaatat 480

catcaagaaa tagaattcct tataaatctt gcaaagacat ttaacataag caagaatggt 540

gcacatgcag gtgttataac atttagttcc agagcagtat tgaatattaa actaaatcaa 600

tactatgac aagaacagtt tgagtttgc attgatgaaa taccgtatat gggttatgtt 660

acacgaatcg atttagcttt acgaaaagct ttagaaatgt ttgatgaaat aaatggagca 720

agaaaggaca ttctcaact tttgtttttg ttaacagatg gtgaacaata tgcaggagac 780

ggcgtttag atgagaatcc tgcaaatgta gcgcaacttg tacgagatag aggtattgaa 840

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tcaagtgaaa aggtat tttt ggccagaaaac tttgatgagt tgataaacia gaat ttttta 960

aagagaataa aagatggcac ctgttttagga aacactgaaa acgagtttaa gcttcataaa 1020

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aaaccaactc tttattctcg tggctttcat agtgtctttc ggatagaatt ggaaaatgat 1140

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gcagtaaaaa tatctcaaca gcgtttaaat aataataca cttatatggt tgaagtaggg 1320

ggtagagttt tttttcaaa agaaaactta tttgcaaat cattttcatc tgtagctgtt 1380

tatgctgcag ataaagaaaa tgttctctca gatgggttca tccggagttt gagcattaag 1440

aatgggaatg aag 1453

<210> 127

<211> 469

<212> DNA

<213> Hydra magnipapillata

<400> 127

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ttgaatgac cttcatttgg gcaaaaaatt ataacagga ttaaaaaacc ttttgaacat 180

gfgatcaata gtttaaaaaa tggatttgc tcaaaaaagga aacaaaaccc ccaagaagat 240
 gctacttgaa tggttattgt tctcctggta aaaaagaat tacgcaaaaa aattaacgaa 300

 ataaaaaatt aacaagacaa caaaacttgt atttttgat actgctttta tttacggata 360
 tttttatata ttgtcacgaa ctttaatttt attctgctaa tgtgatttat gttcaacaa 420
 aacatttttt ttataaaca atgtgaaaga agaaatcaaa gttataaaa 469
 <210> 128
 <211> 1158
 <212> DNA
 <213> Hydra magnipapillata
 <400> 128

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 gacaaaagat actgaaacaa aagaaaatgg ggttgaatct tctgataatc ctagtggact 180

 tttgtgggat tegtgcgaag ctcaagtttc tcagcattgg aaaaaattt ggaagttttt 240
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 cgaaagccta aaacctttaa atttagatcg tcaaaatatt ttagttcatg taacgaacat 360
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 ttgagtttat atggatttgg ctaataaata attgtgttcc agatttatat ctaatacctt 1080
 tatattatc aattaattta agtgtgtatc atttatttta ttcttatata ttaatttgcg 1140
 agatattaga agcaaaaa 1158
 <210> 129

<211> 482
 <212> DNA
 <213> Hydra magnipapillata
 <400> 129

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 gatgcgcgac caaagacagc tcgattgaaa gactctaaaa aaagttcaga acttgtcaag 120
 ttgaatgac cttcatttgg gcaaaaaatt ataaacagga ttaaaaaacc ttttgaacat 180

gtgatcaata gtttaaaaaa tggatttgc tcaaaaaagg aaacaaaacc cccaagaaga 240
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 aataaaaaaa aaacaagaca acaaaacttg tattttttga tactgctttt atttacggat 360
 attttttata tttgtcacga actttaattt tattctgcta atgtgattta tgttcaaaca 420
 aaacattttt ttttataaac aatgttaaaa aagaaatcaa agttataaaa crtnartamr 480
 na 482

<210> 130
 <211> 1389
 <212> DNA
 <213> Hydra magnipapillata
 <400> 130

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gagttgatag cagtaactga taaacggatt gtaaaagact acattatctg gataacagtt 420
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 <211> 2214
 <212> DNA
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 <400> 131
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 aacaaaaaat gtaacagaag tatatttTgga tacgtaattt atgatacgtA cgatcctaca 180
 aatatggaca tgacgtcttt tgctgtatta gattcattaa gattaaacgt ttctaaccAA 240
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 gatatttTgca tagatgtttt agaaatattg cagcaagcat acgaccCAA aatatattca 660
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 aataagcttc tGtttgtag ttatacagct ggagaagatc agacatttaa aaattacttt 900

ttaagcctaa cciatgaaaa ttcgagtaaa tggctgaaag taatTTTTga acgaagcaga 960

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acaatagttt cagatataaa aaaatgttgt tgggattgca tTcTTTgccc ggataacat 1440

attgctgaag gtaaaaactg ttttgactgt ggaaaaaact taattcccaa taaaaataaa 1500

tCagagtgtg tTaaacctaa aagcatttat tggagtTTta acgatagtca aaatccgctg 1560

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aatgtctttg ataaagtaga tattaactt tctaccgttc aaaaagattg tgattcaatt 2040

agctttatga taatttttt atgcttcatt tTagtTTtat cctttttttg cggtattcaa 2100

tCgtttaaag ctcgactct tccaacaaca tacaatgaaa acaaaaacat agcctattct 2160

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<210> 132

<211> 581

<212> DNA

<213> Hydra magnipapillata

<400> 132

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gtattggcaa cactacgcaa actcttccgt gTaaactgtt tGactgtcca aaatcatgtg 180

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 ccacattgag atcgaatggt gctgatcagt taactcaagc taaggctgca agagcaaac 420
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 <211> 834
 <212> DNA
 <213> Hydra magnipapillata
 <400> 133

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 ttaaactatg gtgaaattat agaacacaaa gatttgaaaa gctttggcta ctttcaagc 480
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 cactactatt cagatgaagc acaacatagc tgtgaaaact ctttcaacat caataacgaa 720
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 <210> 134
 <211> 353
 <212> DNA
 <213> Hydra magnipapillata
 <400> 134

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 caagtgattg catgcaatgc cattgcaata taaacattgt gatgtgtaca ataagaacat 180
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 aagtggttta tgcttaaatc tatattgctt tacctggaac aacgggaaaa cgatttcctc 180
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ttaatgttcg gaccagaata cgatctatgt cgcattttta aaggagatc tgaaaaaaa 1620

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<210> 136

<211> 926

<212> DNA

<213> Hydra magnipapillata

<400> 136

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gacgttttta aaaagtggtt ttcaatatca aaagaagaat gtggaacagt ttgttgaca 180

aattctttat gtgtttagc taattataat gcagacgaaa atttatgcga aataattca 240

aaaacacaac tgcaagagca aagacaagga tggatattac tgatgacaaa cacaacaagc 300

gatataaata ttggtccggt gtgtgagcaa agtaatctat gcatgagtaa cgagtgggtc 360

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gccaaagcga gcaaagtatt tcaatcttcg acttatagtt atactaatcg tcctgaaatg 480

gcgattgacg gcgatttaca aacggcatct tgcacacaaa tggatcctat aaactggttt 540

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tatcctgaac gacttgctgg ttataaattg atgtcaagta caaacgatga aaattatgaa 660

catatagtaa cattaagtag agactggcag caaacttata actgttcgaa tagtgctcgc 720

tttattatgg tttccaaagt tcctgataat aaccaatata ttaacattgc cgagattgag 780

gtctgggtat aaattaaaga ttacttgaga tgcaaattha aaactgtttt agactaatga 840

tgtttaacta atgatgttta aaatattcaa gcttgaaatt atgaccaaat tgaacactaa 900

aaagtatatt aataaaatata aaaaaa 926

<210> 137

<211> 1118

<212> DNA

<213> Hydra magnipapillata

<400> 137

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 agaactggca aaaatagtag tcgaggctgt acaagaaaat aataatcagc caataaatac 180

 aaataagatc aaaactcctc gtgaactaag aaacaacaaa acaaaagagt acgtttcaga 240
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 attaaatgat gcaatgagct gtatgtggcc agaatggg 1118

<210> 138

<211> 1413

<212> DNA

<213> Hydra magnipapillata

<400> 138

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 atgtgtatgt catcaaatgg tactgtgcta ataactggtg gtgaagattg caatgcccaa 180
 atctatgata cagaacaaga gaaagttgtc tcagtacttg ctggtcatga attttacata 240

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<210> 139

<211> 5381

<212> DNA

<213> Hydra magnipapillata

<400> 139

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<211> 2139

<212> DNA

<213> Hydra magnipapillata

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<211> 3152

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<213> Hydra magnipapillata

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