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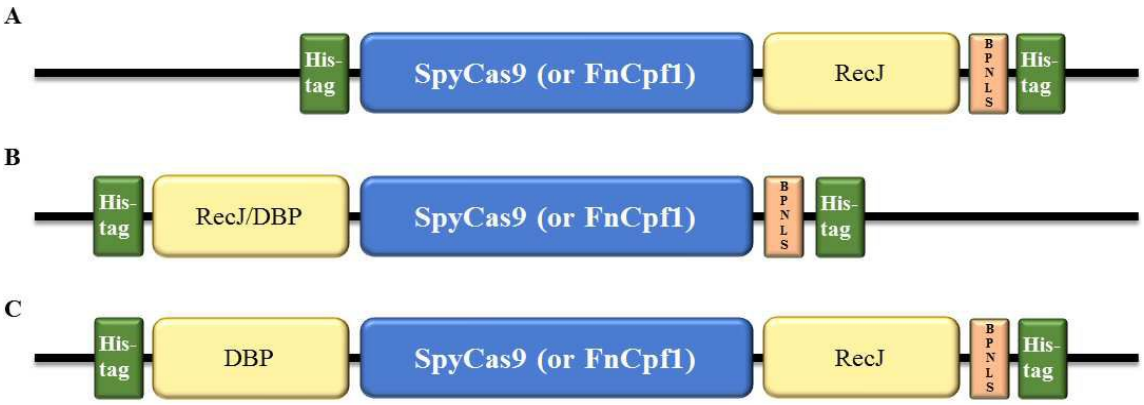
전체 청구항 수 : 총 22 항

(54) 발명의 명칭 **크리스퍼 연관 단백질 및 엑소뉴클레아제를 포함하는 변형된 크리스퍼 연관 단백질**

(57) 요 약

본원 발명은 크리스퍼 연관 단백질 및 엑소뉴클레아제인 RecJ를 포함하는 유전자 편집 기능이 향상된 크리스퍼 플러스 단백질에 관한 것이다. 본원 발명의 일 구체예로 RecJ가 크리스퍼 연관 단백질에 결합된 크리스퍼 플러스 단백질은 종래의 크리스 연관 단백질에 비해 녹아웃 및 녹인 효율이 증가하였다. 따라서, 본 발명의 크리스퍼 플러스는 유전자 편집 효율이 향상된 유전자 가위를 제공하며, 이를 이용할 경우 보다 안정적이고 효율적으로 유전자를 편집할 수 있다. 따라서, 상기 크리스퍼 플러스 단백질은 종래의 크리스퍼 연관 단백질을 대체할 수 있는 효율적인 유전자 편집 기능을 보유한 단백질로서 사업성이 우수할 것으로 예상된다.

대 표 도 - 도12



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명 세 서

청구범위

청구항 1

크리스퍼 연관 단백질 및 엑소뉴클레아제인 RecJ를 포함하는 크리스퍼 플러스 단백질.

청구항 2

제1항에 있어서,

상기 크리스퍼 플러스 단백질은 하기 구조식 1 내지 4에서 선택되는 어느 하나의 구조를 갖는 것인, 크리스퍼 플러스 단백질로서,

<구조식 1>

크리스퍼 연관 단백질-RecJ

<구조식 2>

크리스퍼 연관 단백질-링커 1-RecJ

<구조식 3>

RecJ-크리스퍼 연관 단백질

<구조식 4>

RecJ-링커 1-크리스퍼 연관 단백질

상기 링커 1은 1 내지 20개의 아미노산으로 구성된 것일 수 있다.

청구항 3

제2항에 있어서,

상기 링커 1은 서열번호 17, 18 , 19, 20, 21, 22, 23 또는 24의 아미노산 서열을 가지는 것인, 크리스퍼 플러스 단백질.

청구항 4

제1항에 있어서,

추가적으로 DNA 결합 단백질(DNA binding protein, DBP)를 더 포함하는 것인, 크리스퍼 플러스 단백질.

청구항 5

제4항에 있어서,

상기 DBP는 SSB(single stranded binding protein) 또는 DSB(double stranded binding protein)인 것인, 크리스퍼 플러스 단백질.

청구항 6

제1항에 있어서,

상기 RecJ는 크리스퍼 연관 단백질의 N 말단 또는 C 말단에 결합되는 것인, 크리스퍼 플러스 단백질.

청구항 7

제4항에 있어서,

상기 크리스퍼 플러스 단백질는 하기 구조식 5 내지 7에서 선택되는 어느 하나의 구조를 갖는 것인, 크리스퍼

플러스 단백질로서,

<구조식 5>

DBP-크리스퍼 연관 단백질-RecJ

<구조식 6>

DBP-링커 2-크리스퍼 연관 단백질-RecJ

<구조식 7>

DBP-링커 2-크리스퍼 연관 단백질-링커 1-RecJ

이때, DBP는 SSB 또는 DSB이며,

상기 링커 1은 1 내지 20개의 아미노산으로 구성된 것일 수 있으며,

상기 링커 2는 1 내지 20개의 아미노산으로 구성된 것일 수 있다.

청구항 8

제1항에 있어서,

추가적으로 핵 위치화 시그널(nuclear localization signal)을 더 포함하는 것인, 크리스퍼 플러스 단백질.

청구항 9

제8항에 있어서,

상기 핵 위치화 시그널은 융합 단백질의 C 말단에 위치하는 것인, 크리스퍼 플러스 단백질.

청구항 10

제1항에 있어서,

추가적으로 His tag을 포함하는 것인, 크리스퍼 플러스 단백질.

청구항 11

제1항에 있어서,

상기 크리스퍼 연관 단백질은 Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9, Cas10, Cas12a, Cas12b, Cas12c, Cas12d, Cas12e, Cas13a, Cas13b, Cas13c, Cas13d, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3 및 Csf4로 구성된 군으로부터 선택되는 어느 하나인 것인, 크리스퍼 플러스 단백질.

청구항 12

제1항에 있어서,

상기 RecJ는 서열번호 1, 3, 5, 7, 9, 11, 13 또는 15로 이루어진 군에서 선택되는 어느 하나의 아미노산 서열을 가지는 것인, 크리스퍼 플러스 단백질.

청구항 13

제1항 내지 제12항에 있어서,

상기 크리스퍼 플러스 단백질은 서열번호 31, 41, 49, 53, 57, 71 또는 77로 구성된 군에서 선택되는 어느 하나의 아미노산 서열을 가지는 것인, 크리스퍼 플러스 단백질.

청구항 14

제12항의 크리스퍼 플러스 단백질을 코딩하는 폴리뉴클레오티드.

청구항 15

제14항에 있어서,

상기 폴리뉴클레오티드는 서열번호 32, 42, 50, 54, 58, 72 또는 78로 구성된 군에서 선택되는 어느 하나의 핵산 서열을 가지는 것인, 폴리뉴클레오티드.

청구항 16

제14항의 폴리뉴클레오티드가 적재된 벡터.

청구항 17

제16항에 있어서,

상기 벡터는 플라스미드인, 벡터.

청구항 18

제16항의 벡터가 도입된 숙주 세포.

청구항 19

제1항 내지 제12항의 크리스퍼 플러스 단백질 및 crRNA가 결합된 RNP 복합체.

청구항 20

제19항의 RNP 복합체를 포함하는 유전자 편집용 조성물.

청구항 21

제1항 내지 제12항의 크리스퍼 플러스 단백질 및 crRNA를 유효성분으로 포함하는 유전자 편집용 키트.

청구항 22

제19항에 있어서,

상기 crRNA는 표적 유전자에 상보적으로 결합 가능한 10 내지 30개의 뉴클레오티드로 구성된, 유전자 편집용 키트.

발명의 설명

기 술 분 야

[0001] 본 발명은 엑소뉴클레아제가 크리스퍼 연관 단백질에 결합된 변형된 크리스퍼 연관 단백질 및 이의 용도에 관한 것이다.

배 경 기 술

[0002] 유전체 교정(Genome editing)이란 생명체의 유전정보를 자유롭게 교정하는 기술이다. 생명과학분야의 진보와 유전체 서열 분석 기술의 발전을 통해 우리는 다양한 유전정보에 대해 폭넓게 이해할 수 있게 되었다. 예를 들어 동식물의 번식, 질병과 성장, 다양한 인간 유전질병을 일으키는 유전자 변이, 바이오연료의 생산 등을 위한 유전자에 대한 이해는 이미 확보된 상황이지만 이를 직접적으로 활용하여 생명체를 개선하고, 인간 질병을 치료하는 수준에까지 이르기 위해서는 그 이상의 기술 진보가 필수적이다.

[0003] 유전체 교정 기술은 인간을 포함하여 동물, 식물, 미생물의 유전정보를 변화시켜 그 활용범위를 획기적으로 확장시킬 수 있다. 유전자가위는 원하는 유전정보를 정확히 자를 수 있도록 설계되어 만들어지는 분자 도구로 유전체 교정 기술에서 핵심역할을 하고 있다. 유전자 서열분석 분야를 한 차원 발전시켰던 차세대시퀀싱(Next generation sequencing) 기술과 같이, 유전자가위는 유전정보 활용의 속도와 그 범위를 확장시키고 새로운 산업분야를 창출해 내는 핵심 기술이 되고 있다.

[0004] 지금까지 개발된 유전자 가위는 그 순서에 따라 3세대로 나눌 수 있다. 1세대 유전자 가위는 ZFN(Zinc Finger

Nuclease), 2세대 유전자 가위는 TALEN(Transcription Activator-Like Effector Nuclease), 가장 최근에 연구된 CRISPR(Clustered regularly interspaced short palindromic repeat)-Cas(CRISPR-associated) 9은 3세대 유전자 가위다.

[0005] CRISPRs는 유전자 서열이 밝혀진 박테리아의 대략 40% 및 유전자 서열이 밝혀진 고세균의 90%의 유전체에서 발견되는 여러 짧은 직접 반복을 포함하는 좌위이다. Cas 단백질은 CRISPR RNA(crRNA) 및 trans-activating crRNA(tracrRNA)로 명명된 두 개의 RNA와 복합체를 형성했을 때, 활성 엔도뉴클레아제(endonuclease)를 형성하고, 그렇게 함으로써 파지 또는 플라스미드의 침입에서 외부 유전적 요소를 분해하여 숙주 세포를 보호한다. crRNA는 전달에 외부 침입자로부터 점유되었던 숙주 유전체의 CRISPR 요소로부터 전사된다.

[0006] 이러한 CRISPR-Cas 시스템 유래의 RNA-가이드 뉴클레아제(RNA-guided nuclease)는 유전체를 교정할 수 있는 수단을 제공해준다. 특히, 단일 가이드 RNA(sgRNA)와 Cas 단백질을 이용하여 세포 및 기관의 유전체를 편집할 수 있는 기술과 관련된 연구들이 활발히 진행되어왔다. 최근, Cpf1 단백질(Prevotella 및 Francisella 1 유래)이 CRISPR-Cas 시스템의 또 다른 뉴클레아제 단백질로서 보고되었고(B. Zetsche, et al., 2015), 이에 따라 유전체 교정에 있어서 선택의 폭이 넓어졌다. Cpf1은 5' 돌출부 형태로 절단하는 점, 가이드 RNA의 길이가 더 짧은 점, 시드(seed) 서열 및 절단 위치 사이가 더 길다는 점에서 여러 장점을 가진다. 그럼에도 여전히 보다 우수한 유전자 가위를 개발하기 위하여 많은 연구가 이루어지 있는 실정이다.

선행기술문헌

비특허문헌

[0007] (비특허문헌 0001) B. Zetsche, et al., Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system, Cell, 2015, 163, 759-771.

발명의 내용

해결하려는 과제

[0008] 본 발명자들은 유전체 교정에 있어서 기존에 알려진 크리스퍼 연관 단백질보다 더 효과적인 단백질을 개발하기 위하여 지속적으로 연구한 결과, 표적 핵산 서열을 인식하여 절단하는 효율이 향상될 뿐 아니라, 녹인 효율이 증가된, 엑소뉴클레아제를 포함하는 변형된 크리스퍼 연관 단백질을 개발하여 본 발명을 완성하였다. 따라서, 본 발명의 목적은 표적 핵산 서열을 인식하여 절단하는 효율이 향상된 변형된 크리스퍼 연관 단백질을 제공하는 것이다.

과제의 해결 수단

[0009] 상기 목적을 달성하기 위하여, 크리스퍼 연관 단백질에 엑소뉴클레아제 중 RecJ가 결합된 변형된 크리스퍼 연관 단백질을 제공한다.

발명의 효과

[0010] RecJ가 크리스퍼 연관 단백질에 결합된 크리스퍼 플러스 단백질은 크리스 연관 단백질에 비해 녹아웃(Knockout) 및 녹인(Knockin) 효율이 증가하였다. 따라서, 본원의 변형된 크리스퍼 연관 단백질은 유전자 편집 효율이 향상된 유전자 가위를 제공하며, 이를 이용할 경우 보다 안정적이고 효율적으로 유전자를 편집할 수 있다.

도면의 간단한 설명

[0011] 도 1은 변형된 Cas9 단백질을 체외에서 절단하여 분석한 것이다. 각 RNP의 농도는 25 nM이다. 별표(*)는 약 600 bp에서 나타나는 절단된 템플릿(template)을 의미한다. 각 겔 이미지의 하단 부분에 절단 효율을 표시하였다.

도 2는 SpCas9 및 SpCas9-RecJ에 의한 원형 dsDNA 분해를 확인한 것이다. 선형 dsDNA는 DNA 전기 영동에서 뚜렷하고 두꺼운 dsDNA 크기를 보였다. SpCas9/gRNA C apoproteins를 배양한 후, SpCas9은 시간에 상관없이 단일의 선명하고 두꺼운 dsDNA를 나타내었다. 그러나, SpCas9-RecJ/gRNA C는 60-180분 배양 후에 흐릿하며, 약하고, 얇은, 꼬리가 끌리는 dsDNA형태를 나타내었는데, 이는 DNA 분해를 의미한다.

도 3은 반응 시간에 따른 유전자 편집 효율을 나타낸 것이다. 이는 T7E1 엔도뉴클레아제 활성의 산물이며, 삽입(insertion)-결실률(deletion)(% indel)과 비교하여 측정하였다.

도 4는 CCR5 및 DHCR7에 대한 SpyCas9, SpyCas9-RecJ 및 SpyCas9-T5 간의 HDR 효율을 비교하여 나타낸 것이다. CCR5 및 DHCR7 유전자자리(locus)에서 SpyCas9, SpyCas9-RecJ 및 SpyCas9-T5의 HDR 효율을 나타낸 것이다.

도 5 및 도 6은 유전자 편집 효율을 확인하기 위하여, HEK293 세포에서 SpCas9 및 SpCas9-RecJ의 In vitro 절단 에세이를 수행한 결과를 나타낸다.

도 7은 Cas9 및 Cas9-RecJ이 인간 PD-1 및 CCR5의 유전자에 미치는 온-타겟 효과를 나타낸 것이다.

도 8은 인간 CCR5에 대한 Cas9 및 Cas9-RecJ의 오프 타겟 효과를 확인한 것이다.

도 9는 5개의 오프 타겟팅된 유전자의 In vitro 절단 에세이를 나타낸 것이다.

도 10은 선형 dsDNA를 이용한 In vitro 절단 에세이를 통하여, FnCpf1, FnCpf1-RecJ 및 SpCas9이 가지고 있는 엑소뉴클레아제 기능을 확인한 것이다.

도 11은 선형 dsDNA를 이용하여 FnCpf1 또는 FnCpf1-RecJ 엑소뉴클레아제에 의한 In vitro 절단 에세이 결과를 나타낸 것이다.

도 12는 RecJ를 포함하는 변형된 크리스퍼 연관 단백질의 구조를 나타낸 것이다. A는 SpyCas9 또는 FnCpf1의 C 말단에 RecJ가 융합된 형태이다. B는 SpyCas9 또는 FnCpf1의 N 말단에 RecJ가 결합된 형태이다. C는 SpyCas9 또는 FnCpf1의 C 말단에는 RecJ가 N 말단에는 DBP(DNA Binding Protein)이 결합된 형태이다. 이때, His tag은 6개의 His로 구성되며, BPNLS는 핵 위치화 시그널(nuclear localization signal)을 의미한다.

도 13은 CCR5 및 DHCR7에 대한 SpyCas9의 sgRNA 결합 사이트를 나타낸 것이다. 인간 CCR5(A) 및 인간 DHCR7(B)에 대한 SpyCas9의 sgRNA 결합 영역을 각각 노란색과 핑크색으로 나타낸 것이다. PAM 서열은 녹색으로 표시하였다. 화살표는 뉴클레아제 절단 위치를 나타낸다.

도 14는 CCR5 및 DHCR7에 대한 FnCpf1의 crRNA 결합 사이트를 나타낸 것이다. 인간 CCR5(A) 및 인간 DMNT1(B)에 대한 FnCpf1의 crRNA 결합 영역을 각각 붉은색과 오렌지색으로 나타낸 것이다. PAM 서열은 파란색으로 표시하였다. 화살표는 뉴클레아제 절단 위치를 나타낸다.

도 15 및 도 16은 SpyCas9과 SpyCas9의 C 말단에 다양한 단백질이 결합된 변형된 SpyCas9 사이의 편집 효율을 비교한 것이다. SpyCas9의 C 말단에 GFP, hTdT, 및 엑소뉴클레아제로 알려진 RecJ, RecE, lambda, mungbean, and T5가 결합된 형태이다. 도면에서 나타낸 바와 같이 Knock-out 및 Knock-in 효율이 서로 상이하다.

도 17은 SpyCas9과 SpyCas9-RecJ의 Knock-out 편집 효율을 비교한 것이다. A는 SpyCas9-RecJ의 구조를 나타낸 것이다. B는 대조군, SpyCas9 및 SpyCas9-RecJ 간의 유전자 편집 효율을 나타낸 것이다. C는 CCR5 및 DHCR7 유전자의 편집 정도를 확인하여, SpyCas9 대비 SpyCas9-RecJ의 편집 효율 변화를 백분율로 나타낸 것이다.

도 18은 SpyCas9과 SpyCas9-RecJ의 Knock-in 편집 효율을 비교한 것이다. A는 SpyCas9-RecJ의 구조를 나타낸 것이다. B는 대조군, SpyCas9 및 SpyCas9-RecJ 간의 유전자 편집 효율을 나타낸 것이다. C는 CCR5 및 DHCR7 유전자의 편집 정도를 확인하여, SpyCas9 대비 SpyCas9-RecJ의 편집 효율 변화를 백분율로 나타낸 것이다.

도 19는 및 20은 SpyCas9과 SpyCas9의 N 말단 또는 N/C 양말단에 다양한 단백질이 결합된 변형된 SpyCas9 사이의 편집 효율을 비교한 것이다. 변형된 SpyCas9은 SpyCas9의 N 말단에 RecJ, RecE, GFP, 단일 가닥 DNA 결합 단백질(single stranded DNA binding protein, SSB) 및 이중 가닥 DNA 결합 단백질(double stranded DNA binding protein, DSB)이 결합되어 있다. 또한, 변형된 SpyCas9은 SpyCas9의 C 말단에 RecJ가 결합되어 있고, N 말단에 SSB 또는 DSB이 결합되어 있다. 도면에서 나타낸 바와 같이 Knock-out 및 Knock-in 효율은 서로 상이하다.

도 21은 SpyCas9과 RecJ-SpyCas9 간의 Knock-out 편집 효율을 비교한 것이다. A는 RecJ-SpyCas9의 구조를 나타낸 것이다. B는 대조군, SpyCas9 및 RecJ-SpyCas9의 유전자 편집 효율을 나타낸 것이다. C는 DHCR7 유전자의 편집 정도를 확인하여, SpyCas9 대비 RecJ-SpyCas9의 편집 효율 변화를 백분율로 나타낸 것이다.

도 22는 SpyCas9과 RecJ-SpyCas9의 Knock-in 편집 효율을 비교한 것이다. A는 RecJ-SpyCas9의 구조를 나타낸 것이다. B는 대조군, SpyCas9 및 RecJ-SpyCas9 간의 유전자 편집 효율을 나타낸 것이다. C는 DHCR7 유전자의 편집 정도를 확인하여, SpyCas9 대비 RecJ-SpyCas9의 편집 효율 변화를 백분율로 나타낸 것이다.

도 23은 SpyCas9과 SSB-SpyCas9-RecJ의 Knock-out 편집 효율을 비교한 것이다. A는 SSB-SpyCas9-RecJ의 구조를 나타낸 것이다. B는 대조군, SpyCas9 및 SSB-SpyCas9-RecJ의 유전자 편집 효율을 나타낸 것이다. C는 DHCR7 유전자의 편집 정도를 확인하여, SpyCas9 대비 SSB-SpyCas9-RecJ의 편집 효율 변화를 백분율로 나타낸 것이다.

도 24는 SpyCas9과 DSB-SpyCas9-RecJ 간의 Knock-out 편집 효율을 비교한 것이다. A는 DSB-SpyCas9-RecJ의 구조를 나타낸 것이다. B는 대조군, SpyCas9 및 DSB-SpyCas9-RecJ 간의 유전자 편집 효율을 나타낸 것이다. C는 DHCR7 유전자의 편집 정도를 확인하여, SpyCas9 대비 DSB-SpyCas9-RecJ의 편집 효율 변화를 백분율로 나타낸 것이다.

도 25 및 도 26은 FnCpf1과 FnCpf1의 C 말단에 다양한 단백질이 결합된 변형된 FnCpf1 사이의 편집 효율을 비교한 것이다. FnCpf1의 C 말단에 GFP, hTdT, 및 엑소뉴클레아제로 알려진 RecJ, RecE, lambda, mungbean, 및 T5가 결합된 형태이다. 도면에서 나타낸 바와 같이 Knock-out 및 Knock-in 효율은 서로 상이하다.

도 27은 FnCpf1 및 FnCpf1-RecJ의 Knock-out 편집 효율을 비교한 것이다. A는 FnCpf1-RecJ의 구조를 나타낸 것이다. B는 대조군, FnCpf1 및 FnCpf1-RecJ의 유전자 편집 효율을 나타낸 것이다. C는 CCR5 및 DNMT1 유전자의 편집 정도를 확인하여, FnCpf1 대비 FnCpf1-RecJ의 편집 효율 변화를 백분율로 나타낸 것이다.

도 28은 FnCpf1 및 FnCpf1-RecJ의 Knock-in 편집 효율을 비교한 것이다. A는 FnCpf1-RecJ의 구조를 나타낸 것이다. B는 대조군, FnCpf1 및 FnCpf1-RecJ 간의 유전자 편집 효율을 나타낸 것이다. C는 CCR5 및 DNMT1 유전자의 편집 정도를 확인하여, FnCpf1 대비 FnCpf1-RecJ의 편집 효율 변화를 백분율로 나타낸 것이다.

도 29 및 30은 FnCpf1과 FnCpf1의 N 말단 또는 N/C 양말단에 다양한 단백질이 결합된 변형된 FnCpf1 사이의 편집 효율을 비교한 것이다. 변형된 FnCpf1은 FnCpf1의 N 말단에 RecJ, RecE, GFP, 단일 가닥 DNA 결합 단백질(single stranded DNA binding protein, SSB) 및 이중 가락 DNA 결합 단백질(double stranded DNA binding protein, DSB)이 결합되어 있다. 또한, 변형된 FnCpf1은 FnCpf1의 C 말단에 RecJ가 결합되어 있고, N 말단에 SSB가 결합되어 있다. 도면에서 나타낸 바와 같이 Knock-out 및 Knock-in 효율은 서로 상이하다.

도 31은 FnCpf1 및 RecJ-FnCpf1의 Knock-out 편집 효율을 비교한 것이다. A는 RecJ-FnCpf1의 구조를 나타낸 것이다. B는 대조군, RecJ-FnCpf1 간의 유전자 편집 효율을 나타낸 것이다. C는 CCR5 및 DNMT1 유전자의 편집 정도를 확인하여, FnCpf1 대비 RecJ-FnCpf1의 편집 효율 변화를 백분율로 나타낸 것이다.

도 32는 FnCpf1 및 RecJ-FnCpf1 간의 Knock-in 편집 효율을 비교한 것이다. A는 RecJ-FnCpf1의 구조를 나타낸 것이다. B는 대조군, RecJ-FnCpf1의 유전자 편집 효율을 나타낸 것이다. C는 CCR5 및 DNMT1 유전자의 편집 정도를 확인하여, FnCpf1 대비 RecJ-FnCpf1의 편집 효율 변화를 백분율로 나타낸 것이다.

도 33은 FnCpf1과 SSB-FnCpf1-RecJ의 Knock-out 편집 효율을 비교한 것이다. A는 SSB-FnCpf1-RecJ의 구조를 나타낸 것이다. B는 대조군, FnCpf1 및 SSB-FnCpf1-RecJ의 유전자 편집 효율을 나타낸 것이다. C는 CCR5 및 DNMT1 유전자의 편집 정도를 확인하여, FnCpf1 대비 SSB-FnCpf1-RecJ의 편집 효율 변화를 백분율로 나타낸 것이다.

도 34는 FnCpf1과 SSB-FnCpf1-RecJ의 Knock-in 편집 효율을 비교한 것이다. A는 SSB-FnCpf1-RecJ의 구조를 나타낸 것이다. B는 대조군, FnCpf1 및 SSB-FnCpf1-RecJ의 유전자 편집 효율을 나타낸 것이다. C는 CCR5 및 DNMT1 유전자의 편집 정도를 확인하여, FnCpf1 대비 SSB-FnCpf1-RecJ의 편집 효율 변화를 백분율로 나타낸 것이다.

도 35a, 도 35b 및 도 35c는 SpyCas9 및 SpyCas9의 C 말단에 단백질이 결합된 변형된 SpyCas9에 유도되는 비상동적인 말단 연결에 있어서, ssODN 및 노코다졸(nocodazole)이 미치는 영향을 확인한 것이다. 도 35a는 ssODN 및 노코다졸이 처리되지 않았을 때의 비상동적인 말단 연결 효율을 나타낸다. 도 35b는 ssODN 및 노코다졸이 처리되었을 때의 비상동적인 말단 연결 효율을 나타낸다. 도 35c는 ssODN 및 노코다졸의 존재 유무에 따른 효율을 비교한 것이다. ssODN 및 노코다졸은 Knock-out의 경우, SpyCas9 및 변형된 SpyCas9의 효율에는 큰 영향이 없음을 확인하였다.

도 36a, 도 36b 및 도 36c는 SpyCas9 및 SpyCas9의 N 말단 또는 N/C 양말단에 단백질이 결합된 변형된 SpyCas9에 유도되는 비상동적인 말단 연결에 있어서, ssODN 및 노코다졸이 미치는 영향을 확인한 것이다. 도 36a는 ssODN 및 노코다졸이 처리되지 않았을 때의 비상동적인 말단 연결 효율을 나타낸다. 도 36b는 ssODN 및 노코다졸이 처리되었을 때의 비상동적인 말단 연결 효율을 나타낸다. 도 36c는 ssODN 및 노코다졸의 존재 유무에 따른 효율을 비교한 것이다. ssODN 및 노코다졸은 Knock-out의 경우, SpyCas9 및 변형된 SpyCas9의 효율에는 큰 영향

이 없음을 확인하였다.

도 37a, 도 37b 및 도 37c는 FnCpf1 및 FnCpf1의 C 말단에 단백질이 결합된 변형된 FnCpf1에 유도되는 비상동적인 말단 연결에 있어서, ssODN 및 노코다졸(nocodazole)이 미치는 영향을 확인한 것이다. 도 37a는 ssODN 및 노코다졸이 처리되지 않았을 때의 비상동적인 말단 연결 효율을 나타낸다. 도 37b는 ssODN 및 노코다졸이 처리되었을 때의 비상동적인 말단 연결 효율을 나타낸다. 도 37c는 ssODN 및 노코다졸의 존재 유무에 따른 효율을 비교한 것이다. ssODN 및 노코다졸은 Knock-out의 경우, FnCpf1 및 변형된 FnCpf1의 효율을 향상 시킨다는 점을 확인하였다.

도 38a, 도 38b 및 도 38c는 FnCpf1 및 FnCpf1의 N 말단 또는 N/C 양말단에 단백질이 결합된 변형된 FnCpf1에 유도되는 비상동적인 말단 연결에 있어서, ssODN 및 노코다졸이 미치는 영향을 확인한 것이다. 도 38a는 ssODN 및 노코다졸이 처리되지 않았을 때의 비상동적인 말단 연결 효율을 나타낸다. 도 38b는 ssODN 및 노코다졸이 처리되었을 때의 비상동적인 말단 연결 효율을 나타낸다. 도 38c는 ssODN 및 노코다졸의 존재 유무에 따른 효율을 비교한 것이다. ssODN 및 노코다졸은 Knock-out의 경우, FnCpf1 및 변형된 FnCpf1의 효율에는 큰 영향이 없음을 확인하였다.

도 39a 및 도 39b는 각각의 실험에 대한 타당성을 나타낸 것이다. 최상단에는 on-target 서열을 나타낸 것이며, 미스매치된 핵산은 다른 색으로 표시하였다. GFLAS01은 음성대조군이며; GFLAS02 및 GFLAS05는 SpyCas9이며; GFLAS03, GFLAS04, GFLAS06 및 GFLAS07은 Cas9-RecJ를 이용하여 실험한 결과를 나타낸다.

도 40은 Cas9 및 Cas9-RecJ간의 오프-타겟팅 효율을 비교한 것이다. 5개의 인간 CCR5로 오프-타겟을 테스트하였다. A는 CCR5에 대한 오프-타겟 후보 서열을 나타낸 것이다. B는 딥 시퀀싱(deep sequencing)을 하기 위한 증폭물을 나타낸 것이다.

도 41은 Cas9 및 Cas9-RecJ의 증폭물의 딥 시퀀싱 결과를 나타낸 것이다. Cas9-RecJ은 KCNJ6, CNTPNA2, 및 Ch.5에서는 Cas9에 비해 오프-타겟이 나타나는 비율이 낮다는 것을 확인하였다.

도 42는 SpyCas9의 단백질 서열 및 핵산 서열을 도식화한 것이다.

도 43은 SpyCas9-RecJ의 단백질 서열 및 핵산 서열을 도식화한 것이다.

도 44는 RecJ-SpyCas9의 단백질 서열 및 핵산 서열을 도식화한 것이다.

도 45는 SSB-SpyCas9-RecJ의 단백질 서열 및 핵산 서열을 도식화한 것이다.

도 46은 DSB-SpyCas9-RecJ의 단백질 서열 및 핵산 서열을 도식화한 것이다.

도 47은 FnCpf1의 단백질 서열 및 핵산 서열을 도식화한 것이다.

도 48은 FnCpf1-RecJ의 단백질 서열 및 핵산 서열을 도식화한 것이다.

도 49는 RecJ-FnCpf1의 단백질 서열 및 핵산 서열을 도식화한 것이다.

도 50은 SSB-FnCpf1-RecJ의 단백질 서열 및 핵산 서열을 도식화한 것이다.

도 51은 변형된 크리스퍼 연관 단백질 및 sgRNA를 이용하여 식물체의 유전자를 편집하는 실험 방법의 개요를 나타낸 것이다.

도 52는 유전자 편집을 하기 위해 준비된 식물체를 나타낸 것이다.

도 53은 식물체의 타겟 위치를 도식화한 것이다.

도 54는 차세대 염기서열 분석법(Next-Generation Sequencing, NGS)을 통해 유전자 편집 후의 식물체의 핵산 서열을 분석한 것이다.

도 55는 변형된 크리스퍼 연관 단백질의 삽입-결실 효율을 나타낸 것이다.

도 56은 변형된 크리스퍼 연관 단백질의 삽입-결실 효율을 나타낸 것이다.

발명을 실시하기 위한 구체적인 내용

[0012] 이하 본원 발명을 상세히 설명한다.

[0013] 본 발명은 일 측면으로 크리스퍼 연관 단백질 및 엑소뉴클레아제인 RecJ를 포함하는 변형된 크리스퍼 연관 단백

질을 제공한다. 이때, 상기 변형된 크리스퍼 연관 단백질은 이하에서 크리스퍼 플러스 단백질이라고 지칭할 수 있다.

[0014] 본 명세서에서 사용된 용어, "크리스퍼 플러스 단백질"이란, 크리스퍼 연관 단백질의 유전자 편집 효율이 개선된 크리스퍼 연관 단백질을 지칭한다. 이때, 크리스퍼 연관 단백질의 편집 효율의 개선은 크리스퍼 연관 단백질 이외의 단백질을 결합시킴으로써 달성할 수 있다.

[0015] 상기 크리스퍼 플러스 단백질은 유전자 편집 기능이 향상되었다. 이때, 유전자 편집 기능이란 녹아웃(Knockout) 및 녹인(Knockin) 기능을 의미한다. 녹아웃은 크리스퍼 연관 단백질이 타겟 핵산 서열과 결합하였을 시에, 타겟 핵산의 일부를 결실시키거나, 타겟 핵산의 일부에 뉴클레오티드를 삽입한 경우를 의미한다. 또한, 녹인이란 외래 유전자가 타겟 부위에 삽입되는 것을 의미한다.

[0016] 상기 RecJ는 크리스퍼 연관 단백질의 N 말단 또는 C 말단에 결합될 수 있다. 또한, 크리스퍼 연관 단백질과 RecJ는 직접 결합될 수도 있다. 또한, 상기 크리스퍼 연관 단백질 및 RecJ는 링커 1을 통해 연결되는 것일 수 있다.

[0017] 구체적으로, 상기 크리스퍼 플러스 단백질은 다음 구조 중 어느 하나일 수 있다:

[0018] **<구조식 1>**

[0019] 크리스퍼 연관 단백질-RecJ

[0020] **<구조식 2>**

[0021] 크리스퍼 연관 단백질-링커 1-RecJ

[0022] **<구조식 3>**

[0023] RecJ-크리스퍼 연관 단백질

[0024] **<구조식 4>**

[0025] RecJ-링커 1-크리스퍼 연관 단백질

[0026] 상기 구조식 1 내지 4의 융합 단백질은 N 말단에서 C 말단으로 결합된 것을 나타낸다. 이때, 상기 구조식 1 내지 4에 표시된 링커 1은 1 내지 20개의 아미노산으로 구성된 것일 수 있다. 또한, 상기 링커 1은 5 내지 10개의 아미노산일 수 있으며, 6개, 7개, 8개, 9개, 10개의 아미노산으로 구성될 수 있다. 일 구체예의 링커 1은 서열번호 17, 18, 19, 20, 21, 22, 23 또는 24로 표시되는 아미노산을 가질 수 있다.

[0027] 본 명세서에서 사용된 용어, "크리스퍼 연관 단백질"은 Cas 효소, CRISPR 효소, CRISPR 단백질, Cas 단백질 및 CRISPR Cas는 일반적으로 상호교환적으로 사용될 수 있다.

[0028] 또한, 상기 크리스퍼 연관 단백질은 스트렙토코커스(Streptococcus), 캄필로박터(Campylobacter), 니트라티프락토르(Nitratifractor), 스타필로코커스(Staphylococcus), 파르비바쿨룸(Parvibaculum), 로세부리아(Roseburia), 네이세리아(Neisseria), 글루콘아세토박터(Gluconacetobacter), 아조스피릴룸(Azospirillum), 스파에로카에타(Sphaerochaeta), 락토바실러스(Lactobacillus), 유박테리움(Eubacterium), 코리네박터(Corynebacter), 카르노박테리움(Carnobacterium), 로도박터(Rhodobacter), 리스테리아(Listeria), 팔루디박터(Paludibacter), 클로스트리디움(Clostridium), 라크노스피라세아에(Lachnospiraceae), 클로스트리디아리디움(Clostridiaridium), 렙토트리키아(Leptotrichia), 프란시셀라(Francisella), 레지오넬라(Legionella), 알리사이클로바실러스(Alicyclobacillus), 메타노메티오필러스(Methanomethyophilus), 포르피로모나스(Porphyromonas), 프레보텔라(Prevotella), 박테로이데테스(Bacteroidetes), 헬코코커스(Helcococcus), 레토스피라(Letospira), 데설포비브리오(Desulfovibrio), 데설포나트로눔(Desulfonatronum), 오피투타세아에(Opitutaceae), 투베리바실러스(Tuberibacillus), 바실러스(Bacillus), 브레비바실러스(Brevibacilus), 메틸로박테리움(Methylobacterium) 또는 아시다미노코커스(Acidaminococcus)를 포함하는 속에서 유래된 것일 수 있다.

[0029] 또한, 상기 크리스퍼 연관 단백질은 Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9, Cas10, Cas12a, Cas12b, Cas12c, Cas12d, Cas12e, Cas13a, Cas13b, Cas13c, Cas13d, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3 및 Csf4로 구성된 군으로부터 선택되는 어느 하나 일 수 있다. 일 구체예로 상기 크리스퍼 연관 단백질은 Cas9 또는 Cas12a 일 수 있다. 일 실시

예로 Cas9은 SpyCas9일 수 있다. 또한, Cpf1은 FnCpf1, AsCpf1 또는 LbCpf1 일 수 있으며, 일 실시예로 Cpf1은 FnCpf1 일 수 있다. 상기 크리스퍼 연관 단백질은 종래에 알려진 서열과 95%, 96%, 97%, 98%, 99%의 상동성을 가질 수 있다.

[0030] 이때, 상기 RecJ는 엑소뉴클레아제로 알려진 단백질이다. 이때, 상기 RecJ의 일 구체예로는 에스케리치아(Escherichia) 속 유래의 RecJ(서열번호 1), 스트렙토코코스(Streptococcus) 속 유래의 RecJ(서열번호 3), 락토코코스(Lactococcus) 속 유래의 RecJ(서열번호 5), 바실러스(Bacillus) 속 유래의 RecJ(서열번호 7), 에르리치아(Ehrlichia) 속 유래의 RecJ(서열번호 9), 클라미디아(Chlamydia) 속 유래의 RecJ(서열번호 11), 피네골디아(Finegoldia) 속 유래의 RecJ(서열번호 13), 또는 스트렙토코코스(Streptococcus) 속 유래의 RecJ(서열번호 15)일 수 있다.

[0031] 본 발명은 또 다른 측면으로, 크리스퍼 연관 단백질, RecJ 및 DNA 결합 단백질(DNA binding protein, DBP)를 포함하는 크리스퍼 플러스 단백질 일 수다.

[0032] 상기 RecJ 및 DBP는 크리스퍼 연관 단백질의 N 말단 또는 C 말단에 결합될 수 있다. 또한, 이때, 상기 크리스퍼 연관 단백질, RecJ 및 DBP는 링커을 통해 연결되는 것일 수 있다. 이때, DBP는 SSB(single strand binding protein) 또는 DSB(double strand binding protein) 일 수 있다. 상기 크리스퍼 플러스 단백질의 일시예는 다음 구조 중 어느 하나일 수 있다:

[0033] **<구조식 5>**

[0034] DBP-크리스퍼 연관 단백질-RecJ

[0035] **<구조식 6>**

[0036] DBP-링커 2-크리스퍼 연관 단백질-RecJ

[0037] **<구조식 7>**

[0038] DBP-링커 2-크리스퍼 연관 단백질-링커 1-RecJ

[0039] 상기 구조식 5 내지 7의 융합 단백질은 N 말단에서 C 말단으로 결합된 것이다. 크리스퍼 연관 단백질, RecJ 및 DBP는 직접 결합될 수도 있으며, 링커를 통해 연결될 수 있다. 이때, 링커 1은 상술한 바와 같다. 또한, 상기 구조식 5 내지 7에 표시된 링커 2는 1 내지 20개의 아미노산으로 구성된 것일 수 있다. 또한, 상기 링커 2는 5 내지 10개의 아미노산일 수 있으며, 6개, 7개, 8개, 9개, 10개의 아미노산으로 구성될 수 있다. 일 구체예의 링커 2는 서열번호 17, 18, 19, 20, 21, 22, 23 또는 24로 표시되는 아미노산 서열을 가질 수 있다.

[0040] 본 발명은 또 다른 측면으로, 크리스퍼 연관 단백질 및 RecJ를 포함하는 크리스퍼 플러스 단백질 및 크리스퍼 연관 단백질, RecJ 및 DBP를 포함하는 크리스퍼 플러스 단백질에 추가적으로 추가적으로 핵 위치화 시그널(nuclear localization signal, NLS)을 적어도 하나를 더 포함하는 크리스퍼 플러스 단백질 일 수 있다. 이때, 핵 위치화 시그널은 bpNLS(biparticle nuclear localization sequence)일 수 있다. 적어도 하나 이상의 NLS가 C-말단 또는 N-말단 부착될 수 있다. 이때, 일 구체예에서는 상기 핵 위치화 시그널은 융합 단백질의 C 말단에 위치할 수 있다. 상기 핵 위치화 시그널은 크리스퍼 플러스 단백질의 활성에는 영향을 미치지 않으나, 크리스퍼 플러스 단백질이 세포의 핵내로 이동할 수 있도록 도와주는 펩타이드를 의미한다.

[0041] 또한, 본 발명의 다른 측면은, 상기 크리스퍼 플러스 단백질에 추가적으로 추가적으로 His tag를 더 포함할 수 있다. 이때, 상기 His tag는 크리스퍼 플러스 단백질의 C 말단에 위치할 수 있다. 상기 His tag는 크리스퍼 플러스 단백질의 활성에는 영향을 미치지 않으나, 크리스퍼 플러스 단백질의 분리 정제에 사용될 수 있다.

[0042] 또한, 상기 크리스퍼 플러스 단백질은 서열번호 31, 41, 49, 53, 57, 71 또는 77으로 표시되는 아미노산 서열을 가지는 것 일 수 있다.

[0043] 본 명세서에서 사용된 용어, "PAM"은 프로토스페이서 인접 모티프(protospacer adjacent motif) 또는 PAM-유사 모티프는 크리스퍼 연관 단백질 복합체가 타겟 유전자좌에 결합하는 것을 지시한다.

[0044] 본 발명의 일 구체예에서, 크리스퍼 연관 단백질은 FnCpf1과 관련이 있는 PAM은 5' TTN이며, 여기서 N은 A/C/G 또는 T이다. PAM은 타겟 유전자의 5' 말단의 상류에 위치할 수 있다. Cpf1 패밀리의 T-풍부 PAM은 AT-풍부 게놈의 표적화 및 편집을 가능하게 한다. 반면, Cas9 단백질의 PAM은 타겟 유전자의 3' 말단 하류에 위치할 수 있다.

[0045] 본 발명은 또 다른 측면으로, 상술한 크리스퍼 플러스 단백질을 코딩하는 폴리뉴클레오티드를 제공한다.

[0046] 상기 뉴클레오티드는 신호서열(signal sequence) 또는 리더 서열(leader sequence)을 추가적으로 포함할 수 있다. 여기에서 사용된 용어 "신호서열"은 목적 단백질의 분비를 지시하는 신호펩타이드를 코딩하는 핵산을 의미한다. 상기 신호펩타이드는 숙주 세포에서 번역된 후에 절단된다. 구체적으로, 본원 발명의 신호서열은 ER(endoplasmic reticulum) 막을 관통하는 단백질의 이동을 개시하는 아미노산 서열을 코딩하는 뉴클레오티드일 수 있다.

[0047] 신호서열은 당업계에 그 특징이 잘 알려져 있으며, 통상 16 내지 30개의 아미노산 잔기를 포함하나, 그보다 더 많거나 적은 아미노산 잔기를 포함할 수 있다. 통상적인 신호 펩타이드는 기본 N-말단 영역, 중심 소수성 영역, 및 보다 극성인(polar) C-말단 영역의 세 영역으로 구성된다. 중심 소수성 영역은 미성숙 폴리펩티드가 이동하는 동안 막지질 이중층을 통하여 신호서열을 고정시키는 4 내지 12개의 소수성 잔기를 포함할 수 있다.

[0048] 이때, 상기 크리스퍼 플러스 단백질을 코딩하는 뉴클레오티드의 일 실시예는 서열번호 32, 42, 50, 54, 58, 72 또는 72로 표시되는 서열을 가질 수 있다.

[0049] 본 발명의 또 다른 측면으로, 상기 폴리뉴클레오티드가 적재된 벡터를 제공한다.

[0050] 본원 발명에서 사용된 용어 "벡터"는 숙주 세포에 도입되어 숙주 세포 유전체 내로 재조합 및 삽입될 수 있다. 또는 상기 벡터는 에피좀으로서 자발적으로 복제될 수 있는 뉴클레오티드 서열을 포함하는 핵산 수단으로 이해된다. 상기 벡터는 선형 핵산, 플라스미드, 파지미드, 코스미드, RNA 벡터, 바이러스 벡터 및 이의 유사체들을 포함한다. 바이러스 벡터의 예로는 레트로바이러스, 아데노바이러스, 및 아데노-관련 바이러스를 포함하나 이에 제한되지 않는다. 또한, 상기 플라스미드는 항생제 내성 유전자와 같은 선별 마커를 포함할 수 있고, 플라스미드를 유지하는 숙주 세포는 선택적인 조건하에서 배양될 수 있다.

[0051] 본 발명의 또 다른 측면으로, 상기 벡터가 도입된 숙주 세포를 제공한다.

[0052] 상기 숙주 세포는 벡터를 도입하여 본원의 크리스퍼 플러스 단백질을 생산할 수 있다면 종류에 한정되는 것은 아니다. 일 구체예로 상기 숙주세포는 대장균일 수 있다.

[0053] 본 발명의 또 다른 측면으로, 상기 크리스퍼 플러스 단백질 및 crRNA가 결합된 RNP 복합체를 제공한다.

[0054] 일 구체예에서, 상기 복합체의 핵산 성분는 직접 반복 서열에 연결된 가이드 서열을 포함할 수 있다. 상기 직접 반복 서열은 하나 이상의 줄기 루프(stem loop) 또는 최적화된 2차 구조를 포함할 수 있다. 바람직한 구현예에서, 직접 반복은 최소한의 길이가 16 nt이고 단일 줄기 루프를 가질 수 있다. 추가 구현예에서, 직접 반복은 길이가 16 nt보다 더 길며, 바람직하게는 17 nt 초과이고, 하나 초과의 줄기 루프 또는 최적화된 2차 구조를 가질 수 있다. 바람직한 구현예에서, 직접 반복은 변형되어 하나 이상의 단백질-결합 RNA 압타머를 포함할 수 있다.

[0055] 이때, 상기 crRNA는 타겟 유전자에 상보적으로 결합할 수 있는 핵산 서열을 갖는다. 이때, 타겟 유전자는 세포 내 DNA 분자에 포함될 수 있다. 세포는 원핵 세포 또는 진핵 세포일 수 있다. 세포는 포유동물 세포일 수 있다. 포유동물 세포는 비인간 영장류, 소, 돼지, 설치류 또는 마우스 세포일 수 있다. 세포는 비포유동물 진핵 세포, 예컨대 가금류, 어류 또는 새우일 수 있다. 세포는 또한 식물 세포일 수 있다. 식물 세포는 카사바, 옥수수, 수수, 밀, 담배, 애기장대, 채소 또는 쌀 일 수 있다.

[0056] 본 발명에 의해 세포에 도입된 변형은, 세포 및 세포 의 자손이 생물학적 생성물, 예컨대 항체, 전분, 알코올 또는 기타 다른 원하는 세포 산출물의 개선된 생성을 위하여 변경되도록 하는 것일 수 있다. 본 발명에 의해 세포에 도입된 변형은 세포 및 세포의 자손이 생성된 생물학적 생성물을 변화시키는 변경을 포함하도록 하는 것일 수 있다.

[0057] 또한, 본 발명의 또 다른 측면으로, 상기 RNP 복합체를 유효성분으로 포함하는 유전자 편집용 조성물 또는 유전자 편집용 키트를 제공한다.

[0058] 이하, 본원 발명을 하기 실시예에 의하여 더욱 상세하게 설명한다. 단, 하기 실시예는 본원 발명을 예시하기 위한 것일 뿐, 본원 발명의 범위가 이들만으로 한정되는 것은 아니다.

[0059] **I. 크리스퍼 플러스 단백질의 제조 및 활성 확인**

[0060] **제조예 1. Cpf1 및 RecJ외의 다른 변형된 단백질이 결합된 변형된 크리스퍼 연관 단백질 제조**

[0061] 이콜라이 단백질 발현 벡터 pET28a (addgene, Watertown, MA, USA) 를 NotI 제한 효소를 사용하여서 FnCpf1

(3,902 bp)의 정방향 프라이머 (CGTCGACAAGCTTGCGGCCGCATGTCAATTTATCAAGAATT) 와 역방향 프라이머 (TGGTGGTGCTCGAGTGCGGCCGCTTgGTTATTCCTATTCTGCAC) 를 이용하여 pY002 (addgene, Watertown, MA, USA)를 중합효소 연쇄반응으로 얻은 산물을 깁슨 어셈블리 킷트 (NEB, Ipswich, MA, USA) 방법으로 50도에 25분 인큐베이션으로 통하여 클로닝하였다. 결과적으로 얻어진 pET28a-FnCpf1의 말단에 PspXI 제한효소를 사용하여서 RecJ (1,731 bp), RecE (2,598 bp), SSB (348 bp)는 이콜라이 지놈에서 증폭하였고, mungbean (1,065 bp)은 숙주 나물에서 mRNA 산물을 얻어서 cDNA로 전환한 다음 코딩 영역을 얻을 수 있었다. T5 (828 bp), DSB (267 bp)는 GenScript (Piscataway, NJ, USA)에서 합성하여서 사용하였다. GFP (714 bp) 서열은 생명공학연구원에서 개발한 pCTV-GFP 벡터에서 증폭하여서 사용하였고, hTdT (1,527 bp)는 GenScript에서 DNTT(NM_004088) ORF Clone을 주문하여서 증폭하였다. 증폭된 중합효소반응 산물은 깁슨 어셈블리 방법을 통해서 Cpf1의 말단에 삽입되었다.

표 1

[0062]

name	sequence	size	function
RecJF1	GCGGCCGCACTCGACctgcagGTGAAACAACAGATACAACTTCGT	45	RecJ full-length in back
RecJR1	ccggccttttcgtggccgccggccttttctgcagAATTGGCCAGATATTGTCGATGATA	60	RecJ full-length in back
RecJR2	TCAGTGGTGGTGGTGGTGctttttctttttgcctggccggccttttcgtggccgc	60	RecJ full-length in back
MungbeanF1	CGGCCGCACTCGACctgcagATGCAAACGTTACAGATGAGT	41	Mungbean Nuclease in back
MungbeanR1	cgtggccgccggccttttctgcagACCATTGTAAGAGATAGGTC	44	Mungbean Nuclease in back
T5exonuF1	CGGCCGCACTCGACctgcagATGGCTTCCCGTCGTAATCTAATG	44	T5 exonuclease in back
T5exonuR5	gtggccgccggccttttctgcagTTGTTCTGCAATCTCCAAAATA	45	T5 exonuclease in back
SSBF1	GCGGCaGCACTCGACctgcagATGGCCAGCAGAGGCGTAAACAAGG	46	SSB isolation in back
SSBR1	tcgtggccgccggccttttctgcagACGACCACCCAGCATCTGCATGGT	49	SSB isolation in back
RecEF1	GCGGGCGCACTCGACctgcagATGAGCACAAAACCACTCTTCC	43	RecE in back
RecER1	ttcgtggccgccggccttttctgcagGTCATTTGCATATTCCTTAGCCCA	50	RecE in back
DSBF1	AGCGGCCGCACTCGACctcgagATGGCTAAAAAAGAAATGGTTGAATT	21	with DSBR1 in back
DSBR1	ctttttcgtggccgccggccttttctcgagTTTAGAGAAAACTGTGTCA	37	with DSBF1 in back
T5exoF5	GGTGGACAGCAAATGGGTCGCGGATCCATGGCTTCCCGTCGTAATCTAA	49	T5 full-length in back
T5exoR5	GCTTGTCGACGGAGCTCGAATTCGGATCCTTGTTCTGCAATCTCCAAA	48	T5 full-length in back
pAGROF2	gtctcagctgggaggcgacgaaATGAGTAAAGGAGAAGAACTTTT	45	GFP in back
pAGROR2	gcggaggggttggatcaaagtgaaAATAACCTCTCCTTCTTTTTC	45	GFP in back
HsTdTF2	GCGGCCGCACTCGACctcgagATGGATCCACCACGAGCGTCCCAC	45	HsTdT full length in back
HsTdTR2	ttcgtggccgccggccttttctcgagGGCATTTCTTTCCCACGGTTCAATAT	52	HsTdT full length in back

[0063] **제조예 2. 타겟 sgRNA 또는 crRNA의 제작**

[0064] **제조예 2.1. CCR5 및 DHCR7을 타겟팅하는 sgRNA의 제작**

[0065] CCR5 및 DHCR7을 타겟팅하며, SpyCas9와 함께 이용될 수 있는 sgRNA를 제작하였다. sgRNA 결합사이트는 도면 13에 나타낸 바와 같다. 인간 CCR5(A) 및 인간 DHCR7(B)에 대한 SpyCas9의 sgRNA 결합 영역을 각각 노란색과 핑크색으로 나타낸 것이다. PAM 서열은 녹색으로 표시하였다. 화살표는 뉴클레아제 절단 위치를 나타낸다.

[0066] **제조예 2.2. CCR5 및 DHCR7을 타겟팅하는 crRNA의 제작**

[0067] CCR5 및 DHCR7을 타겟팅하며, FnCpf1과 함께 이용될 수 있는 crRNA를 제작하였다. crRNA 결합사이트는 도면 14에 나타낸 바와 같다. 인간 CCR5(A) 및 인간 DMNT1(B)에 대한 FnCpf1의 crRNA 결합 영역을 각각 붉은색과 오렌지색으로 나타낸 것이다. PAM 서열은 파란색으로 표시하였다. 화살표는 뉴클레아제 절단 위치를 나타낸다.

[0068] **실시예 1. Cas9 및 RecJ가 결합된 변형된 크리스퍼 연관 단백질 제조**

[0069] **실시예 1.1. SpCas9 제조**

[0070] 이콜라이 단백질 발현 벡터 pET28a를 NotI 제한 효소를 사용하여서 SpCas9(4,104 bp)의 정방향 프라이머(CCGTCGACAAGCTTGCGGCCGCATGGACAAGAAGTACAGCATCGGCCTGGA) 와 역방향 프라이머(TGAGCCAGCTGGGCGGCGACGCGGCCGCACTCGACctcgagaaaaggccggcggccacgaaaaaggccggccaggcaaaaaagaaaaagCACCACCACCACCACCACTGA) 를 이용하여 pET28a/Cas9-cys (addgene, Watertown, MA, USA)에서 중합효소 연쇄반응으로 얻은 산물을 깁슨 어셈블리 킷트 (NEB, Ipswich, MA, USA) 방법으로 50도에 25분 인큐베이션으로 통하여 클로닝하였다. 서열 정보는 도42-도50에 명시하고 있다.

[0071] **실시예 1.2. RecJ이 결합된 SpCas9의 제조**

[0072] 결과적으로 얻어진 pET28a-SpCas9의 말단에 PspXI 제한효소를 사용하고 SpCas9 앞쪽에는 BamHI 제한 효소를 사용하여서 RecJ (1,731 bp), RecE (2,598 bp), SSB (348 bp)는 이콜라이 지놈에서 증폭하였고, mungbean (1,065 bp)은 숙주 나물에서 mRNA 산물을 얻어서 cDNA로 전환한 다음 코딩 영역을 얻을 수 있었다. T5 (828 bp), DSB (267 bp)는 GenScript (Piscataway, NJ, USA)에서 합성하여서 사용하였다. GFP (714 bp) 서열은 생명공학연구원에서 개발한 pCTV-GFP 벡터에서 증폭하여서 사용하였고, hTdT (1,527 bp)는 GenScript에서 DNTT(NM_004088) ORF Clone을 주문하여서 증폭하였다. 증폭된 중합효소반응 산물은 깁슨 어셈블리 방법을 통해서 50도에 25분 인큐베이션을 통해서 SpCas9의 말단과 상단에 각각 삽입되었다.

표 2

[0073]

name	sequence	size	function
RecJF2	GGACAGCAAATGGGTCGCGGATCCGTGAAACAACAGATACAACTTCGT	45	RecJ full-length in front
RecJR2	TTGTCGACGGAGCTCGAATTCGGATCCAATTGGCCAGATATTGTCGATGATA	60	RecJ full-length in front
MungbeanF2	GGACAGCAAATGGGTCGCGGATCCATGCAAACGTTACAGATGAGT	41	Mungbean Nuclease in front
MungbeanR2	TTGTCGACGGAGCTCGAATTCGGATCCACCATTGTAAGAGATAGGTC	44	Mungbean Nuclease in front
T5exonuF2	GGACAGCAAATGGGTCGCGGATCCATGGCTTCCCGTCGTAATCTAATG	44	T5 exonuclease in front
T5exonuR6	TTGTCGACGGAGCTCGAATTCGGATCCTTGTTCTGCAATCTCCAAAATA	45	T5 exonuclease in front
SSBF2	GGACAGCAAATGGGTCGCGGATCCATGGCCAGCAGAGGCGTAAACAAGG	46	SSB isolation in front
SSBR2	TTGTCGACGGAGCTCGAATTCGGATCCACGACCACCCAGCATCTGCATGGT	49	SSB isolation in front

RecEF2	GGACAGCAAATGGGTCGCGGATCCATGAGCACAAAACCACTCTTCC	43	RecE in front
RecER2	TTGTCGACGGAGCTCGAATTCGGATCCGTCATTTGCATATTCCTTAGCCCA	50	RecE in front
DSBF2	GGACAGCAAATGGGTCGCGGATCCATGGCTAAAAAAGAAATGGTT	21	with DSBR1 in front
DSBR2	TTGTCGACGGAGCTCGAATTCGGATCCTTTAGAGAAAACTGTGT	37	with DSBF1 in front
pAGROF3	gtctcagctgggaggcgacgaaATGAGTAAAGGAGAAGAACTTTT	45	GFP in front
pAGROR3	TTGTCGACGGAGCTCGAATTCGGATCCAATAACCTCTCCTTCTTTTTC	45	GFP in front
HsTdTF3	GCGGCCGCACTCGACctcgagATGGATCCACCACGAGCGTCCCAC	45	HsTdT full length in front
HsTdTR3	TTGTCGACGGAGCTCGAATTCGGATCCGGCATTTCTTTCCCACGGTTCAATAT	52	HsTdT full length in front
RecJF1	GCGGCCGCACTCGACctgcagGTGAAACAACAGATACAACTTCGT	45	RecJ full-length in back
RecJR1	ccggccttttt cgtggccgccggccttttctgcagAATTGGCCAGATATTGTCGATGATA	60	RecJ full-length in back
RecJR2	TCAGTGGTGGTGGTGGTGctttttctttttgcctggccggccttttt cgtggccgc	60	RecJ full-length in back
MungbeanF1	CGGCCGCACTCGACctgcagATGCAAACGTTACAGATGAGT	41	Mungbean Nuclease in back
MungbeanR1	cgtggccgccggcctttt ctgcagACCATTGTAAGAGATAGGTC	44	Mungbean Nuclease in back
T5exonuF1	CGGCCGCACTCGACctgcagATGGCTTCCCGTCGTAATCTAATG	44	T5 exonuclease in back
T5exonuR5	gtggccgccggcctttt ctgcagTTGTTCTGCAATCTCCAAAATA	45	T5 exonuclease in back
SSBF1	GCGGCaGCACTCGACctgcagATGGCCAGCAGAGGCGTAAACAAGG	46	SSB isolation in back
SSBR1	tcgtggccgccggcctttt ctgcagACGACCACCCAGCATCTGCATGGT	49	SSB isolation in back
RecEF1	GCGGGCGCACTCGACctgcagATGAGCACAAAACCACTCTTCC	43	RecE in back
RecER1	ttcgtggccgccggcctttt ctgcagGTCATTTGCATATTCCTTAGCCCA	50	RecE in back
DSBF1	AGCGGCCGCACTCGACctcgagATGGCTAAAAAAGAAATGGTTGAATT	21	with DSBR1 in back
DSBR1	ctttttcgtggccgccggcctttt ctcgagTTTAGAGAAAACTGTGTCA	37	with DSBF1 in back
T5exoF5	GGTGGACAGCAAATGGGTCGCGGATCCATGGCTTCCCGTCGTAATCTAA	49	T5 full-length in back
T5exoR5	GCTTGTCGACGGAGCTCGAATTCGGATCCTTGTTCTGCAATCTCCAAA	48	T5 full-length in back
pAGROF2	gtctcagctgggaggcgacgaaATGAGTAAAGGAGAAGAACTTTT	45	GFP in back
pAGROR2	gcggaggggttggatcaaagtgaaAATAACCTCTCCTTCTTTTTC	45	GFP in back
HsTdTF2	GCGGCCGCACTCGACctcgagATGGATCCACCACGAGCGTCCCAC	45	HsTdT full length in back
HsTdTR2	ttcgtggccgccggcctttt ctcgagGGCATTTCTTTCCCACGGTTCAATAT	52	HsTdT full length in back

[0074] **실시예 2. Cpf1 및 RecJ가 결합된 변형된 크리스퍼 연관 단백질 제조**

[0075] **실시예 2.1. FnCpf1 제조**

[0076] 이콜라이 단백질 발현 벡터 pET28a를 NotI 제한 효소를 사용하여서 FnCpf1 (3,902 bp)의 정방향 프라이머 (CGTCGACAAGCTTGCGGCCGCATGTCAATTTATCAAGAATT) 와 역방향 프라이머 (TGGTGGTGCTCGAGTGCGGCCGCTTgGTTATTCCTATTCTGCAC) 를 이용하여 pY002 (addgene, Watertown, MA, USA) 을 중합효소 연쇄반응으로 얻은 산물을 깁슨 어셈블리 킷트 (NEB, Ipswich, MA, USA) 방법으로 50도에 25분 인큐베이션으로 통하여 클로닝하였다. 서열 정보는 도42-도50에 명시하고 있다.

[0077] **실시예 2.2. RecJ이 결합된 변형된 FnCpf1의 제조**

[0078] 해당 플라스미드를 지닌 대장균 세포를 카나마이신 항생제가 포함된 LB 배지에 접종하여 37℃에서 밤새 배양한다. 다음 날 배양액을 LB 20 mL에 접종하여 2~3시간 키운 뒤 이를 LB 1L에 넣어 OD_{600}이 0.5~0.7이 될 때까지 37℃에서 배양한다. 1 mM IPTG를 넣고 18℃에서 20시간 배양한다. 다음 날 배양액을 4000 rpm에서 30분간 원심분리하여 상층액은 버리고 펠릿에 용해 완충액(lysis buffer, 5 mM imidazole, 20 mM Tris, 500 mM sodium chloride, 1 mM DTT, 1 mM PMSF, pH 8.0)을 넣어 현탁한다. 현탁액을 초음파-파쇄(sonication)한 후 15000 xG에서 1시간 동안 원심분리하여 상층액만을 분리하고 히스티딘 태깅 컬럼을 사용한 FPLC에 주입하여 단백질을 분리한다. 분리한 단백질 분획을 다시 탈염(desalting) 컬럼을 사용한 FPLC에 주입하여 단백질을 분리 후 해당 분획을 농축한다. 농축된 단백질을 Bradford assay로 정량한 뒤 -80℃에 분주하여 보관한다. 변형된 FnCpf1의 구체예를 도 12에 나타내었다.

[0079] **실험예 1. 변형된 Cas9 단백질의 절단 효율 확인 : 선형 dsDNA**

[0080] 단백질과 gRNA는 몰농도 비율이 1:1.2가 되도록 준비하였다. 반응 부피 20 μL로 하여 단백질 25 nM에 gRNA 30 nM을 섞은 후 20분 상온에 두고 RNP가 형성되도록 하였다. SpCas9-BPNLS의 분자량을 기준으로 각 단백질의 분자량을 반영하여 넣어줄 단백질의 양을 계산하였다. SpCas9-BPNLS의 분자량은 166.580 kDa이며 25 nM에 해당하는 양은 83.5 g이다. gRNA는 CCR5 유전자를 타겟으로하며 약 33,500 g/mol의 농도를 가지고 있고 30 nM에 해당하는 양은 20 ng이다. 타겟 유전자인 CCR5 주형의 크기는 약 1.4 kb이다.

[0081] 1.5 kb 크기의 타겟 유전자인 CCR5 주형 DNA 500 ng을 RNP 복합체와 섞은 후 37℃에서 1시간 반응시켰다. 2% agarose gel에 전기영동한 후 겔 이미지를 수득하였다. 이를 이용하여 주형 DNA와 RNP 복합체가 작용하고 남은 주형 DNA의 비율을 계산하여, 단백질의 뉴클레아제 활성 효율을 수득하였다. 절단 후에는 약 600 bp 정도의 밴드가 나타났다(도 1).

[0082] **실험예 2. 변형된 Cas9 단백질의 절단 효율 확인 : 원형 dsDNA**

[0083] TAAGG 또는 AAAC 오버행을 가지는 목표서열 20개의 정방향과 역방향 단일 가닥 올리고뉴클레오타이드 2개로 이루어져 있으며, 95℃에서 5분 후, 55℃에서 10분의 인큐베이션하여 올리고뉴클레오타이드가 이차구조를 가지지 않도록 하면서 20개의 정방향과 역방향의 올리고뉴클레오타이드의 단일 가닥을 상보적으로 결합시켜서 20개 베이스페어를 가지는 이중 가닥의 선형으로 정렬시켰다. 이중 가닥의 올리고뉴클레오타이드는 Cas9에 대한 gRNA 지지체를 포함하는 클로닝 벡터의 2개 BsaI 사이트 (A↓TAGGTGAGACCGCAGGTCTCG↓GTTTT)에 클로닝하였다.

[0084] 결과적으로, sgRNA는 pGEM_447 vector(서열번호 102)를 목표로하는 20개의 목표서열 (목표서열; AAGCCCGGCCGAAATGACCA) 이 T7 프로모터와 sgRNA 지지체 사이에 위치하는 클로닝 벡터에서 T7 프로모터를 주형으로 하는 정방향 프라이머 (정방향 프라이머 서열; ATTCTAATACGACTCACTATAGG)와 sgRNA 지지체의 마지막 20 개의 서열을 주형으로 하는 역방향 프라이머 (역방향 프라이머 서열; GCACCGACTCGGTGCCACTT)를 사용하여서 중합효소 연쇄방반응으로 얻어지는 PCR 산물을 MEGAshortscript™ High Yield Transcription Kit(Invitrogen, Grand Island, NY)를 이용하여 RNA 산물인 sgRNA 산물을 얻었다. RNA 산물은 DNase I (NEB, Ipswich, MA)을 이용하여 dsDNA 주형을 제거하기 위해 절단하였고, MEGAclear™ 전사 Clean-Up kit (Invitrogen, Grand Island, NY)를 이용하여 정제하였다.

[0085] 절단 시험 전의 Cas9-sgRNA 복합체는 NEB2.1 완충액(NEB, Ipswich, MA)을 이용하여 37℃에서 10분간 배양하였다. 절단 시험은 20 μL의 반응 내 0.2-1.6 μg Cas9 단백질, 500 ng sgRNA 및 500 ng dsDNA 기질로 구성되어 있으며, 37℃에서 0 내지 180분 동안 수행되었다. 절단된 dsDNA는 40분 동안 DNA 전기 영동으로 확인하였다(도

2).

[0086] **실험예 3. 시간에 따른 변형된 Cas9 단백질의 편집 효율 확인**

[0087] HEK293T 세포를 10% 태아 소혈청인 FBS와 P/S가 함유된 DMEM 배지에서 37℃, 5% CO_2 배양기에서 유지시켰다. 트랜스펙션 전날 150,000 세포를 24 well 플레이트로 옮겼다. Cas9 변이 단백질을 세포에 트렌스펙션 시키기 위해 각각 정제된 Cas9 변이체 단백질을 25 ㎕ Opti-MEM 혈청 환원 배지에 첨가한 후 120 ng의 가이드 RNA를 첨가하였다. Cas9 단백질에 대한 가이드 RNA의 몰비는 거의 1.2 : 1의 비율로 유지하였다. 혼합물을 부드럽게 두드린 후 잘 혼합하여 10분 동안 실온에서 배양하였다. 다른 튜브에는 2 ㎕의 lipofectamine 3000을 25 ㎕ Opti-MEM 혈청 환원 배지에 넣어주었고, 희석된 25 ㎕의 시약은 가이드 RNA와 Cas9 단백질 복합체에 넣고 15분 동안 상온에서 배양한 뒤 총 50 ㎕의 혼합물을 세포에 넣어주었다.

[0088] 게놈 DNA 추출은 제조사의 지시에 따라 PureLink Genomic DNA 키트를 사용하여 진행하였다. 간단하게 세포를 PBS로 1회 세척하고 트립신으로 37℃에서 5분 동안 인큐베이션하였다. 그 후, 세포를 얻기 위해 1.2 ml의 성장 배지를 세포에 첨가 한 후 상온, 250xg에서 5분 동안 스핀다운시켰다. 게놈상 인간 DHCR7의 유전자 증폭을 위해, 두 개의 상이한 PCR 프라이머 쌍이 사용되었다. 전방 프라이머 1: 5'-CAGTAGAGCAGGCATGTTGAGT-3'와 역방향 프라이머 1 : 5'-GTGAAGGTGTATCAAACGCTGA-3' 를 사용하였고, 이는 T7E1 분석 후 크기가 각각 427 및 205의 절단된 2개의 DNA 밴드를 나타낸다. 반면, 다음의 다른 프라이머 쌍을 사용할 때는 절단된 단일 DNA 단편만 생성된다 : 정방향 프라이머 2 : 5'-GGGAAACCACTGGCCTTGG-3'; 역방향 프라이머 2 : 5'-GAGCCAGGATCCATGTCCCA-3'.

[0089] 게놈 DNA를 증폭 후 Wizard® SV 젤 및 PCR 클린업 시스템을 사용하여 정제하였다. 200 ng의 DNA를 PCR 튜브에 넣고 NEB 완충액 2번을 1 ㎕와 증류수 9 ㎕를 넣었다. 이후, 혼합물을 다음 조건을 사용하여 PCR 기계에서 재구성하였다. 98℃에서 5분 동안 변성 후 -2℃/초로 85℃ 온도를 낮추고 -0.1℃/초로 25℃까지 온도를 낮춘다. 이후 이형이중가닥의 변성을 막기 위해 4℃로 유지시킨다. 1 ㎕의 T7E1 엔도뉴클레아제를 혼합물에 첨가하고 37℃에서 60분 동안 배양한다.

[0090] 유전자 변형의 분석을 위해, 2% 겔을 사용하여 이미지를 얻고, Alphaimager 2200 이미지 분석 소프트웨어를 분석에 사용하였다. 유전자 변형에 대한 비율은 1- [(1-fraction cleaved) 1/2]식을 이용하여 구했다. 그 결과를 도 3에 나타내었다.

[0091] **실험예 4. 인간 유전자 CCR5 및 DHCR7에 대한 SpyCas9, SpyCas9-RecJ 및 SpyCas9-T5의 HDR(Homology Dependent Repair) 효율 분석**

[0092] HEK293T 세포를 10% 태아 소혈청인 FBS와 P/S가 함유된 DMEM 배지에서 37℃, 5% CO_2 배양기에서 유지시켰다.

HEK293T 세포를 $3x10^6$ 밀도로 10 cm 배양접시에 배양하였다. 세포의 증식 정도는 70% 이하로 유지하였다. 이후 형질전환 16시간 전 200 ng/ml의 노코다졸(Sigma)을 세포에 처리하였고, 동기화된 세포를 유전자 도입 실험에 사용하였다.

[0093] 외부 유전자인 ssODN(single-stranded oligodeoxynucleotides)을 도입시키기 위해 $2x10^5$의 HEK293T 세포를 형질전환 실험에 이용하였다. 각각 33 pmole의 SpCas9, SpCas9-RecJ, SpCas9-T5 단백질과 66 pmole의 CCR5 sgRNA를 이용하였다. 도입 유전자로는 ssODN 20 pmole을 사용하였다. 형질전환을 위해 Lonza사의 4D-Nucleofector 기기를 이용한 전기천공법을 사용하였다. 24시간 후 invitrogen사의 PureLink™ Genomic DNA Mini Kit를 사용하여 genomic DNA를 추출하였다.

[0094] 편집된 부분을 가지고 있는 gDNA와 그렇지 않은 gDNA 샘플을 이용하여 표적 부위를 증폭하였다. 이후 일루미나의 프로토콜대로 PCR과 정제 과정을 거친 후 MiniSeq 장비를 이용하여 표적 부위에 대한 딥 시퀀싱(deep sequencing) 분석을 진행하였다. HDR 분석 결과는 도 4에 나타내었다. 이때, 분석에 이용된 프라이서 서열은 표 3에 기재된 바와 같다.

표 3

[0095]

Proteins	Genes	Adapter primer sequences (5' -3')
SpyCas9	CCR5	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGAGGGCAACTAAATACATTCT (서열번호 81)
		GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAACACCAGTGAGTAGAGCGG (서열번호 82)
	DHCR7	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGTTTGAGCAACAGTTCTCC (서열번호 83)
		GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGACGATGTCCACCACAG (서열번호 84)

FnCpf1	CCR5	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGTATTTCTGTTCAGATCAC (서열번호 85)
		GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCCCATCAATTATAGAAAGCC (서열번호 86)
	DNMT1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTGCACACAGCAGGCCTTTG (서열번호 87)
		GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCCAATAAGTGGCAGAGTGC (서열번호 88)

[0096] **실험예 5. SpCas9 및 SpCas9-RecJ에 의한 오프-타겟 효과 확인**

[0097] SpCas9과 SpCas9-RecJ 단백질을 이용하여 인간 CCR5 유전자를 표적으로 했을 때 표적 부분 외 다른 부분에 대한 영향을 알아보기 위해 스페이서(spacer) 서열 부분이 유사한 다음의 4가지 유전자를 선택하였고 서열은 하기 표 4와 같다.

표 4

[0098]

Target	Gene	Spacer sequence
On	CCR5	TGACATCAATTATTATACAT (서열번호 89)
off#1	ADCY5	TGACATCAATTATTATAgAT (서열번호 90)
off#2	KCNJ6	TGACATCAcTTATTATgCAT (서열번호 91)
off#3	CNTNAP2	TGACATaAATTATTcTACAT (서열번호 92)
off#4	Chr. 5 N/A	TGAaATCAATTATcATAgAT (서열번호 93)

[0099] Mock, SpCas9, SpCas9-RecJ를 처리한 gDNA로부터 표적 외 부분 ADCY5, KCNJ6, CNTNAP2, Chr.5 N/A에 대한 각각의 프라이머를 이용하여 증폭시켰다. 이후 정제 과정과 제조사의 지시에 따라 시퀀싱 라이브러리를 제조하였고 일루미나 MiniSeq 장비를 이용하여 표적 부위에 대한 딥 시퀀싱 분석을 진행하였다. 그 결과를 도 5 및 도 6에 나타내었다. 이때, 분석에 이용된 프라이서 서열은 표 5에 기재된 바와 같다.

표 5

[0100]

Gene	Target specific primer sequences	Adapter primer sequences
ADCY5	GTCCCATGACAGGCGTGTAT	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCAAGAGTGTAGAGGAGGACAG
	GCTCCCACCTTAGTGCTCTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAAAAGGAGATGCTTGGCAC
KCNJ6	TGGAGCCATTGGTTTGCATC	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAAGACCAAAAAACCTCATGGAT
	GCCTGGCCAAGTTTCAGTTA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTTTGAGGAAGACAAGTCATGT
CNTNAP2	TGCAGTGCAGACTCTTTCCA	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGCAGACTCTTTCCATACTGT
	AAGGACACAGGGCAACTGAA	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAAATCAAATTTCATGTGTCCA
Chr. 5 N/A	TGTGGAACGAGTGGTGACAG	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCAGAAGTTCCTGAAATTATCC
	GACCAAACCACATTCTTCTCAC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACATGAGAGATAGTGATATGT

[0101] **실시예 6. Cas9 및 Cas9-RecJ의 PD-1 및 CCR5에 대한 온-타겟 효과 확인**

[0102] 인간 PD-1 및 CCR5의 동일한 유전자 자리에 대한 Cas9 및 Cas9-RecJ 아포단백질(apoproteins)을 지질 매개 형질감염(lipid mediated transfection) 방법으로 HEK293 세포에 형질감염 시켰다. 게놈 DNA 추출을 위해 0시간 내지 48시간의 다른 시점에서 세포를 수득하였다. 이때, 0 시간은 대조군이다. CCR5를 확인하기 위한 544 bp 밴드 및 PD-1을 확인하기 위한 524 bp 밴드를 생성하기 위하여 PCR을 수행하였다. T7E1 엔도뉴클레아제 어세이를 수행할 경우, CCR5에서는 274 bp 및 271 bp의 작은 단편이 나타나고, PD-1에서는 319 bp 및 205 bp의 작은 단편이 나타났다. 이러한 것을 통하여 삽입-결실율(indel)을 측정할 수 있었다. 이에 대한 결과를 도 7에 나타내었다.

[0103] **실험예 7. Cas9 및 Cas9-RecJ에 의한 오프-타겟 효과 확인**

[0104] 인간 CCR5 sgRNA와 Cas9 및 Cas9-RecJ를 이용시 5개의 오프 타겟팅된 유전자를 나타낸 것이다. ADCY5 641bps; KCNJ6, 566 bps; CNTPNA2, 300 bps; Chr.5, 605bps. 오프-타겟 효과를 도 8에 나타내었다.

[0105] 또한, 5개의 오프-타겟된 유전자의 In vitro 절단 에세이를 분석하였다. 예상된 절단된 DNA의 크기는 ADCY5에서 377 bp 및 264 bp이고; KCNJ6에서 352 bp 및 214 bp이며; CNTPNA2에서 183 bp 및 117 bp이며; Ch. 5에서 355 bp 및 250 bp이다. 절단은 오프 타겟팅 효과를 확인하기에는 명확하지 않았다. In vitro 에세이를 근거로, SpCas9-RecJ의 오프 타겟팅 정도는 SpCas9과 차이가 없었다. 이에 대한 결과를 도 9에 나타내었다.

[0106] **실험예 8. 변형된 FnCpf1 단백질의 절단 효율 확인**

[0107] 단백질과 gRNA는 몰농도 비율이 1:1.2가 되도록 준비하였다. 반응 부피 20 μL로 하여 단백질 50 nM에 crRNA 60 nM을 섞은 후 20분 상온에 두고 RNP가 형성되도록 하였다. crRNA는 CCR5 유전자를 타겟으로하며, 타겟 유전자인 CCR5 주형의 크기는 약 1.4 kb이다.

[0108] 1.5 kb 크기의 타겟 유전자인 CCR5 주형 DNA 500 ng을 RNP 복합체와 섞은 후 37℃에서 1시간 반응시킨다. 2% agarose gel에 전기영동한 후 겔 이미지를 수득하였다. 이를 이용하여 주형 DNA와 RNP 복합체가 작용하고 남은 주형 DNA의 비율을 계산하여, 단백질의 뉴클레아제 활성 효율을 얻었다.

[0109] FnCpf1, FnCpf1-RecJ, 또는 SpCas9에 의한 선형 dsDNA가 절단됨을 확인하기 위한 실험을 수행하였다. 선형 dsDNA는 PCR을 통해 증폭되고 정제되었다. Cas9 sgRNA 및 Cpf1 sgRNA 사이트는 서로 근접한 곳에 위치한다. SpCas9 및 FnCpf1 단백질에 사용된 sgRNA는 각각 5'-NGG-3' PAM(protospacer adjacent motif)이 상단(upstream)에는 존재하며, 5'-TTTN-3' PAM이 하단(downstream)에 존재한다. 절단되기 전의 온전한 dsDNA는 1,200 bp 위치에서 단일 밴드로 관찰되었다. 상기 dsDNA는 절단된 후 두개 모두 600 bp 밴드에 위치하였다. 그러나, FnCpf1-RecJ를 이용할 경우, RecJ의 엑소뉴클레아제 활성에 의해 상기 절단된 밴드가 사라짐을 확인하였다(도 10).

[0110] FnCpf1 또는 FnCpf1-RecJ 엑소뉴클레아제에 의한 선형 dsDNA In vitro 절단 에세이를 수행하였다. 선형 dsDNA는 FnCpf1 또는 FnCpf1-RecJ에 의해 절단되었다. 선형 dsDNA는 플라스미드로부터 PCR을 이용하여 증폭되고 분리되었다. sgRNA는 FnCpf1 단백질을 타겟 사이트로 위치시키기 위해 사용되었으며, 5'-TTTN-3' PAM이 하단(downstream)에 존재한다. 절단되기 전의 온전한 dsDNA는 1,200 bp 위치에서 단일 밴드로 관찰되었다. 상기 dsDNA는 절단되면 두개 모두 600 bp 밴드에 위치하였다. 그러나, FnCpf1-RecJ를 이용할 경우, RecJ의 엑소뉴클레아제 활성에 의해 상기 절단된 밴드가 사라졌다. 반응 시간을 증가시킴에 따라, FnCpf1 및 FnCpf1-RecJ 반응 혼합물에서 원래의 밴드가 흐려짐을 확인하였다(도 11).

[0111] **실험예 9. 변형된 Cas9 단백질의 편집 효율 확인**

[0112] HEK293T 세포를 10% 태아 소혈청인 FBS와 P/S가 함유된 DMEM 배지에서 37℃, 5% CO_2 배양기에서 유지시켰다. 녹인 실험의 효율을 높이기 위해 HEK293T 세포를 $3x10^6$ 밀도로 10 cm 배양접시에 배양한 후 형질전환 16시간 전 200 ng/ml의 노코다졸(Sigma)을 세포에 처리하였고, 동기화된 세포를 유전자 도입 실험에 사용하였다. 사용한 외부 유전자인 ssODN(single-stranded oligodeoxynucleotides)의 서열은 다음과 같다:

[0113] CCR5: 5' TGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTAT

[0114] ACATATGCATCGGAGCCCTGCCAAAAAATCAATGTGAAGCAAATCGCAGCCC

[0115] DHCR7: 5' ATGGGCCCCAGTGTGACTGCCTGCATCCGTCCTCGCAGGGAGGTGGACTGGT

[0116] TTTGAATTCCACTGGCGAGCGTCATCTTCCTACTGCTGTTCGCCCCCTTCATCG

[0117] 외부 유전자인 ssODN(single-stranded oligodeoxynucleotides)을 도입시키기 위해 $2x10^5$의 HEK293T 세포를 형질전환 실험에 이용하였다. 각각 33 pmole의 단백질과 66 pmole의 sgRNA를 이용하였다. 도입 유전자로는 ssODN 20 pmole을 사용하였다. 형질전환을 위해 Lonza사의 4D-Nucleofector 기기를 이용한 전기천공법을 사용하였다. 24시간 후 invitrogen사의 PureLink™ Genomic DNA Mini Kit를 사용하여 genomic DNA를 추출하였다.

[0118] 편집된 부분을 가지고 있는 gDNA와 그렇지 않은 gDNA 샘플을 이용하여 표적 부위를 증폭하였다. 이후 일루미나의 프로토콜대로 PCR과 정제 과정을 거친 후 MiniSeq 장비를 이용하여 표적 부위에 대한 딥 시퀀싱(deep sequencing) 분석을 진행하였다. 시퀀싱을 위해 사용된 어댑터 프라이머 서열은 다음과 같다.

[0119] CCR5 (F): 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGAGGGCAACTAAATACATTCT

[0120] CCR5 (R): 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAACACCAGTGAGTAGAGCGG

[0121] DHCR7 (F): 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGTTTGAGCAACAGTTCTCC

[0122] DHCR7 (R): 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGACGATGTCCACCACAG

[0123] SpyCas9과 SpyCas9의 C 말단에 다양한 단백질이 결합된 변형된 SpyCas9의 CCR5 및 DHCR7에 대한 유전자 편집 효율을 도 15 및 도 16에 나타내었다. SpyCas9의 C 말단에 GFP, hTdT, 및 엑소뉴클레아제로 알려진 RecJ, RecE, lambda, mungbean, and T5가 결합된 형태이다. 도면에서 나타낸 바와 같이 Knock-out 및 Knock-in 효율이 서로 상이하였다.

[0124] 사용한 변형된 Cas9-RecJ의 구조 및 이의 Knock-out 및 Knock-in 효율을 도 17 및 도 18에 나타내었다. 도 17A는 SpyCas9-RecJ의 구조를 나타낸 것이다. 도 17B는 대조군, SpyCas9 및 SpyCas9-RecJ 간의 유전자 편집 효율을 나타낸 것이다. 도 17C는 CCR5 및 DHCR7 유전자의 편집 정도를 확인하여, SpyCas9 대비 SpyCas9-RecJ의 편집 효율 변화를 백분율로 나타낸 것이다.

[0125] 도 18A는 SpyCas9-RecJ의 구조를 나타낸 것이다. 도 18B는 대조군, SpyCas9 및 SpyCas9-RecJ 간의 유전자 편집 효율을 나타낸 것이다. 도 18C는 CCR5 및 DHCR7 유전자의 편집 정도를 확인하여, SpyCas9 대비 SpyCas9-RecJ의 편집 효율 변화를 백분율로 나타낸 것이다.

[0126] 또한, SpyCas9과 SpyCas9의 N 말단 또는 N/C 양말단에 다양한 단백질이 결합된 변형된 SpyCas9의 DHCR7에 대한 유전자 편집 효율을 도 19는 및 20에 나타내었다. 변형된 SpyCas9은 SpyCas9의 N 말단에 RecJ, RecE, GFP, 단일 가닥 DNA 결합 단백질(SSB) 및 이중 가락 DNA 결합 단백질(DSB)이 결합되어 있다. 또한, 변형된 SpyCas9은 SpyCas9의 C 말단에 RecJ가 결합되어 있고, N 말단에 SSB 또는 DSB이 결합되어 있다. 도면에서 나타낸 바와 같이 Knock-out 및 Knock-in 효율이 서로 상이하였다.

[0127] 도 21A는 RecJ-SpyCas9의 구조를 나타낸 것이다. 도 21B는 대조군, SpyCas9 및 RecJ-SpyCas9 간의 유전자 편집 효율을 나타낸 것이다. 도 21C는 DHCR7 유전자의 편집 정도를 확인하여, SpyCas9 대비 RecJ-SpyCas9의 편집 효율 변화를 백분율로 나타낸 것이다.

[0128] 도 22A는 RecJ-SpyCas9의 구조를 나타낸 것이다. 도 22B는 대조군, SpyCas9 및 RecJ-SpyCas9 간의 유전자 편집 효율을 나타낸 것이다. 도 22C는 CCR5 및 DHCR7 유전자의 편집 정도를 확인하여, SpyCas9 대비 RecJ-SpyCas9의 편집 효율 변화를 백분율로 나타낸 것이다.

[0129] 도 23A는 SSB-SpyCas9-RecJ의 구조를 나타낸 것이다. 도 23B는 대조군, SpyCas9 및 SSB-SpyCas9-RecJ 간의 유전자 편집 효율을 나타낸 것이다. 도 23C는 DHCR7 유전자의 편집 정도를 확인하여, SpyCas9 대비 SSB-SpyCas9-RecJ의 편집 효율 변화를 백분율로 나타낸 것이다.

[0130] 도 24A는 DSB-SpyCas9-RecJ의 구조를 나타낸 것이다. 도 24B는 대조군, SpyCas9 및 DSB-SpyCas9-RecJ 간의 유전자 편집 효율을 나타낸 것이다. 도 24C는 DHCR7 유전자의 편집 정도를 확인하여, SpyCas9 대비 DSB-SpyCas9-RecJ의 편집 효율 변화를 백분율로 나타낸 것이다.

[0131] **실험예 10. 변형된 Cpf1 단백질의 편집 효율 확인**

[0132] HEK293T 세포를 10% 태아 소혈청인 FBS와 P/S가 함유된 DMEM 배지에서 37℃, 5% CO_2 배양기에서 유지시켰다. 녹인 실험의 효율을 높이기 위해 HEK293T 세포를 $3x10^6$ 밀도로 10 cm 배양접시에 배양한 후 형질전환 16시간 전 200 ng/ml의 노코다졸(Sigma)을 세포에 처리하였고, 동기화된 세포를 유전자 도입 실험에 사용하였다. 사용한 외부 유전자인 ssODN(single-stranded oligodeoxynucleotides)의 서열은 다음과 같다.

[0133] CCR5: 5' AATTCTCTGAGGCTTTCTTTTAAATATACATAAGGAACTTTCGGAGTGAAGGG

[0134] AGAGTTTCATATGGTCAATAACTTGATGCATGTGAAGGGGAGATAAAAAGGTT

[0135] DNMT1: 5' TGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGC

[0136] ACCTGCCGAATTCTCAGCTGCTCACTTGAGCCTCTGGGTCTAGAACCCTCTGG

[0137] 외부 유전자인 ssODN(single-stranded oligodeoxynucleotides)을 도입시키기 위해 $2x10^5$의 HEK293T 세포를 형

질전환 실험에 이용하였다. 각각 33 pmole의 단백질과 66 pmole의 sgRNA를 이용하였다. 도입 유전자로는 ssODN 20 pmole을 사용하였다. 형질전환을 위해 Lonza사의 4D-Nucleofector 기기를 이용한 전기천공법을 사용하였다. 24시간 후 invitrogen사의 PureLink™ Genomic DNA Mini Kit를 사용하여 genomic DNA를 추출하였다.

[0138] 편집된 부분을 가지고 있는 gDNA와 그렇지 않은 gDNA 샘플을 이용하여 표적 부위를 증폭하였다. 이후 일루미나의 프로토콜대로 PCR과 정제 과정을 거친 후 MiniSeq 장비를 이용하여 표적 부위에 대한 딥 시퀀싱(deep sequencing) 분석을 진행하였다. 시퀀싱을 위해 사용된 어댑터 프라이머 서열은 다음과 같다.

[0139] CCR5 (F): 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGTATTTCTGTTCAGATCAC

[0140] CCR5 (R): 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCCCATCAATTATAGAAAGCC

[0141] DNMT1 (F): 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTGCACACAGCAGGCCTTTG

[0142] DNMT1 (R): 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCCAATAAGTGGCAGAGTGC

[0143] FnCpf1과 FnCpf1의 C 말단에 다양한 단백질이 결합된 변형된 FnCpf1의 CCR5 및 DNMT1 유전자에 대한 유전자 편집 효율을 도 25 및 도 26에 나타내었다. FnCpf1의 C 말단에 GFP, hTdT, 및 엑소뉴클레아제로 알려진 RecJ, RecE, lambda, mungbean, 및 T5이 결합된 형태이다. 도면에서 나타낸 바와 같이 Knock-out 및 Knock-in 효율이 서로 상이하다.

[0144] 도 27A는 FnCpf1-RecJ의 구조를 나타낸 것이다. 도 27B는 대조군, FnCpf1 및 FnCpf1-RecJ 간의 유전자 편집 효율을 나타낸 것이다. 도 27C는 CCR5 및 DNMT1 유전자의 편집 정도를 확인하여, FnCpf1 대비 FnCpf1-RecJ의 편집 효율 변화를 백분율로 나타낸 것이다.

[0145] 도 28A는 FnCpf1-RecJ의 구조를 나타낸 것이다. 도 28B는 대조군, FnCpf1 및 FnCpf1-RecJ 간의 유전자 편집 효율을 나타낸 것이다. 도 28C는 CCR5 및 DNMT1 유전자의 편집 정도를 확인하여, FnCpf1 대비 FnCpf1-RecJ의 편집 효율 변화를 백분율로 나타낸 것이다.

[0146] FnCpf1과 FnCpf1의 N 말단 또는 N/C 양말단에 다양한 단백질이 결합된 변형된 FnCpf1의 유전자 편집 효율을 도 29 및 도 30에 나타내었다. 변형된 FnCpf1은 FnCpf1의 N 말단에 RecJ, RecE, GFP, 단일 가닥 DNA 결합 단백질(single stranded DNA binding protein, SSB) 및 이중 가락 DNA 결합 단백질(double stranded DNA binding protein, DSB)이 결합되어 있다. 또한, 변형된 FnCpf1은 FnCpf1의 C 말단에 RecJ가 결합되어 있고, N 말단에 SSB이 결합되어 있다. 도면에서 나타낸 바와 같이 Knock-out 및 Knock-in 효율이 서로 상이하였다.

[0147] 도 31A는 RecJ-FnCpf1의 구조를 나타낸 것이다. 도 31B는 대조군, RecJ-FnCpf1 간의 유전자 편집 효율을 나타낸 것이다. 도 31C는 CCR5 및 DNMT1 유전자의 편집 정도를 확인하여, FnCpf1 대비 RecJ-FnCpf1의 편집 효율 변화를 백분율로 나타낸 것이다.

[0148] 도 32A는 RecJ-FnCpf1의 구조를 나타낸 것이다. 도 32B는 대조군, RecJ-FnCpf1 간의 유전자 편집 효율을 나타낸 것이다. 도 32C는 CCR5 및 DNMT1 유전자의 편집 정도를 확인하여, FnCpf1 대비 RecJ-FnCpf1의 편집 효율 변화를 백분율로 나타낸 것이다.

[0149] 도 33A는 SSB-FnCpf1-RecJ의 구조를 나타낸 것이다. 도 33B는 대조군, FnCpf1 및 SSB-FnCpf1-RecJ 간의 Knock-out 유전자 편집 효율을 나타낸 것이다. 도 33C는 CCR5 및 DNMT1 유전자의 편집 정도를 확인하여, FnCpf1 대비 SSB-FnCpf1-RecJ의 편집 효율 변화를 백분율로 나타낸 것이다.

[0150] 도 34A는 SSB-FnCpf1-RecJ의 구조를 나타낸 것이다. 도 34B는 대조군, FnCpf1 및 SSB-FnCpf1-RecJ 간의 Knock-in 유전자 편집 효율을 나타낸 것이다. 도 34C는 CCR5 및 DNMT1 유전자의 편집 정도를 확인하여, FnCpf1 대비 SSB-FnCpf1-RecJ의 편집 효율 변화를 백분율로 나타낸 것이다.

[0151] **실험예 11. 변형된 크리스퍼 연관 단백질의 편집 효율에 미치는 조건 확인**

[0152] ssODN 및 노코다졸(Nocodazole)의 존재 유무에 따른 변형된 Cas9 단백질의 활성 변화를 확인하기 위한 실험을 수행하였다.

[0153] 크리스퍼 플러스 매개 녹아웃을 확인하기 위하여, 노코다졸이 처리되지 않은 세포를 사용하였다. 녹인의 효율을 증가시키기 위하여, HEK293T 세포를 10-cm 배양 접시에 3 x 10^6 세포 밀도로 접종하였다. 상기 세포를 노코다졸(200 ng/ml)로 16시간 배양한 후, 전기 영동을 수행하였다. 하기 표 6에 기재한 ssODN 주형을 녹인을 위해 사용

하였다:

[0154] 크리스퍼 플러스 단백질을 세포에 트랜스펙션하기 전에, 분리정제된 SpyCas9 또는 FnCpf1 변이체 단백질(33 pmol) 및 sgRNA 또는 crRNA(66 pmol)을 상온에서 20분간 인큐베이션시켜 RNP를 복합체를 형성하였다. HDR 주형(20 pmol ssODN 또는 2 pmol dsDNA)을 RNP 복합체에 첨가하였다. Lonza를 이용하여 HEK293T 세포에 뉴클레오펙션(Nucleofection)을 수행하였다. 그 후, 각각의 뉴클레오펙션은 20 μl의 뉴클레오펙션 시약에 있는 2 x 10^5 세포와 RNP:DNA 10 μl을 혼합였다.

표 6

[0155]

Proteins	Genes	ssODN donor sequences (5' -3')
SpyCas9	CCR5	TGGAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTAT (서열번호 94) ACATATGCATCGGAGCCCTGCCAAAAAATCAATGTGAAGCAAATCGCAGCCC (서열번호 95)
	DHCR7	ATGGGCCCCAGTGTGACTGCCTGCATCCGTCCTCGCAGGGAGGTGGACTGGT (서열번호 96) TTTGAATTCCACTGGCGAGCGTCATCTTCCTACTGCTGTTCGCCCCCTTCATCG (서열번호 97)
FnCpf1	CCR5	AATTCTCTGAGGCTTTCTTTTAAATATACATAAGGAACTTTCGGAGTGAAGGG (서열번호 98) AGAGTTTCATATGGTCAATAACTTGATGCATGTGAAGGGGAGATAAAAAGGTT (서열번호 99)
	DNMT1	TGGCCCTGGGGCCGTTTCCCTCACTCCTGCTCGGTGAATTTGGCTCAGCAGGC (서열번호 100) ACCTGCCGAATTCTCAGCTGCTCACTTGAGCCTCTGGGTCTAGAACCCTCTGG (서열번호 101)

[0156] **실험예 11.1. 변형된 Cas9의 조건 확인**

[0157] 도 35a 및 도 36a는 ssODN 및 노코다졸이 처리되지 않았을 때의 비상동적인 말단 연결 효율을 나타낸다. 도 35b 및 도 36b는 ssODN 및 노코다졸이 처리되었을 때의 비상동적인 말단 연결 효율을 나타낸다. 도 35c 및 도 36c는 ssODN 및 노코다졸의 존재 유무에 따른 효율을 비교한 것이다. ssODN 및 노코다졸은 Knock-out의 경우, SpyCas9 및 변형된 SpyCas9의 효율에는 큰 영향이 없음을 확인하였다.

[0158] **실험예 11.2. 변형된 Cpf1의 조건 확인**

[0159] 크리스퍼 플러스 매개 녹아웃을 확인하기 위하여, 노코다졸이 처리되지 않은 세포를 사용하였다. 녹인의 효율을 증가시키기 위하여, HEK293T 세포를 10-cm 배양 접시에 3 x 10^6 세포 밀도로 접종하였다. 상기 세포를 노코다졸(200 ng/ml)로 16시간 배양한 후, 전기 영동을 수행하였다. 상기 표 6에 기재한 ssODN 주형을 녹인을 위해 사용하였다:

[0160] 크리스퍼 플러스 단백질을 세포에 트랜스펙션하기 전에, 분리정제된 SpyCas9 또는 FnCpf1 변이체 단백질(33 pmol) 및 sgRNA 또는 crRNA(66 pmol)을 상온에서 20분간 인큐베이션시켜 RNP를 복합체를 형성하였다. HDR 주형(20 pmol ssODN 또는 2 pmol dsDNA)을 RNP 복합체에 첨가하였다. Lonza를 이용하여 HEK293T 세포에 뉴클레오펙션(Nucleofection)을 수행하였다. 그 후, 각각의 뉴클레오펙션은 20 μl의 뉴클레오펙션 시약에 있는 2 x 10^5 세포와 RNP:DNA 10 μl을 혼합하였다.

[0161] FnCpf1 및 FnCpf1의 C 말단에 단백질이 결합된 변형된 FnCpf1에 유도되는 비상동적인 말단 연결에 있어서, ssODN 및 노코다졸(nocodazole)이 미치는 영향을 확인한 것을 도 37에 나타내었다. 도 37a는 ssODN 및 노코다졸이 처리되지 않았을 때의 비상동적인 말단 연결 효율을 나타낸다. 도 37b는 ssODN 및 노코다졸이 처리되었을 때의 비상동적인 말단 연결 효율을 나타낸다. 도 37c는 ssODN 및 노코다졸의 존재 유무에 따른 효율을 비교한 것이다. ssODN 및 노코다졸은 Knock-out의 경우, FnCpf1 및 변형된 FnCpf1의 효율을 향상 시킨다는 점을 확인하였다.

[0162] FnCpf1 및 FnCpf1의 N 말단 또는 N/C 양말단에 단백질이 결합된 변형된 FnCpf1에 유도되는 비상동적인 말단 연결에 있어서, ssODN 및 노코다졸이 미치는 영향을 도 38에 나타내었다. 도 38a는 ssODN 및 노코다졸이 처리되지 않았을 때의 비상동적인 말단 연결 효율을 나타낸다. 도 38b는 ssODN 및 노코다졸이 처리되었을 때의 비상동적인 말단 연결 효율을 나타낸다. 도 38c는 ssODN 및 노코다졸의 존재 유무에 따른 효율을 비교한 것이다. ssODN 및 노코다졸은 Knock-out의 경우, FnCpf1 및 변형된 FnCpf1의 효율에는 큰 영향이 없음을 확인하였다.

[0163] **실험예 12. 크리스퍼 플러스 단백질의 유전자 편집 효능 검증**

[0164] **제조예 1. 식물 유전자 편집을 위한 용액 조성물 제조**

[0165] 종자 파종 배지의 조성은 다음과 같다.

표 7

[0166]

종자 파종 배지	1 L
1/2 MS Powder	2.2 g
Sucrose	10 g
Plant agar	7 g
pH	5.7 (1N NaOH 또는 1N HCl 로 측정함)

[0167] Enzyme solution의 조성은 다음과 같다. 단, 사용 직전에 만들어 사용함을 원칙으로 한다.

표 8

[0168]

Enzyme Solution	20 mL
1.0% Cellulase R10	200 mg
0.5% Macerozyme R10	100 mg
0.4 M Mannitol	10 mL (0.8 M Mannitol Stock solution)
20 mM MES, pH 5.7	4 mL (100 mM MES Stock solution, pH 5.7)
20 mM KCl	200 μL (2 M KCl Stock solution)
위 시약들 조합한 후 60도에서 10분간 배양한 후 아래 시약을 조합한다.	
10 mM $CaCl_2 \cdot 2H_2O$	200 μL (1 M $CaCl_2 \cdot 2H_2O$ Stock solution)
0.1 % BSA	200 μL (10% BSA Stock solution)

[0169] MMG solution의 조성은 다음과 같다. 단, 사용 직전에 만들어 사용함을 원칙으로 한다.

표 9

[0170]

MMG Solution	10 mL
0.4 M Mannitol	5 mL (0.8 M Mannitol Stock solution)
4 mM MES, pH 5.7	400 μL (0.1 M MES Stock solution, pH5.7)
15 mM $MgCl_2$	150 μL (1 M $MgCl_2$ Stock solution)
Nuclease-free water	4.45 mL

[0171] PEG4000 solution의 조성은 다음과 같다. 단, 사용 직전에 만드는 것을 원칙으로 한다.

표 10

[0172]

PEG Solution	5 mL
0.2 M Mannitol	1.25 mL (0.8 M Mannitol Stock solution)
40%W/V PEG-4000	2 g (Polyethylene glycol 4000)
100 mM $CaCl_2 \cdot 2H_2O$	500 μL (1 M $CaCl_2$*?*$2H_2O$ Stock solution)
Nuclease-free water	1.5 mL

[0173] W5 solution의 조성은 다음과 같다.

표 11

[0174]

W5 Solution	50 mL
154 mM NaCl	3.85 mL (2 M NaCl Stock solution)
125 mM $CaCl_2 \cdot 2H_2O$	6.25 mL (1 M $CaCl_2 \cdot 2H_2O$ Stock solution)
5 mM KCl	125 μL (2 M KCl Stock solution)
2 mM MES, pH 5.7	500 μL (0.1 M MES Stock solution)
Nuclease-free water	39.275 mL

[0175] 상기 Enzyme Solution, MMG Solution, PEG Solution, W5 Solution은 모두 0.45 μm Syringe-filter로 필터링하였다..

[0176] **실험예 12.1. 실험 식물체의 준비**

[0177] 도 51에 나타낸 바와 같이 실험을 수행하였다. 구체적으로, 담배 종자 파종 및 원형질체 분리를 다음과 같이 수행하였다. 먼저, 담배 종자를 50% Clorox로 1분간 처리하였다. 그 후, Clean bench로 옮겨 멸균수로 5번 씻어, 필터페이퍼에서 20분간 건조하였다. 그 후, 멸균된 종자를 종자파종 배지에 올려 일주일간 배양한 후 마젠타박스에 옮겨 3주동안 키웠다. 배양 빛조건은 광배양 16시간, 암배양 8시간이며, 온도는 25℃ 내지 28℃에서 키웠다. 이때, 식물은 4~6주 자란 잎을 사용해야하며, 8주 이상 자란 잎은 사용하지 않음을 원칙으로 한다.

[0178] 4~6주 자란 후, 담배 어린잎을 유리판에 올려 잎병과 잎끝부분을 자른 후 잎 안쪽부분만을 잘랐다. 0.5 mm 이하로 자르고, 자른 잎은 Enzyme solution 10 mL에 올려 상온에서 암 상태로 3~4시간 orbital shaker(50 rpm) 위에 올려서 배양하였다(도 52).

[0179] 배양 후 W5 solution 10mL을 첨가한 후 조심스럽게 잘 섞어주었다. 그 후, Cell strainer(70 μm)를 이용해서 enzyme solution 내에 존재하는 원형질체를 걸러주었다. 그 후, 걸러진 원형질체를 100 g으로 6분 원심분리하였다. 상층액을 버리고 MMG solution을 첨가해서 조심스럽게 원형질체 pellet을 풀어 주었다. 그 후, Ice에서 10 ~ 30분 정도 방치하였다. 이 중 일부를 hemocytometer 이용해 현미경으로 원형질체의 수를 계수하였다. MMG solution을 더 첨가하여 원형질체의 농도가 2 X 10^6 cells/mL이 되도록 희석하였다.

[0180] **실험예 12.2. 크리스퍼 플러스 단백질을 이용한 식물체 유전자 편집**

[0181] 2 mL e-tube에 sgRNA와 Cas9 protein 그리고 NEB buffer 3.1을 최종 volume 20 μL 가 되게 한 후 상온에서10분간 반응시켰다. Folding을 방지함으로써 DNA binding 효율을 높여 주기 위하여, gRNA는 사용 전에 95℃에서 3분, ice에서 3분 놓아준다.

표 12

[0182]

SgRNA	2.5ug	5ug	10ug
Cas9	10ug	20ug	30ug
10x NEB buffer (3.1)	2 μL	2 μL	2 μL
Nuclease-free water	Up to 20 μL		

[0183] E-tube (2 mL)에 원형질체 200 μL (5 X 10^5 cells)와 PFT3 sgRNA 서열은 CAAGGGCTTCTAAAGCTTGCAAA과 PFT1 sgRNA 서열 GGCTTTTGAGAATTCTAATG 이 사용되었다. PFT3은 담배의 알파 1,3-푸코스트랜스퍼래이즈의 3번 도메인을 목표하고 PFT1은 담배의 알파 1,3-푸코스트랜스퍼래이즈의 4번 도메인을 목표하였다. Cas9 protein (volume 20 μL)을 넣고 잘 섞은 후, Clean bench에서 10분간 배양하였다. 앞에서 배양한 volume과 동일한 PEG solution 220 μL를 넣고 조심스럽게 섞어주었다. 상온에서 15분간 배양 후, W5 solution 840 μL을 첨가하여 잘 혼합하였다. 100 g에서 2분간 원심분리한 후, 상등액을 제거하였다. 형질전환한 원형질체에 W5 Solution 1000 μL을 넣어 재현탁하였다. 100g에서 2분간 원심분리한 후, 상등액을 제거하였다. W5 Solution 1000 μL을 넣어 Resuspension한 후, 6 또는 12 well plate에 넣어 30℃ 암배양기에서 48시간 배양 후 수거하였다. 수거된 세포는 DNA를 추출 후 타겟 시퀀스를 PCR 한 후 NGS 분석에 들어간다.

[0184] **실험예 12.3. NGS 분석**

[0185] 분석장비(일루미나제품, MiniSeq (SY-420-1001), Miniseq mid output kit (300 cycles, FC-420-1004))를 이용하여 분석하였다. Amplicon primers 조건은 아래와 같으며, 타겟 specific 시퀀스 앞 뒤로 붙여서 제작하였다.

[0186] 타겟 PFT1에 사용한 시퀀스 정보는 다음과 같다:

[0187] F:5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-ACTTCTCTTGGGCTGAGTATGA

[0188] R:5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-CAGTAAGTTTGGATATTTGAAA

[0189] 타겟 PFT3에 사용한 시퀀스 정보는 다음과 같다:

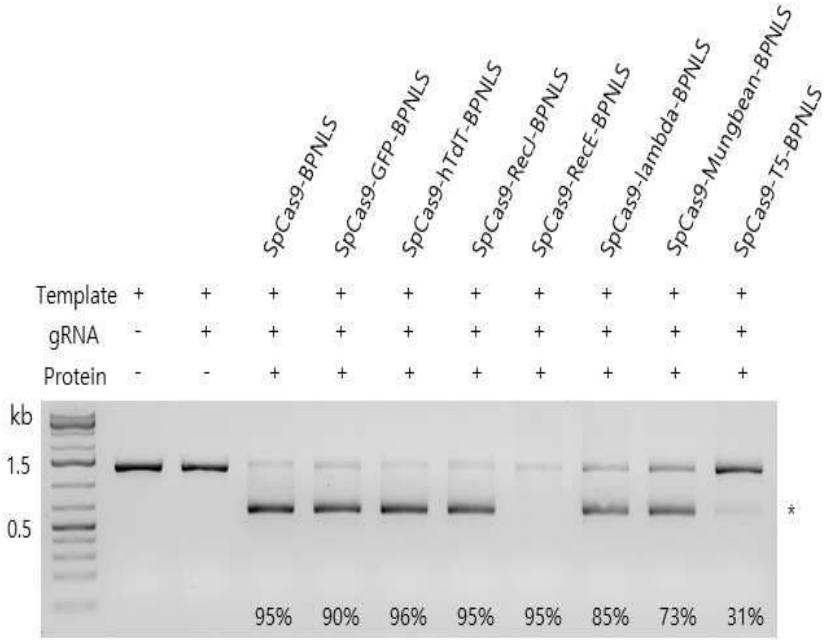
[0190] F:5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-GCATTTGGTGTAGGTTTAGGCT

[0191] R:5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-AATTCTGAAAATCCAAGTCTAT

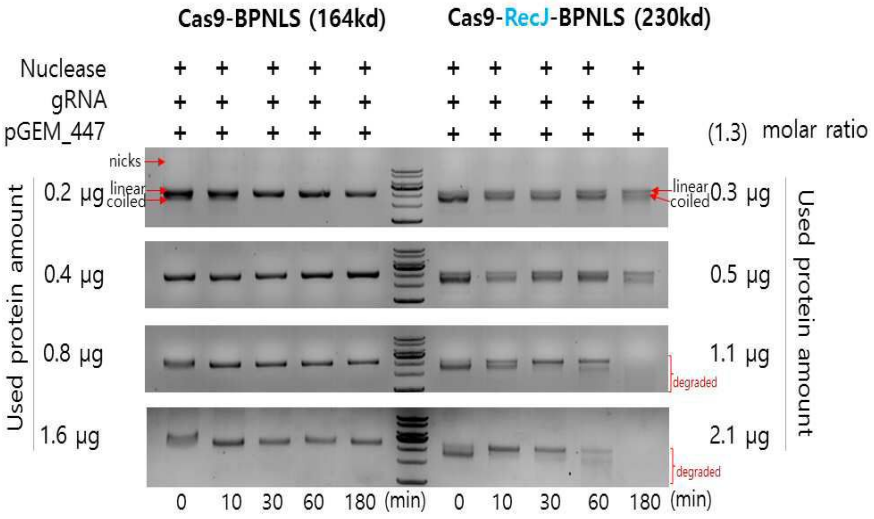
[0192] 타겟 영역을 amplicon primer를 이용해 1차 PCR을 수행하였다. 생산된 PCR product는 반드시 clean-up을 수행하였다. 타겟 영역에 amplicon이 포함된 DNA에 index를 붙여서 library를 만들었다. 수행한 2차 PCR을 Bioanalyzer로 QC를 수행 후 시퀀싱을 수행하였다.

도면

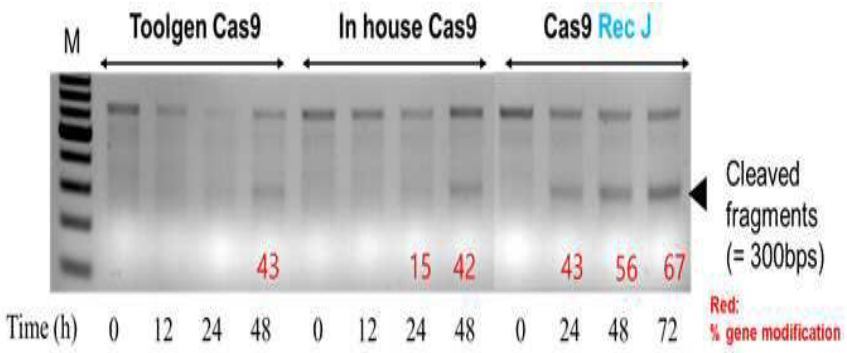
도면1



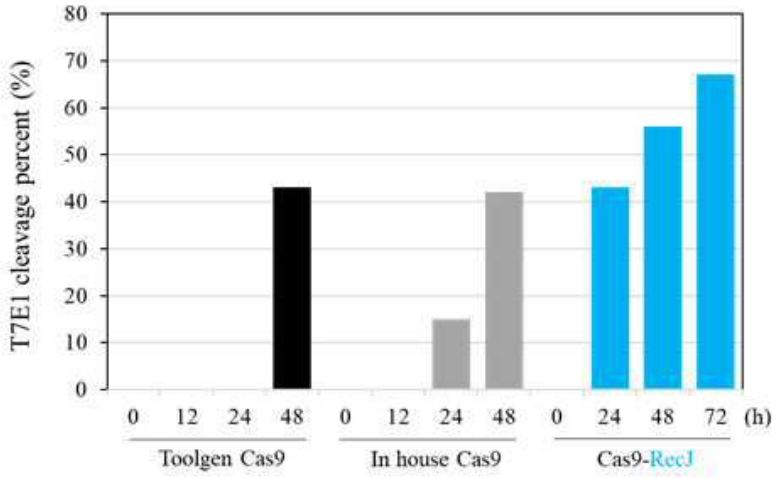
도면2



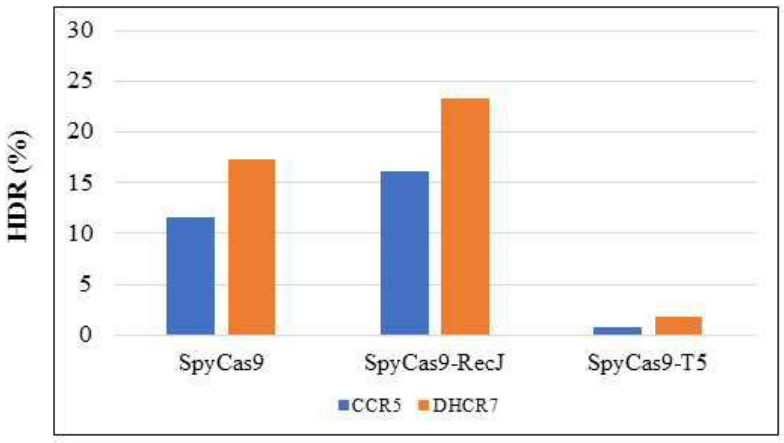
도면3a



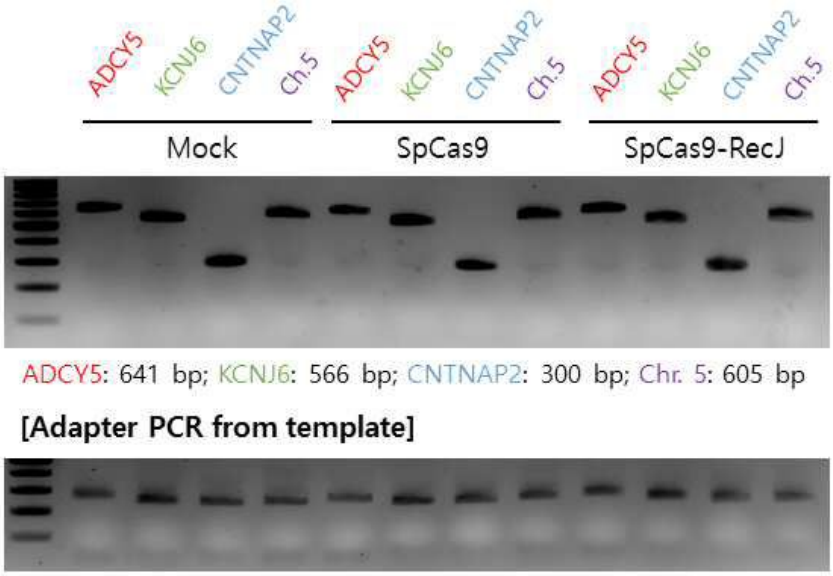
도면3b



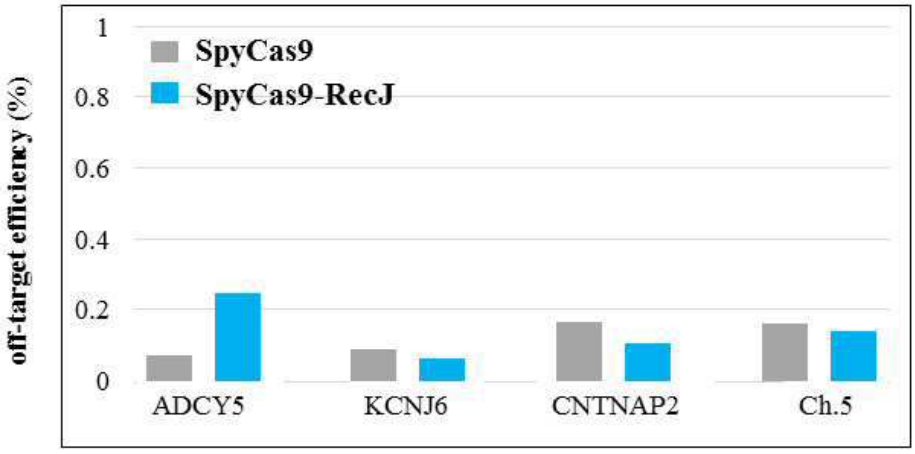
도면4



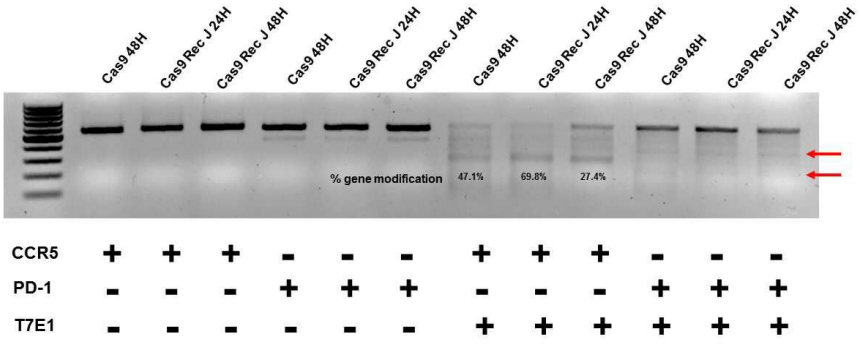
도면5


ADCY5
KCNJ6
CNTNAP2
Ch.5
ADCY5
KCNJ6
CNTNAP2
Ch.5
ADCY5
KCNJ6
CNTNAP2
Ch.5
Mock
SpCas9
SpCas9-RecJ
ADCY5: 641 bp; KCNJ6: 566 bp; CNTNAP2: 300 bp; Chr. 5: 605 bp
[Adapter PCR from template]

도면6

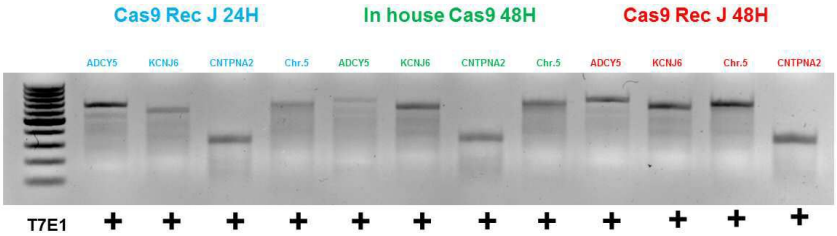

off-target efficiency (%)
1
0.8
0.6
0.4
0.2
0
SpyCas9
SpyCas9-RecJ
ADCY5
KCNJ6
CNTNAP2
Ch.5

도면7

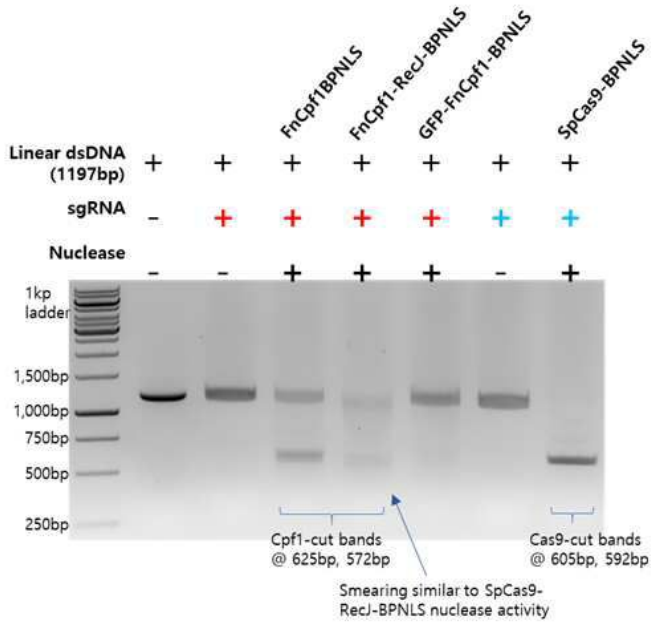

Cas9 48H
Cas9 Rec J 24H
Cas9 Rec J 48H
Cas9 48H
Cas9 Rec J 24H
Cas9 Rec J 48H
Cas9 48H
Cas9 Rec J 24H
Cas9 Rec J 48H
Cas9 48H
Cas9 Rec J 24H
Cas9 Rec J 48H
% gene modification
47.1%
69.8%
27.4%
CCR5 + + + - - - + + + - - -
PD-1 - - - + + + - - - + + +
T7E1 - - - - - - + + + + + +

도면8

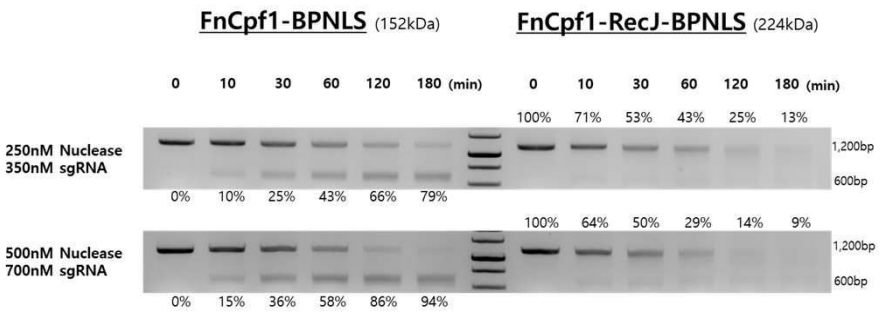
도면9



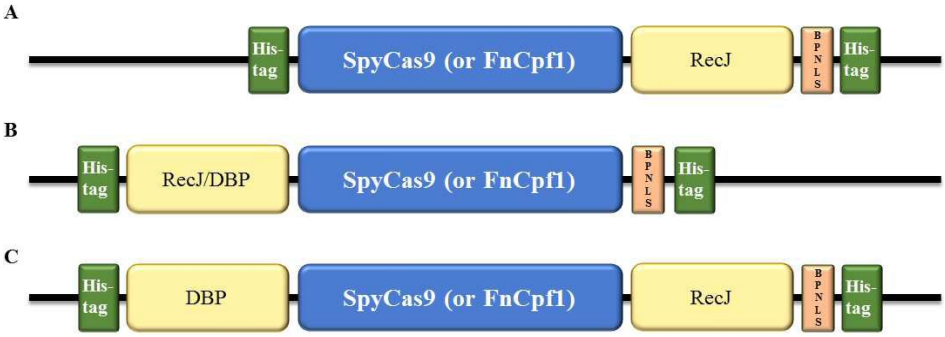
도면10



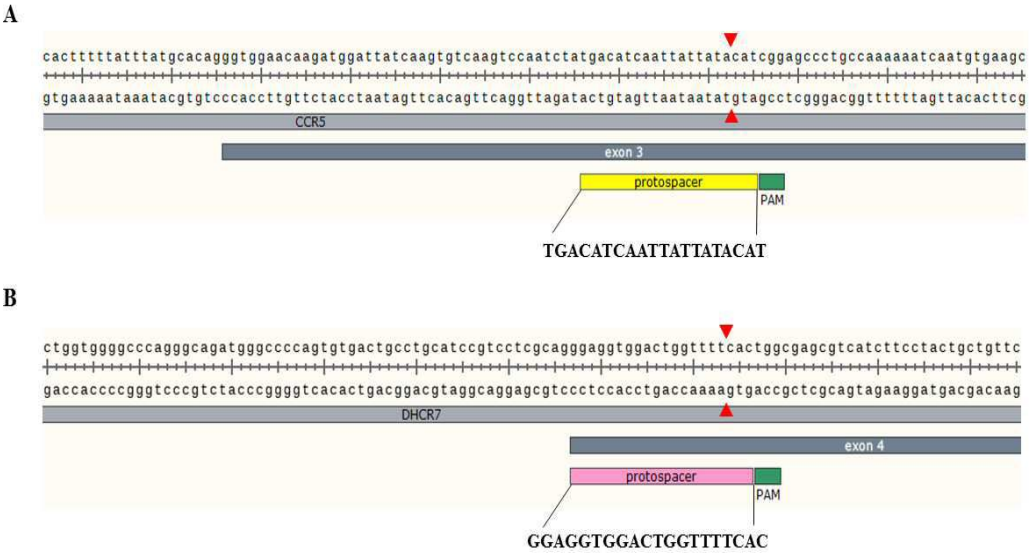
도면11



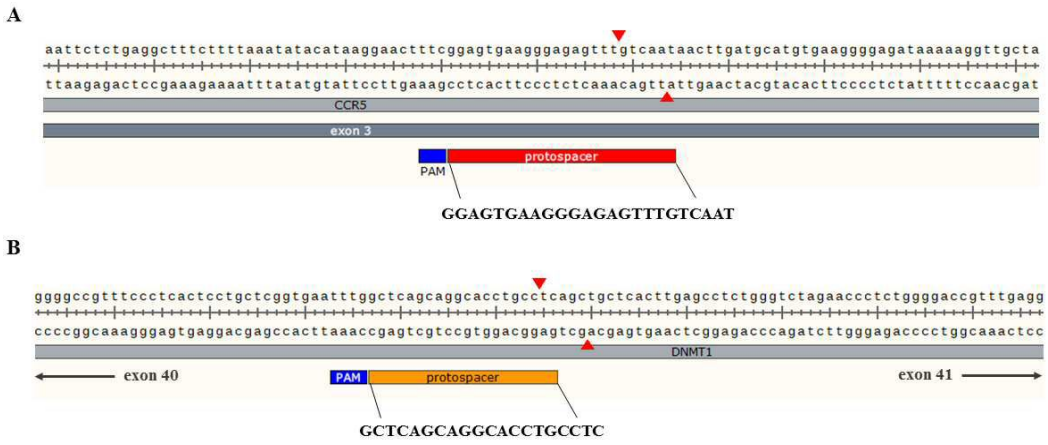
도면12



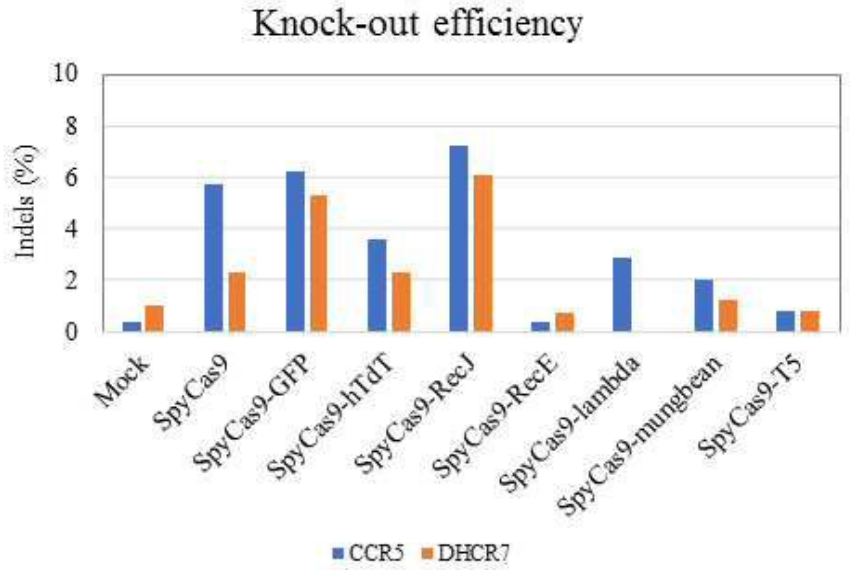
도면13



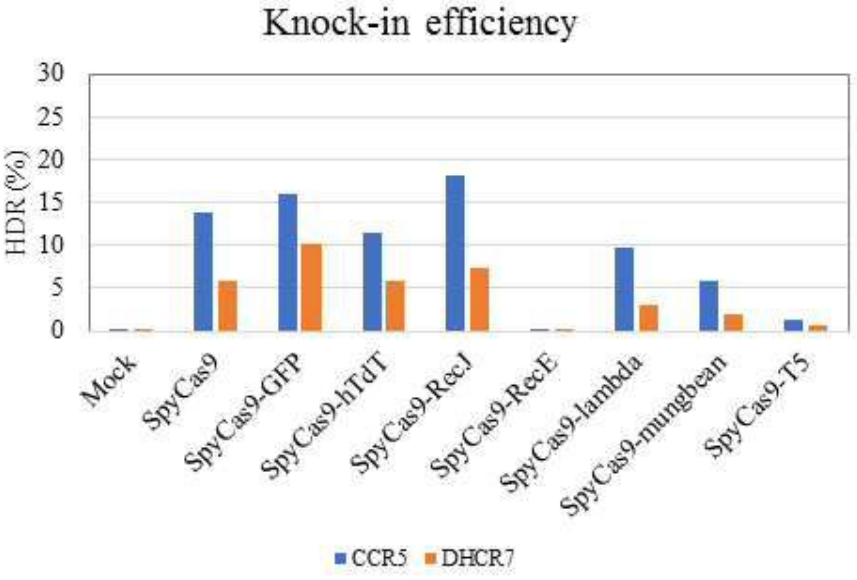
도면14



도면15


Knock-out efficiency
Indels (%)
10
8
6
4
2
0
Mock
SpyCas9
SpyCas9-GFP
SpyCas9-hTdT
SpyCas9-RecJ
SpyCas9-RecE
SpyCas9-lambda
SpyCas9-mungbean
SpyCas9-T5
CCR5
DHCR7

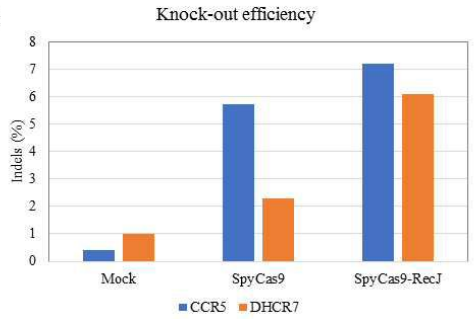
도면16


Knock-in efficiency
HDR (%)
30
25
20
15
10
5
0
Mock
SpyCas9
SpyCas9-GFP
SpyCas9-hTdT
SpyCas9-RecJ
SpyCas9-RecE
SpyCas9-lambda
SpyCas9-mungbean
SpyCas9-T5
CCR5
DHCR7

도면17

A

B



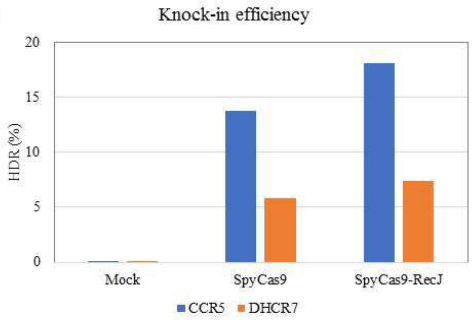
C

Percent change in efficiency of SpyCas9-RecJ over SpyCas9		
	CCR5	DHCR7
SpyCas9-RecJ	+30%	+170%

도면18

A

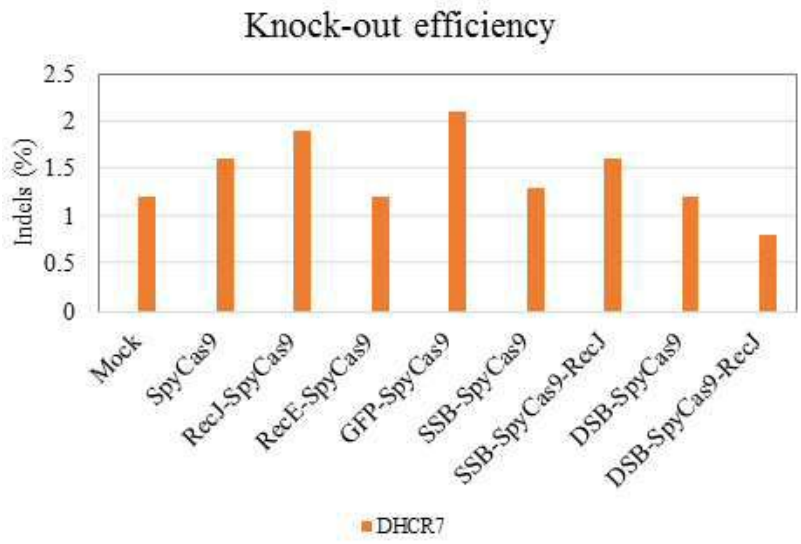
B



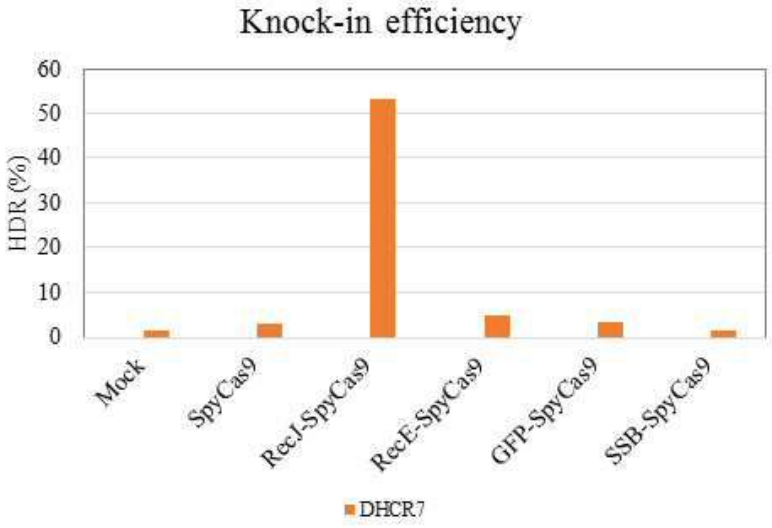
C

Percent change in efficiency of SpyCas9-RecJ over SpyCas9		
	CCR5	DHCR7
SpyCas9-RecJ	+30%	+30%

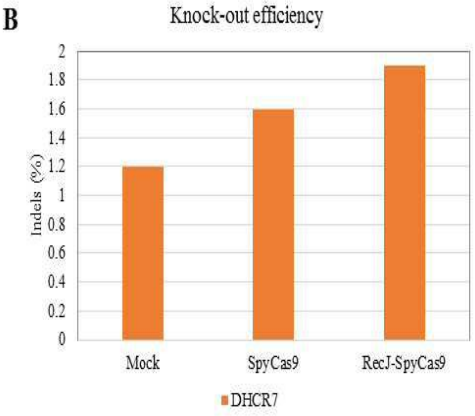
도면19



도면20



도면21



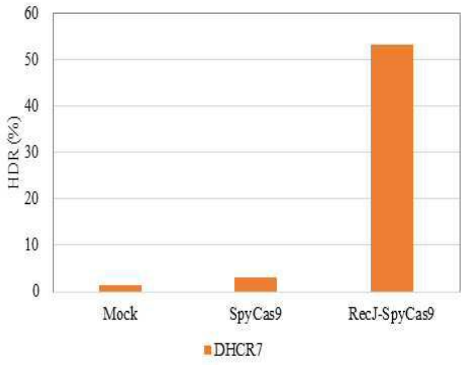
C

Percent change in efficiency of RecJ-SpyCas9 over SpyCas9	
	DHCR7
RecJ-SpyCas9	+20%

도면22

A

B



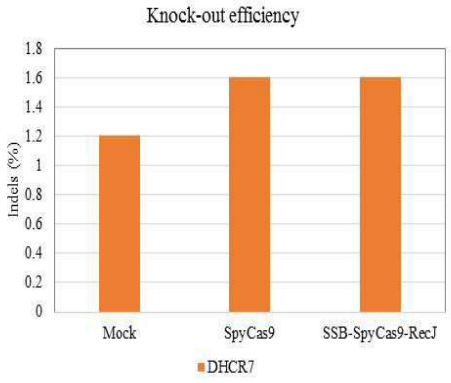
C

Percent change in efficiency of RecJ-SpyCas9 over SpyCas9	
	DHCR7
RecJ-SpyCas9	+1770%

도면23

A

B



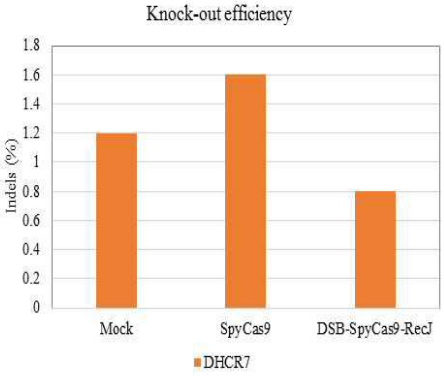
C

Percent change in efficiency of SSB-SpyCas9-RecJ over SpyCas9	
	DHCR7
SSB-SpyCas9-RecJ	0%

도면24

A

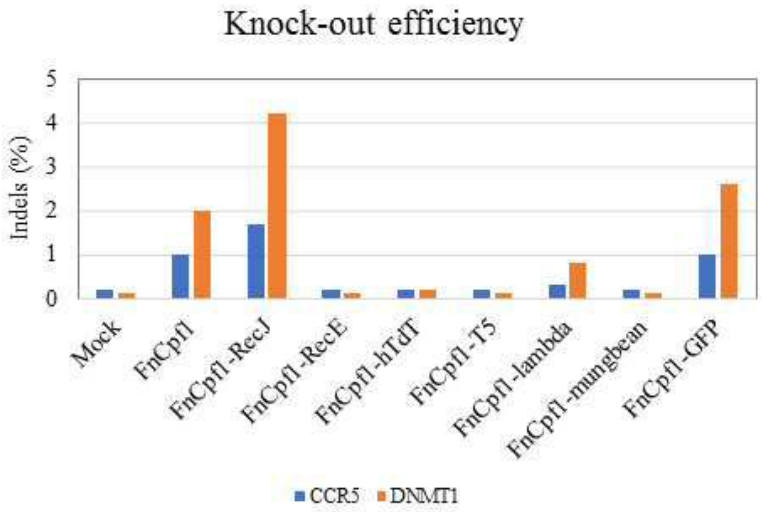
B



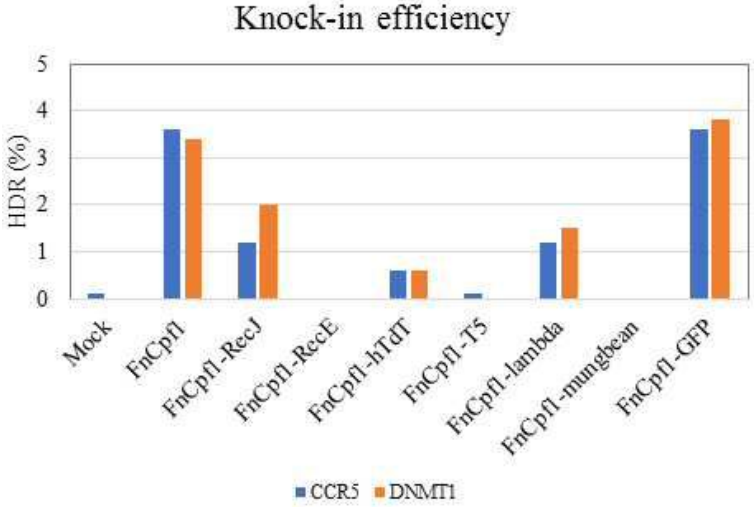
C

Percent change in efficiency of DSB-SpyCas9-RecJ over SpyCas9	
	DHCR7
DSB-SpyCas9-RecJ	-50%

도면25



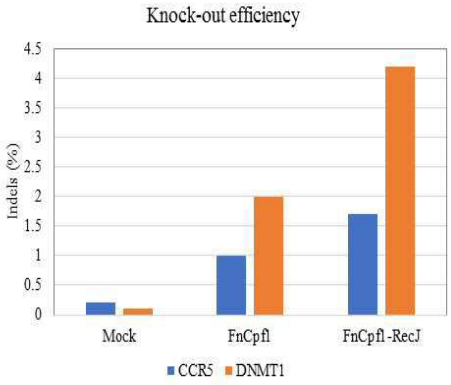
도면26



도면27

A

B



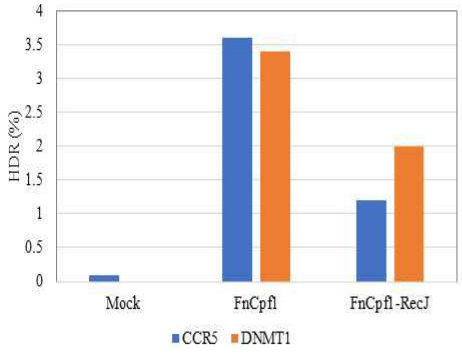
C

Percent change in efficiency of FnCpf1-RecJ over FnCpf1		
	CCR5	DNMT1
FnCpf1-RecJ	+70%	+110%

도면28

A

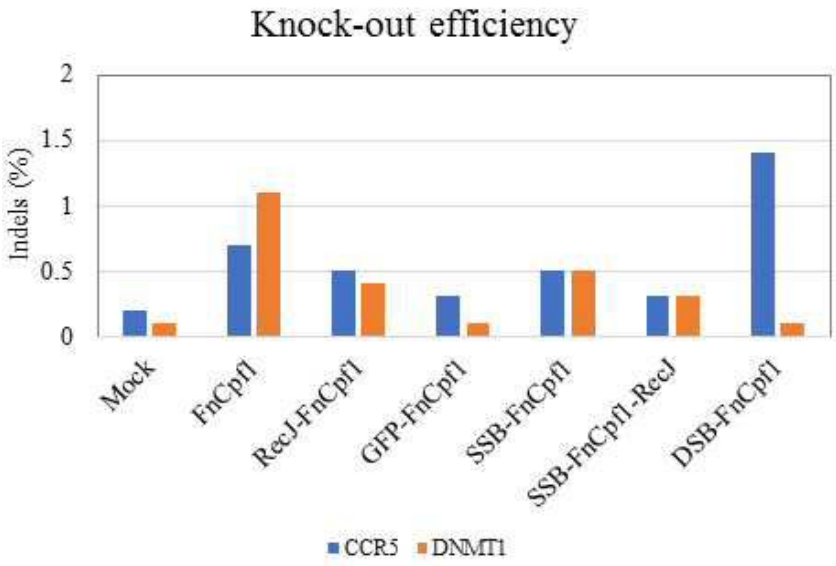
B



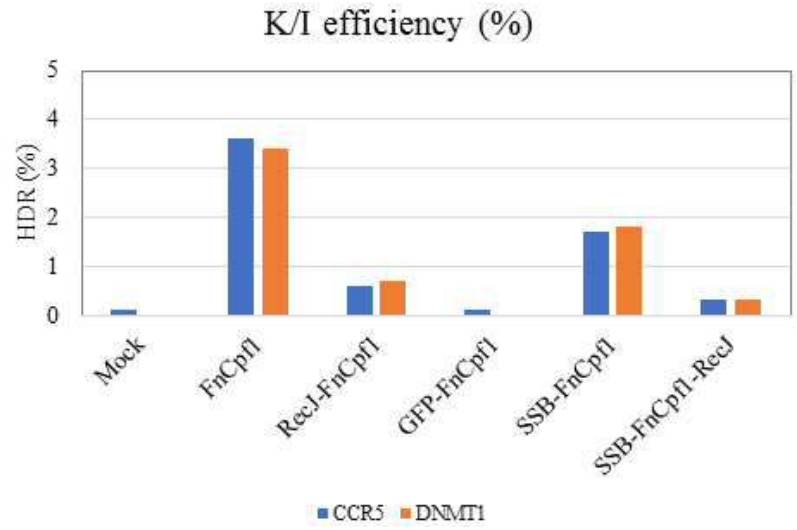
C

Percent change in efficiency of FnCpf1-RecJ over FnCpf1		
	CCR5	DNMT1
FnCpf1-RecJ	-70%	-40%

도면29



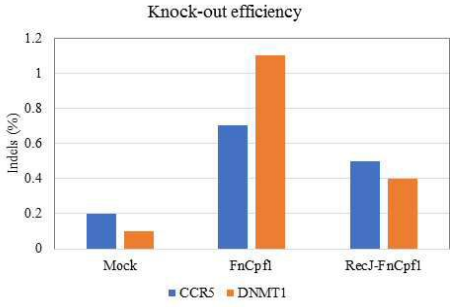
도면30



도면31

A

B



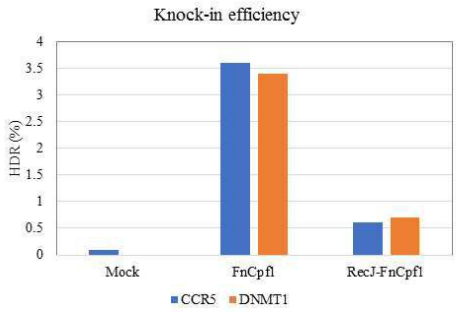
C

Percent change in efficiency of RecJ-FnCpf1 over FnCpf1		
	CCR5	DNMT1
RecJ-FnCpf1	-30%	-60%

도면32

A

B



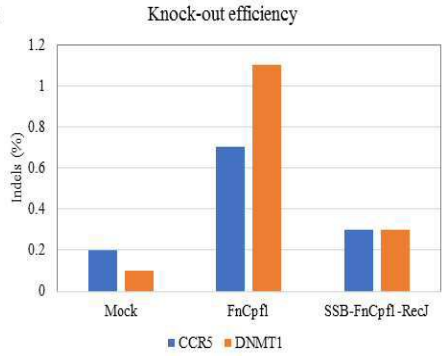
C

Percent change in efficiency of RecJ-FnCpf1 over FnCpf1		
	CCR5	DNMT1
RecJ-FnCpf1	-80%	-80%

도면33

A

B



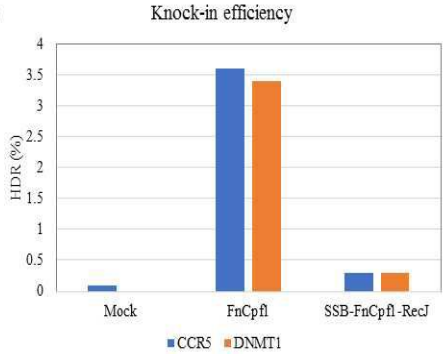
C

Percent change in efficiency of SSB-FnCpf1-RecJ over FnCpf1		
	CCR5	DNMT1
SSB-FnCpf1-RecJ	-60%	-70%

도면34

A

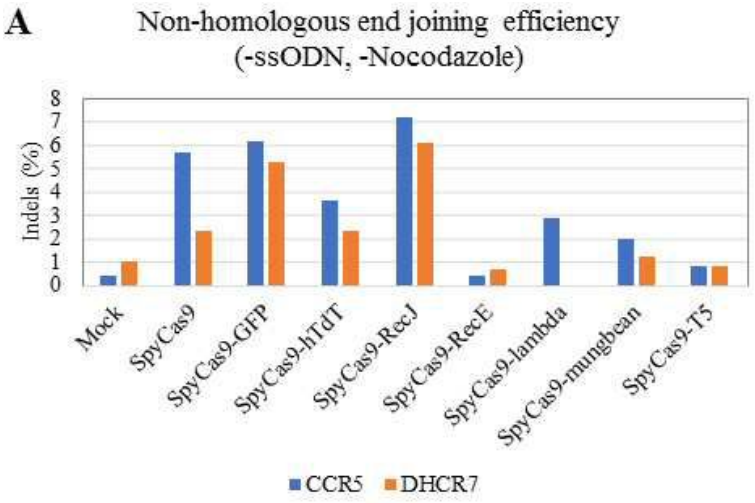
B



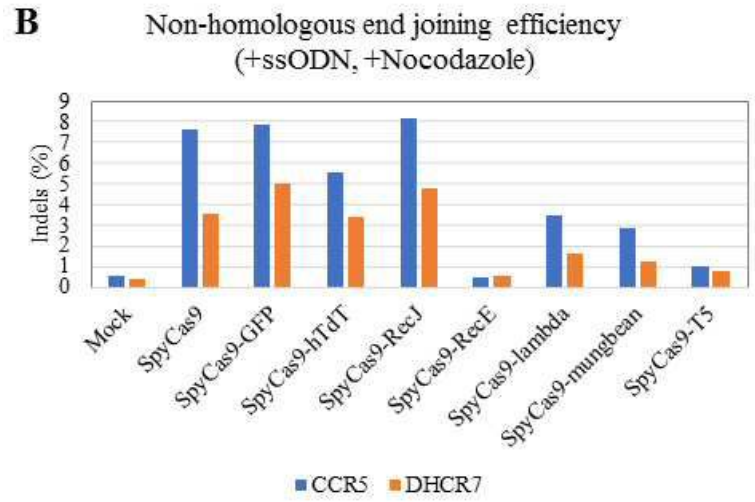
C

Percent change in efficiency of SSB-FnCpf1-RecJ over FnCpf1		
	CCR5	DNMT1
SSB-FnCpf1-RecJ	-90%	-90%

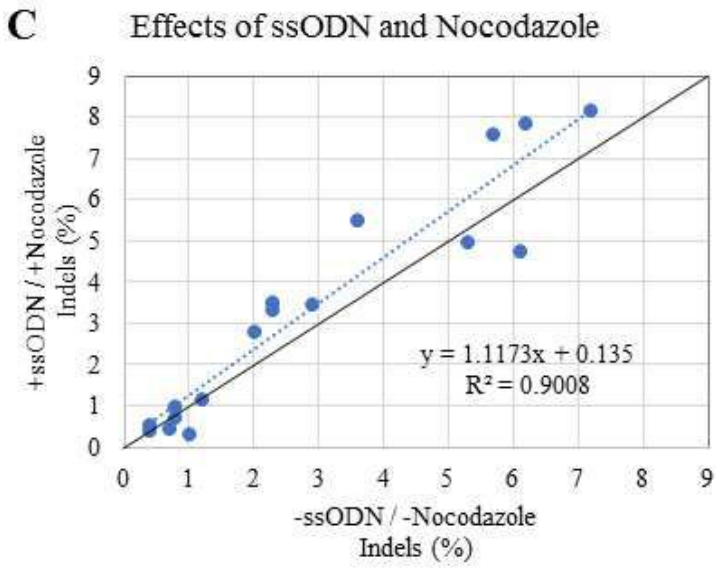
도면35a



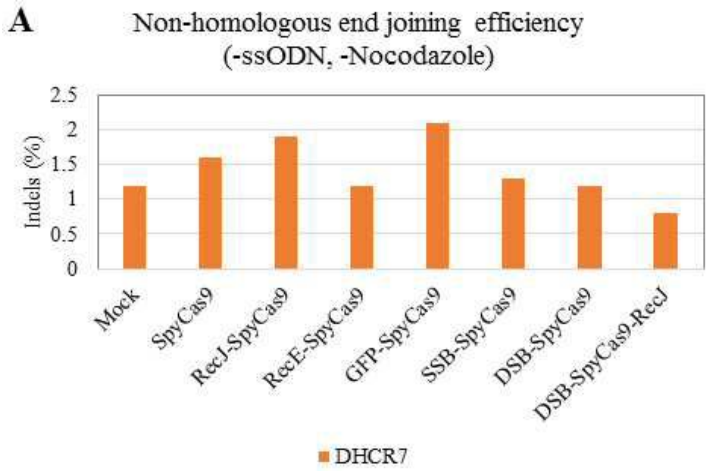
도면35b



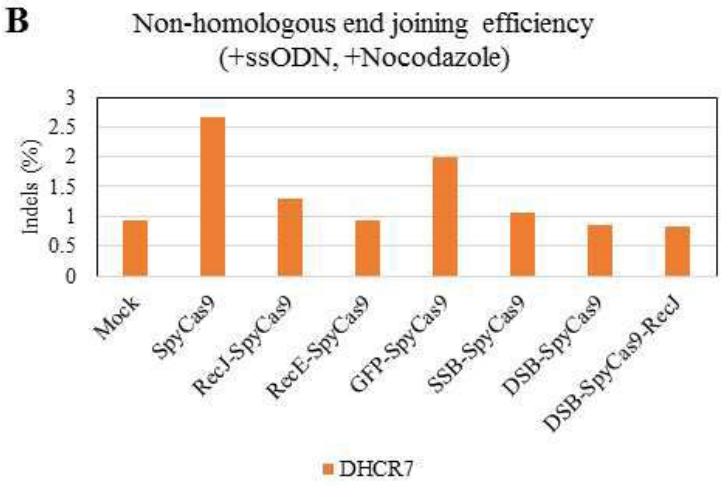
도면35c



도면36a



도면36b



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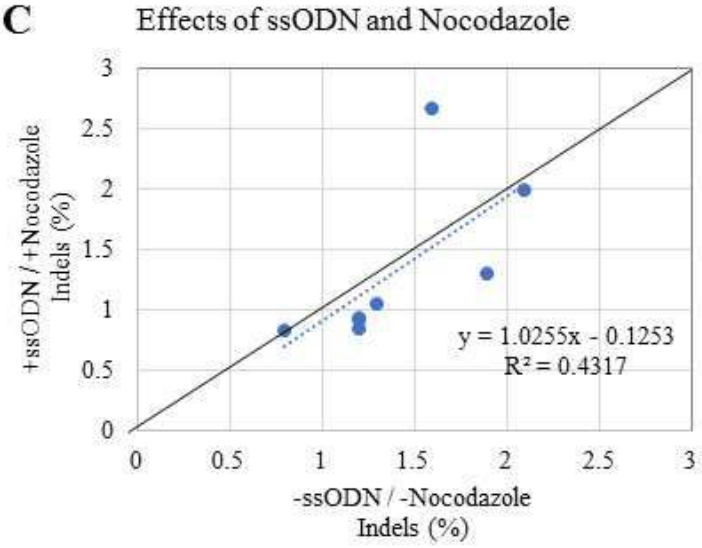
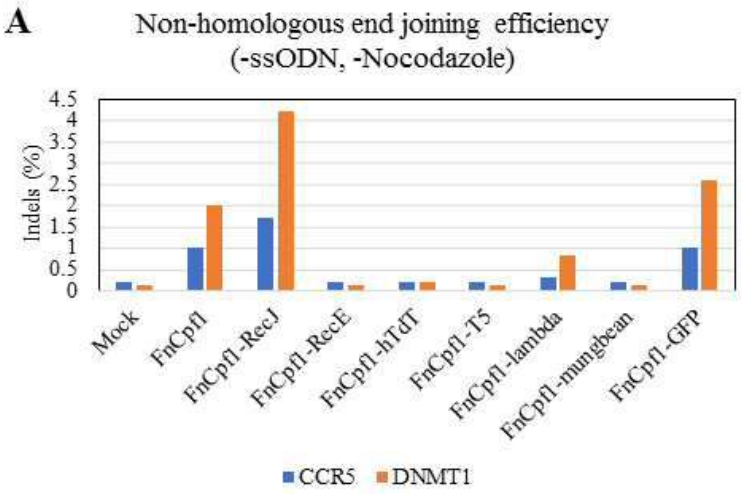
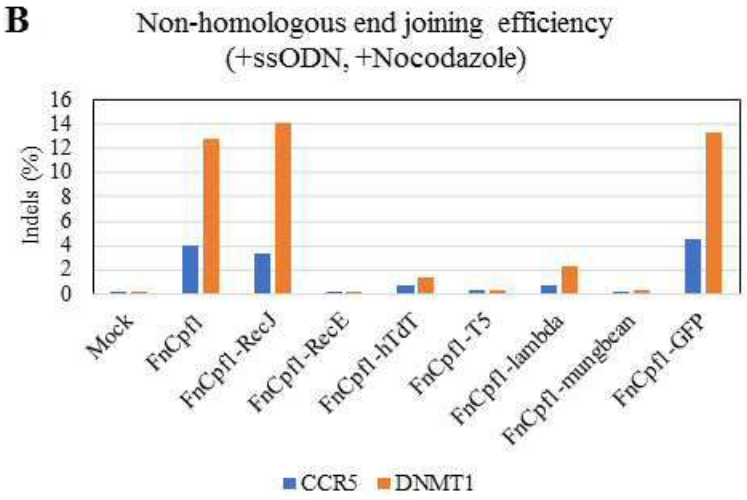


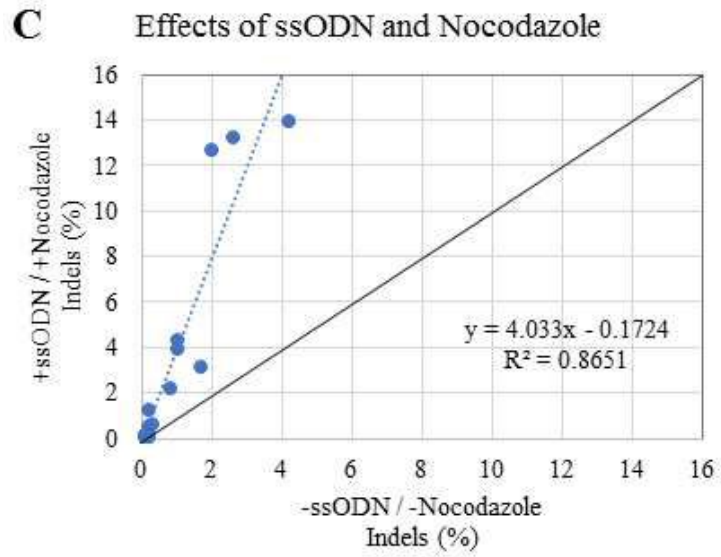
도면37a



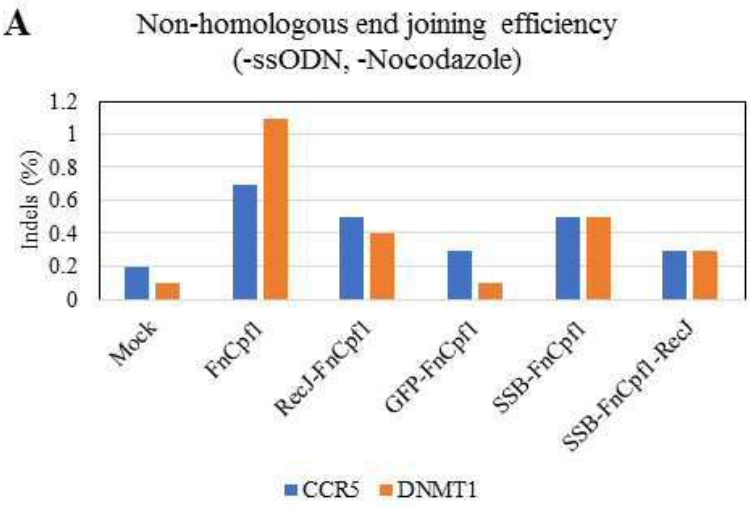
도면37b



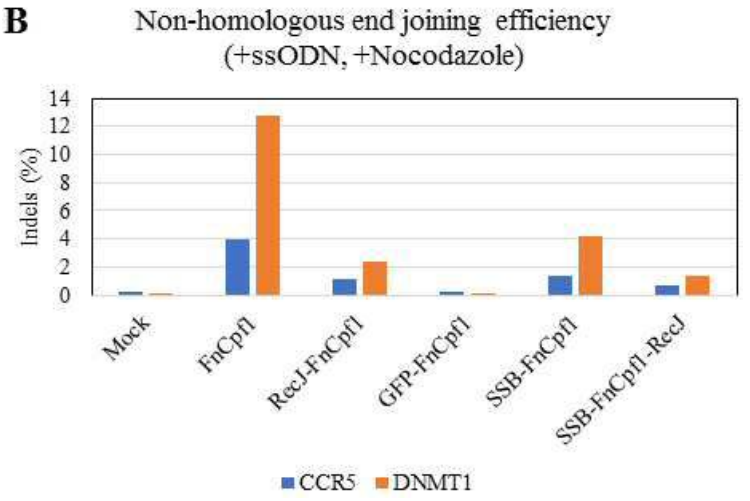
도면37c



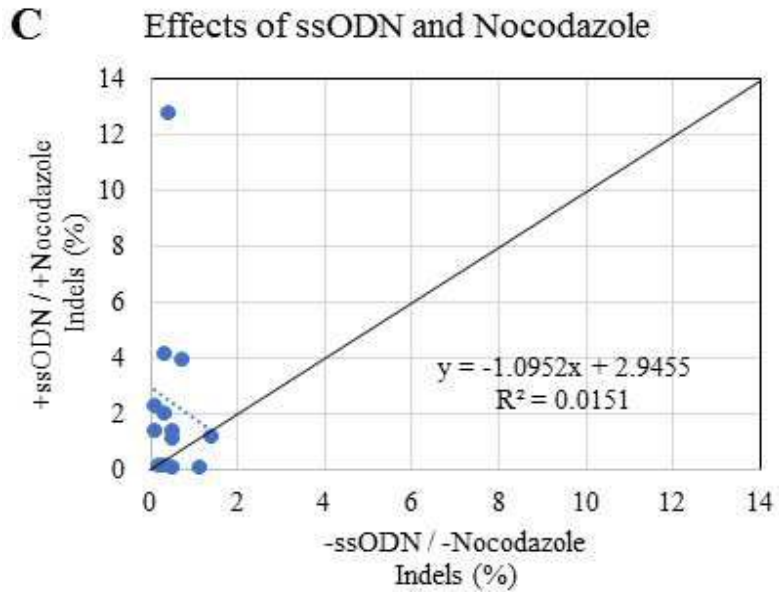
도면38a



도면38b

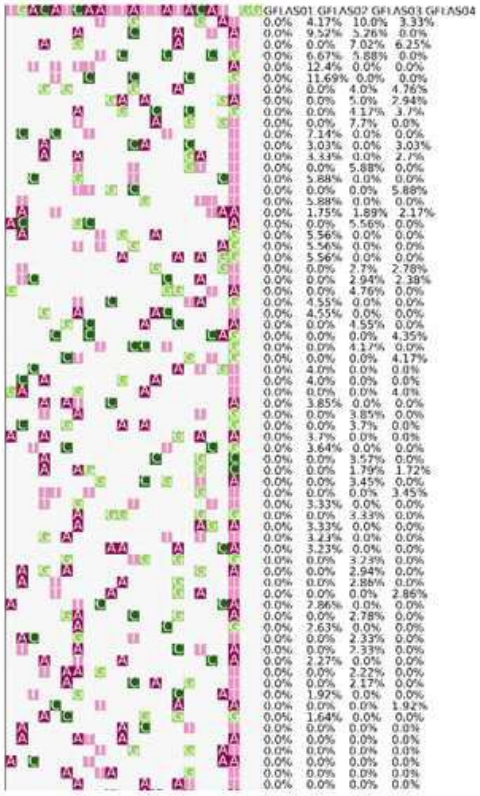


도면38c



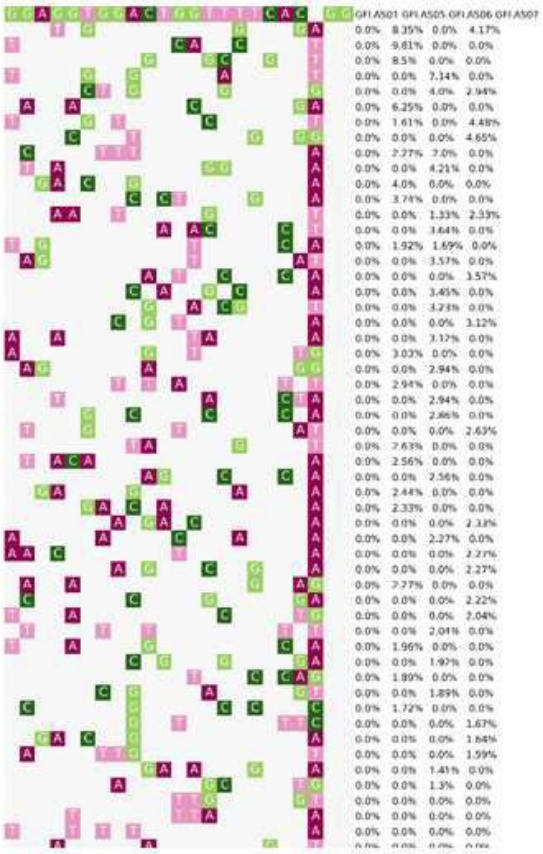
도면39a

Off-Target effect of Crispr on CCR5



도면39b

Off-Target effect of Crispr on DHCR7



도면40

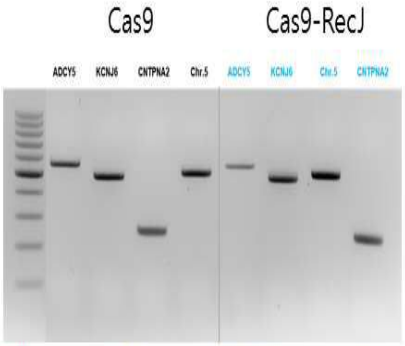
Human CCR 5 off-target candidate

A

Human CCR 5 off-target sequence list

Target	Gene	Spacer sequence
On	CCR5	TGACATCAATTATTATACAT
Off #1	ADCY5	TGACATCAATTATTATAgAT
Off #2	KCNJ6	TGACATCAcTTATTATgCAT
Off #3	CNTNAP2	TGACATaAATTATTcTACAT
Off #4	Chr. 5 N/A	TGAaATCAATTATcATAgAT

B



ADCY5: 641 bp; KCNJ6: 566 bp; CNTNAP2: 300 bp; Chr. 5: 605 bp

도면41

off-targets of CCR5	Cas9		Cas9-RecJ	
	reads (372,767)	percent (%)	reads (406,009)	percent (%)
ADCY5	269	0.0007	1009	0.0025
KCNJ6	339	0.0009	254	0.0006
CNTPNA2	624	0.0017	437	0.0011
Ch.5	601	0.0016	573	0.0014

(by amplicon deep sequencing)

도면42

SpyCas9

Nucleotide sequence

ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACTGA

Amino acid sequence

MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSEFELRRQACGRMDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDAAALDLQKDQQDLRRREVDETADLPALERLPPLLRRYVASRGVRSAQELSRSVKGHPQLIKSLKDVQAVEELRSVVKGMLPNVQQLSGVDKNAVELVNAFRGTRNVVGDFDADQAISTALSVLLAMRSLGCSNIDYLVPNERFDGVFGLSPEVVDQAHAKRGAQALNT

도면43

SpyCas9-RecJ

Nucleotide sequence

ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACTGA

Amino acid sequence

MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSEFELRRQACGRMDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDAAALDLQKDQQDLRRREVDETADLPALERLPPLLRRYVASRGVRSAQELSRSVKGHPQLIKSLKDVQAVEELRSVVKGMLPNVQQLSGVDKNAVELVNAFRGTRNVVGDFDADQAISTALSVLLAMRSLGCSNIDYLVPNERFDGVFGLSPEVVDQAHAKRGAQALNTQPTRKEIEQGMQVEALTCEKLERSRDTLPGGLAMYHPEWHQQVVVGLASRIKERFHRPVIAFAPAGDGTLKSGSRGIQGLHMVRDALERLDTLPPSLLQGEVVSDGPLSPAEMTMEVAQLLRDAGPWGQMPPEPLPDGHPRLLQQRLVGERHLKVMVEPVQGGPLLDIGRAPNVDTALWPDNGVRBVQLAYYLDINHPGNRSLQIIEDNIWPLKQKPAATKKAGQAKKKKHHHHHH.

도면44

도면45

도면46

도면47

FnCpf1

Nucleotide sequence

ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCATGTCAATTTATCAAGAATTTGTTAATAAATATAGTTTAAGTAAAA
CTCTAAGATTTGAGTTAATCCCACAGGGTAAAACACTTGAAAACATAAAAGCAAGAGGTTTGATTTTAGATGATGAGAAAAGAGCTAAAGACTACAAAAAGGCTAAACAAATAATTGATAAATATCATCAGTTTTTTATAGAGGAGATATTAAGTTCGGTTTGTATTAGCGAAGATTTATTACA
AAACTATTCTGATGTTTATTTTAAACTTAAAAAGAGTGATGATGATAATCTACAAAAAGATTTTAAAAGTGCAAAAGATACGATAAAGAAACAAATATCTGAATATATAAAGGACTCAGAGAAATTTAAGAATTTGTTTAATCAAAACCTTATCGATGCTAAAAAAGGGCAAGAGTCAGATTTA
ATTCTATGGCTAAAGCAATCTAAGGATAATGGTATAGAACTATTTAAAGCCAATAGTGATATCACAGATATAGATGAGGCGTTAGAAATAATCAAATCTTTTAAAGGTTGGACAACTTATTTTAAGGGTTTTCATGAAAATAGAAAAAATGTTTATAGTAGCAATGATATTCCTACATCTATTATT
TATAGGATAGTAGATGATAATTTGCCTAAATTTCTAGAAAATAAAGCTAAGTATGAGAGTTTAAAAGACAAAGCTCCAGAAGCTATAAACTATGAACAAATTAAAAAAGATTTGGCAGAAGAGCTAACCTTTGATATTGACTACAAAACATCTGAAGTTAATCAAAGAGTTTTTTCACTTGATG
AAGTTTTTGAGATAGCAAACTTTAATAATTATCTAAATCAAAGTGGTATTACTAAATTTAATACTATTATTGGTGGTAAATTTGTAAATGGTGAAAATACAAAGAGAAAAGGTATAAATGAATATATAAATCTATACTCACAGCAAATAAATGATAAAACACTCAAAAAAATATAAAATGAGTGTT
TTATTTAAGCAAATTTTAAGTGATACAGAATCTAAATCTTTTGTAATTGATAAGTTAGAAGATGATAGTGATGTAGTTACAACGATGCAAAGTTTTTATGAGCAAATAGCAGCTTTTAAAACAGTAGAAGAAAAAATCTATTAAAGAAACACTATCTTTATTATTTGATGATTTAAAAGCTCAAAAA
CTTGATTTGAGTAAAATTTATTTTAAAAATGATAAATCTCTTACTGATCTATCACAACAAGTTTTTGATGATTATAGTGTTATTGGTACAGCGGTACTAGAATATATAACTCAACAAATAGCACCTAAAAATCTTGATAACCCTAGTAAGAAAGAGCAAGAATTAATAGCCAAAAAAACTGAAAA
AGCAAAATACTTATCTCTAGAAACTATAAAGCTTGCCTTAGAAGAATTTAATAAGCATAGAGATATAGATAAACAGTGTAGGTTTGAAGAAATACTTGCAAACTTTGCGGCTATTCCGATGATATTTGATGAAATAGCTCAAAACAAAGACAATTTGGCACAGATATCTATCAAATATCAAAAT
CAAGGTAAAAAAGACCTACTTCAAGCTAGTGCGGAAGATGATGTTAAAGCTATCAAGGATCTTTTAGATCAAACTAATAATCTCTTACATAAACTAAAAAATATTTCATATTAGTCAGTCAGAAGATAAGGCAAATATTTTAGACAAGGATGAGCATTTTTATCTAGTATTTGAGGAGTGCTACTT
TGAGCTAGCGAATATAGTGCCTCTTTATAACAAAATTAGAAACTATATAACTCAAAAGCCATATAGTGATGAGAAATTTAAGCTCAATTTTGAGAACTCGACTTTGGCTAATGGTTGGGATAAAAATAAAGAGCCTGACAATACGGCAATTTTATTTATCAAAGATGATAAATATTATCTGGGT
GTGATGAATAAGAAAAATAACAAAATATTTGATGATAAAGCTATCAAAGAAAATAAAGGCGAGGGTTATAAAAAAATTGTTTATAAACTTTTACCTGGCGCAAATAAAATGTTACCTAAGGTTTTCTTTTCTGCTAAATCTATAAAATTTTATAATCCTAGTGAAGATATACTTAGAATAAGAAA
TCATTCCACACATACAAAAAATGGTAGTCCTCAAAAAGGATATGAAAAATTTGAGTTTAATATTGAAGATTGCCGAAAATTTATAGATTTTTATAAACAGTCTATAAGTAAGCATCCGGAGTGGAAAGATTTTGGATTTAGATTTTCTGATACTCAAAGATATAATTCTATAGATGAATTTTATAG
AGAAGTTGAAAATCAAGGCTACAAACTAACTTTTGAAAATATATCAGAGAGCTATATTGATAGCGTAGTTAATCAGGGTAAATTGTACCTATTCCAAATCTATAATAAAGATTTTTCAGCTTATAGCAAAGGGCGACCAAATCTACATACTTTATATTGGAAAGCGCTGTTTGATGAGAGAAAT
CTTCAAGATGTGGTTTATAAGCTAAATGGTGAGGCAGAGCTTTTTTATCGTAAACAATCAATACCTAAAAAAATCACTCACCCAGCTAAAGAGGCAATAGCTAATAAAAACAAAGATAATCCTAAAAAAGAGAGTGTTTTTGAATATGATTTAATCAAAGATAAACGCTTTACTGAAGATAAGT
TTTTCTTTCACTGTCCTATTACAATCAATTTTAAATCTAGTGGAGCTAATAAGTTTAATGATGAAATCAATTTATTGCTAAAAGAAAAAGCAAATGATGTTCATATATTAAGTATAGATAGAGGTGAAAGACATTTAGCTTACTATACTTTGGTAGATGGTAAAGGCAATATCATCAAACAAGATA
CTTTCAACATCATTGGTAATGATAGAATGAAAACAAACTACCATGATAAGCTTGCTGCAATAGAGAAAGATAGGGATTCAGCTAGGAAAGACTGGAAAAAGATAAATAACATCAAAGAGATGAAAGAGGGCTATCTATCTCAGGTAGTTCATGAAATAGCTAAGCTAGTTATAGAGTATAAT
GCTATTGTGGTTTTTGAGGATTTAAATTTTGGATTTAAAAGAGGGCGTTTCAAGGTAGAGAAGCAGGTCTATCAAAAGTTAGAAAAAATGCTAATTGAGAAACTAAACTATCTAGTTTTCAAAGATAATGAGTTTGATAAAACTGGGGGAGTGCTTAGAGCTTATCAGCTAACAGCACCTTTTG
AGACTTTTAAAAAGATGGGTAAACAAACAGGTATTATCTACTATGTACCAGCTGGTTTTACTTCAAAAATTTGTCCTGTAACTGGTTTTGTAAATCAGTTATATCCTAAGTATGAAAGTGTCAGCAAATCTCAAGAGTTCTTTAGTAAGTTTGACAAGATTTGTTATAACCTTGATAAGGGCTATTT
TGAGTTTAGTTTTGATTATAAAAACTTTGGTGACAAGGCTGCCAAAGGCAAGTGGACTATAGCTAGCTTTGGGAGTAGATTGATTAACTTTAGAAATTCAGATAAAAATCATAATTGGGATACTCGAGAAGTTTATCCAACTAAAGAGTTGGAGAAATTGCTAAAAGATTATTCTATCGAATAT
GGGCATGGCGAATGTATCAAAGCAGCTATTTGCGGTGAGAGCGACAAAAAGTTTTTTGCTAAGCTAACTAGTGTCCTAAATACTATCTTACAAATGCGTAACTCAAAAACAGGTACTGAGTTAGATTATCTAATTTCACCAGTAGCAGATGTAAATGGCAATTTCTTTGATTCGCGACAGGCG
CCAAAAAATATGCCTCAAGATGCTGATGCCAATGGTGCTTATCATATTGGGCTAAAAGGTCTGATGCTACTAGGTAGGATCAAAAATAATCAAGAGGGCAAAAAACTCAATTTGGTTATCAAAAATGAAGAGTATTTTGAGTTCGTGCAGAATAGGAATAACCAAGCGGCCGCACTCGAGAA
AAGGCCGGCGGCCACGAAAAAGGCCGGCCAGGCAAAAAAGAAAAAGTCGACACCACCACCACCACCACTGAGATCCGGCTGCTAA

Amino acid sequence

MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSEFELRRQACGRMSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFIEEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFNQNLIDAKKGQESDLILWLKQSKDN
GIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENRKNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDYKTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQINDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDV
VTTMQSFYEQIAAFKTVEEKSIKETLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPSKKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNKDNLAQISIKYQNQGKKDLLQASAEDDVKAIKDLLDQTNNLLHKLKIFHISQ
SEDKANILDKDEHFYLVFEECYFELANIVPLYNKIRNYITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAILFIKDDKYYLGVMNKKNNKIFDDKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFYNPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSD
TQRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTLYWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLIKDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSIDRGERHLAYYTLVDGKGNIIKQDT
FNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQVVHEIAKLVIEYNAIVVFEDLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVFKDNEFDKTGGVLRAYQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKSQEFFSKFDKICYNLDKGYFEFSFDYKNFGD
KAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYPTKELEKLLKDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVADVNGNFFDSRQAPKNMPQDADANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNNQAAALEKRPAATKKAGQAKKKKSTP
PPPPLRSGC

도면48

FnCpf1-RecJ

Nucleotide sequence

ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCATGTCAATTTATCAAGAATTTGTTAATAAATATAGTTTAAGTAAAA
CTCTAAGATTTGAGTTAATCCCACAGGGTAAAACACTTGAAAACATAAAAGCAAGAGGTTTGATTTTAGATGATGAGAAAAGAGCTAAAGACTACAAAAAGGCTAAACAAATAATTGATAAATATCATCAGTTTTTTATAGAGGAGATATTAAGTTCGGTTTGTATTAGCGAAGATTTATTACA
AAACTATTCTGATGTTTATTTTAAACTTAAAAAGAGTGATGATGATAATCTACAAAAAGATTTTAAAAGTGCAAAAGATACGATAAAGAAACAAATATCTGAATATATAAAGGACTCAGAGAAATTTAAGAATTTGTTTAATCAAAACCTTATCGATGCTAAAAAAGGGCAAGAGTCAGATTTA
ATTCTATGGCTAAAGCAATCTAAGGATAATGGTATAGAACTATTTAAAGCCAATAGTGATATCACAGATATAGATGAGGCGTTAGAAATAATCAAATCTTTTAAAGGTTGGACAACTTATTTTAAGGGTTTTCATGAAAATAGAAAAAATGTTTATAGTAGCAATGATATTCCTACATCTATTATT
TATAGGATAGTAGATGATAATTTGCCTAAATTTCTAGAAAATAAAGCTAAGTATGAGAGTTTAAAAGACAAAGCTCCAGAAGCTATAAACTATGAACAAATTAAAAAAGATTTGGCAGAAGAGCTAACCTTTGATATTGACTACAAAACATCTGAAGTTAATCAAAGAGTTTTTTCACTTGATG
AAGTTTTTGAGATAGCAAACTTTAATAATTATCTAAATCAAAGTGGTATTACTAAATTTAATACTATTATTGGTGGTAAATTTGTAAATGGTGAAAATACAAAGAGAAAAGGTATAAATGAATATATAAATCTATACTCACAGCAAATAAATGATAAAACACTCAAAAAAATATAAAATGAGTGTT
TTATTTAAGCAAATTTTAAGTGATACAGAATCTAAATCTTTTGTAATTGATAAGTTAGAAGATGATAGTGATGTAGTTACAACGATGCAAAGTTTTTATGAGCAAATAGCAGCTTTTAAAACAGTAGAAGAAAAAATCTATTAAAGAAACACTATCTTTATTATTTGATGATTTAAAAGCTCAAAAA
CTTGATTTGAGTAAAATTTATTTTAAAAATGATAAATCTCTTACTGATCTATCACAACAAGTTTTTGATGATTATAGTGTTATTGGTACAGCGGTACTAGAATATATAACTCAACAAATAGCACCTAAAAATCTTGATAACCCTAGTAAGAAAGAGCAAGAATTAATAGCCAAAAAAACTGAAAA
AGCAAAATACTTATCTCTAGAAACTATAAAGCTTGCCTTAGAAGAATTTAATAAGCATAGAGATATAGATAAACAGTGTAGGTTTGAAGAAATACTTGCAAACTTTGCGGCTATTCCGATGATATTTGATGAAATAGCTCAAAACAAAGACAATTTGGCACAGATATCTATCAAATATCAAAAT
CAAGGTAAAAAAGACCTACTTCAAGCTAGTGCGGAAGATGATGTTAAAGCTATCAAGGATCTTTTAGATCAAACTAATAATCTCTTACATAAACTAAAAAATATTTCATATTAGTCAGTCAGAAGATAAGGCAAATATTTTAGACAAGGATGAGCATTTTTATCTAGTATTTGAGGAGTGCTACTT
TGAGCTAGCGAATATAGTGCCTCTTTATAACAAAATTAGAAACTATATAACTCAAAAGCCATATAGTGATGAGAAATTTAAGCTCAATTTTGAGAACTCGACTTTGGCTAATGGTTGGGATAAAAATAAAGAGCCTGACAATACGGCAATTTTATTTATCAAAGATGATAAATATTATCTGGGT
GTGATGAATAAGAAAAATAACAAAATATTTGATGATAAAGCTATCAAAGAAAATAAAGGCGAGGGTTATAAAAAAATTGTTTATAAACTTTTACCTGGCGCAAATAAAATGTTACCTAAGGTTTTCTTTTCTGCTAAATCTATAAAATTTTATAATCCTAGTGAAGATATACTTAGAATAAGAAA
TCATTCCACACATACAAAAAATGGTAGTCCTCAAAAAGGATATGAAAAATTTGAGTTTAATATTGAAGATTGCCGAAAATTTATAGATTTTTATAAACAGTCTATAAGTAAGCATCCGGAGTGGAAAGATTTTGGATTTAGATTTTCTGATACTCAAAGATATAATTCTATAGATGAATTTTATAG
AGAAGTTGAAAATCAAGGCTACAAACTAACTTTTGAAAATATATCAGAGAGCTATATTGATAGCGTAGTTAATCAGGGTAAATTGTACCTATTCCAAATCTATAATAAAGATTTTTCAGCTTATAGCAAAGGGCGACCAAATCTACATACTTTATATTGGAAAGCGCTGTTTGATGAGAGAAAT
CTTCAAGATGTGGTTTATAAGCTAAATGGTGAGGCAGAGCTTTTTTATCGTAAACAATCAATACCTAAAAAAATCACTCACCCAGCTAAAGAGGCAATAGCTAATAAAAACAAAGATAATCCTAAAAAAGAGAGTGTTTTTGAATATGATTTAATCAAAGATAAACGCTTTACTGAAGATAAGT
TTTTCTTTCACTGTCCTATTACAATCAATTTTAAATCTAGTGGAGCTAATAAGTTTAATGATGAAATCAATTTATTGCTAAAAGAAAAAGCAAATGATGTTCATATATTAAGTATAGATAGAGGTGAAAGACATTTAGCTTACTATACTTTGGTAGATGGTAAAGGCAATATCATCAAACAAGATA
CTTTCAACATCATTGGTAATGATAGAATGAAAACAAACTACCATGATAAGCTTGCTGCAATAGAGAAAGATAGGGATTCAGCTAGGAAAGACTGGAAAAAGATAAATAACATCAAAGAGATGAAAGAGGGCTATCTATCTCAGGTAGTTCATGAAATAGCTAAGCTAGTTATAGAGTATAAT
GCTATTGTGGTTTTTGAGGATTTAAATTTTGGATTTAAAAGAGGGCGTTTCAAGGTAGAGAAGCAGGTCTATCAAAAGTTAGAAAAAATGCTAATTGAGAAACTAAACTATCTAGTTTTCAAAGATAATGAGTTTGATAAAACTGGGGGAGTGCTTAGAGCTTATCAGCTAACAGCACCTTTTG
AGACTTTTAAAAAGATGGGTAAACAAACAGGTATTATCTACTATGTACCAGCTGGTTTTACTTCAAAAATTTGTCCTGTAACTGGTTTTGTAAATCAGTTATATCCTAAGTATGAAAGTGTCAGCAAATCTCAAGAGTTCTTTAGTAAGTTTGACAAGATTTGTTATAACCTTGATAAGGGCTATTT
TGAGTTTAGTTTTGATTATAAAAACTTTGGTGACAAGGCTGCCAAAGGCAAGTGGACTATAGCTAGCTTTGGGAGTAGATTGATTAACTTTAGAAATTCAGATAAAAATCATAATTGGGATACTCGAGAAGTTTATCCAACTAAAGAGTTGGAGAAATTGCTAAAAGATTATTCTATCGAATAT
GGGCATGGCGAATGTATCAAAGCAGCTATTTGCGGTGAGAGCGACAAAAAGTTTTTTGCTAAGCTAACTAGTGTCCTAAATACTATCTTACAAATGCGTAACTCAAAAACAGGTACTGAGTTAGATTATCTAATTTCACCAGTAGCAGATGTAAATGGCAATTTCTTTGATTCGCGACAGGCG
CCAAAAAATATGCCTCAAGATGCTGATGCCAATGGTGCTTATCATATTGGGCTAAAAGGTCTGATGCTACTAGGTAGGATCAAAAATAATCAAGAGGGCAAAAAACTCAATTTGGTTATCAAAAATGAAGAGTATTTTGAGTTCGTGCAGAATAGGAATAACCAAGCGGCCGCACTCGACCT
GCAGGTGAAACAACAGATACAACTTCGTCGCCGTGAAGTCGATGAAACGGCAGACTTGCCCGCTGAATTGCCTCCCTTGCTGCGCCGTTTATACGCCAGCCGGGGCGTGCGCAGTGCGCAAGAACTGGAACGCAGTGTTAAAGGTATGTTGCCCTGGCAGCAACTGAGCGGCGTCGAAA
AGGCCGTTGAGATCCTTTACAACGCTTTTCGCGAAGGAACGCGGATTATTGTGGTCGGCGATTTTGACGCCGACGGCGCGACCAGCACGGCTCTAAGCGTGCTGGCGATGCGCTCGCTTGGTTGCAGCAATATCGACTATCTGGTACCAAACCGTTTCGAAGACGGTTACGGCTTAAGCCC
GGAAGTAGTCGATCAGGCCCATGCCCGTGGCGCGCAGTTAATTGTCACGGTGGATAACGGTATTTCCTCCCATGCGGGCGTTGAACACGCTCGCTCGTTGGGCATTCCGGTTATTGTTACCGATCACCATTTGCCGGGCGAAACATTACCCGCAGCGGAAGCGATCATTAACCCTAACTTGC
GCGACTGTAATTTCCCGTCGAAATCACTGGCAGGCGTGGGTGTGGCGTTTTATCTGATGCTGGCGCTGCGCACCTTTTTGCGCGATCAGGGCTGGTTTGATGAGCGTGGCATCGCAATTCCTAACCTGGCAGAACTGCTGGATCTGGTCGCGCTGGGAACAGTGGCGGACGTCGTGCCGCT
GGACGCTAATAATCGCATTCTGACCTGGCAGGGGATGAGTCGCATCCGTGCCGGAAAGTGCCGTCCAGGGATTAAAGCGCTGCTGGAAGTGGCAAACCGTGATGCACAAAAACTCGCCGCCAGCGATTTAGGTTTTGCGCTGGGGCCACGTCTCAATGCTGCCGGACGACTGGACGATAT
GTCCGTCGGTGTGGCGCTCTTGCTGTGCGACAACATCGGCGAAGCGCGCGTGCTGGCAAATGAACTCGATGCGCTAAACCAGACGCGAAAAGAGATCGAACAAGGAATGCAAGTTGAAGCCCTGACCCTGTGCGAGAAACTGGAGCGAAGTCGCGACACGCTACCCGGCGGGCTGGCA
ATGTATCACCCCGAATGGCATCAGGGCGTTGTCGGTATTCTGGCTTCGCGCATCAAAGAGCGTTTTCACCGTCCGGTTATCGCCTTTGCGCCAGCAGGTGATGGTACGCTGAAAGGTTCAGGTCGCTCCATTCAGGGGCTGCATATGCGTGATGCACTGGAGCGATTAGACACACTCTACCC
TGGCATGATACTGAAGTTTGGCGGTCATGCGATGGCGGCGGGTTTGTCGCTGGAAGAGGATAAATTCGAACTCTTTCAACAACGGTTTGGCGAGCTGGTTACCGAGTGGCTGGACCCTTCGCTATTGCAAGGCGAAGTGGTGTCAGACGGCCCGTTAAGCCCGGCCGAAATGACCATGGA
AGTGGCGCAGCTGCTGCGCGATGCTGGCCCGTGGGGGCAGATGTTCCCGGAGCCGCTGTTTGATGGTCATTTCCGTCTGCTGCAACAGCGGCTGGTGGGCGAACGTCATTTGAAAGTCATGGTCGAACCGGTCGGCGGCGGTCCGCTGCTGGATGGTATTGCTTTTAATGTCGATACCGCC
CTCTGGCCGGATAACGGCGTGCGCGAAGTGCAACTGGCTTACAAGCTCGATATCAACGAGTTTCGCGGCAACCGCAGCCTGCAAATTATCATCGACAATATCTGGCCAATTCTGCAGAAAAGGCCGGCGGCCACGAAAAAGGCCGGCCAGGCAAAAAAGAAAAAGCACCACCACCACC
ACCACTGA

Amino acid sequence

MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSEFELRRQACGRMSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFIEEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFNQNLIDAKKGQESDLILWLKQSKDN
GIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENRKNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDYKTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQINDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDV
VTTMQSFYEQIAAFKTVEEKSIKETLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPSKKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNKDNLAQISIKYQNQGKKDLLQASAEDDVKAIKDLLDQTNNLLHKLKIFHISQ
SEDKANILDKDEHFYLVFEECYFELANIVPLYNKIRNYITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAILFIKDDKYYLGVMNKKNNKIFDDKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFYNPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSD
TQRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTLYWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLIKDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSIDRGERHLAYYTLVDGKGNIIKQDT
FNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQVVHEIAKLVIEYNAIVVFEDLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVFKDNEFDKTGGVLRAYQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKSQEFFSKFDKICYNLDKGYFEFSFDYKNFGD
KAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYPTKELEKLLKDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVADVNGNFFDSRQAPKNMPQDADANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNNQAAALDLQVKQQIQLRRREVDETAD
LPAELPPLLRRLYASRGVRSAQELERSVKGMLPWQQLSGVEKAVEILYNAFREGTRIIVVGDFDADGATSTALSVLAMRSLGCSNIDYLVPNRFEDGYGLSPEVVDQAHARGAQLIVTVDNGISSHAGVEHARSLGIPVIVTDHHLPGETLPAAEAIINPNLRDCNFPSKSLAGVGVAFYLMLALRTFLRDQ
GWFDERGIAIPNLAELLDLVALGTVADVVPLDANNRILTWQGMSRIRAGKCRPGIKALLEVANRDAQKLAASDLGFALGPRLNAAGRLDDMSVGVALLLCDNIGEARVLANELDALNQTRKEIEQGMQVEALTLCEKLERSRDTLPGGLAMYHPEWHQGVVGILASRIKERFHRPVIAFAPAGDGTLKG
SGRSIQGLHMRDALERLDTLYPGMILKFGGHAMAAGLSLEEDKFELFQQRFGELVTEWLDPSLLQGEVVSDGPLSPAEMTMEVAQLLRDAGPWGQMFPEPLFDGHFRLLQQRLVGERHLKVMVEPVGGGPLLDGIAFNVDTALWPDNGVREVQLAYKLDINEFRGNRSLQIIDNIWPILQKRPAATK
KAGQAKKKKHHHHHH

도면49

RecJ-FnCpf1

Nucleotide sequence

ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGCGGATCCGTGAAACAACAGATACAACTTCGTCGCCGTGAAGTCGATGAAACGGCAGACTTGCCCGCTGAATTGCCTCCCTTGCTG
CGCCGTTTATACGCCAGCCGGGGCGTGCGCAGTGCGCAAGAACTGGAACGCAGTGTTAAAGGTATGTTGCCCTGGCAGCAACTGAGCGGCGTCGAAAAGGCCGTTGAGATCCTTTACAACGCTTTTCGCGAAGGAACGCGGATTATTGTGGTCGGCGATTTTGACGCCGACGGCGCGACC
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SSB-FnCpf1-RecJ

Nucleotide sequence

Amino acid sequence

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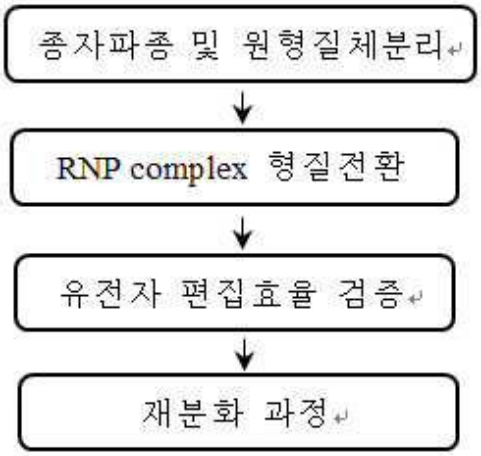

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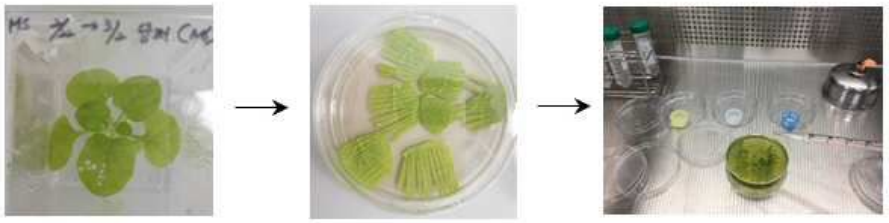

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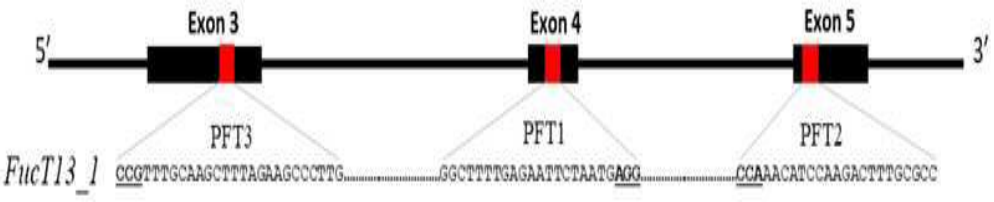

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Conditions	Samples	Total Sequences	With both indicator sequences	More than minimum frequency	Insertions	Deletions	Indel frequency	Indel frequency (%)
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	Cas9	80766	80073	76594	279	574	853 (1.1%)	1.1
	Cas9	73419	72721	69201	879	924	1803 (2.6%)	2.6
	Cas9	95494	94584	90408	629	1353	1982 (2.2%)	2.2
	Cas9:RecJ	102906	101894	97056	2315	2601	4916 (5.1%)	5.1
	Cas9:RecJ	125519	124292	118522	3190	3504	6694 (5.6%)	5.6
	Cas9:RecJ	102660	101069	94971	7330	9381	16711 (17.6%)	17.6
13_1 PFT1	con	196230	144327	138573	7	472	479 (0.3%)	0.3
	Cas9	125076	79885	75976	822	1190	2012 (2.6%)	2.6
	Cas9	38597	21658	20137	711	571	1282 (6.4%)	6.4
	Cas9:RecJ	227861	160703	153332	7741	6873	14614 (9.5%)	9.5
	Cas9:RecJ	198048	152119	144225	11924	8799	20723 (14.4%)	14.4

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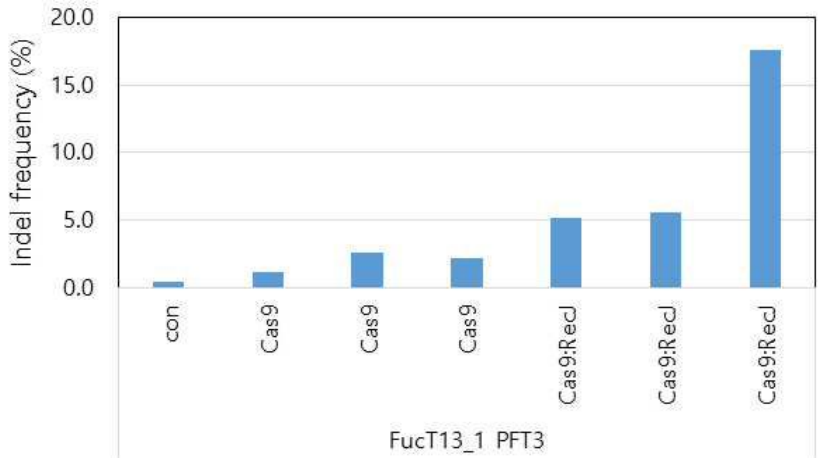

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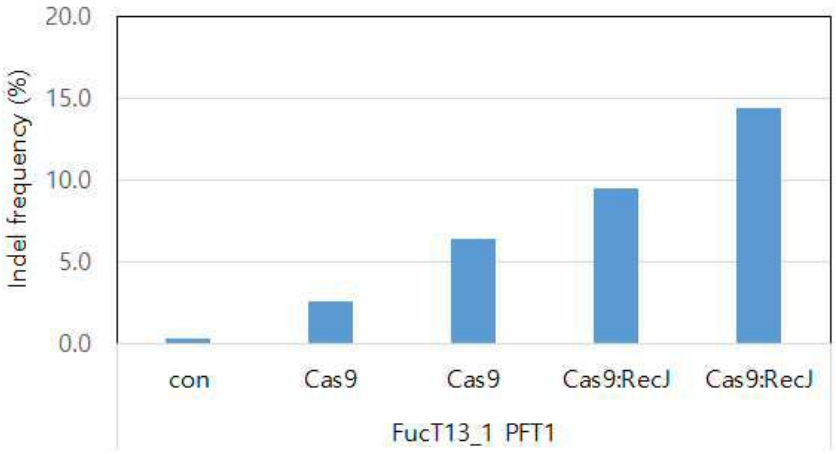

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<130> FPD201811-0042c

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Leu Pro Trp Gln Gln Leu Ser Gly Val Glu Lys Ala Val Glu Ile Leu
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Ala Tyr Leu Leu His Asp Met Asp Lys Ala Val Glu Arg Ile Arg Gln
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Arg Thr Met Gly Ala Gly Asn Ala His Leu Lys Leu Lys Ile Ser Lys

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Gly Glu Ala Ser Phe Glu Val Val Ala Phe Gly Gln Gly Arg Trp Ala

515 520 525

Thr Glu Phe Ser Gln Thr Lys Asn Leu Glu Leu Ala Val Lys Leu Ser

530 535 540

Val Asn Gln Trp Asn Gly Gln Thr Ala Leu Gln Leu Met Met Val Asp

545 550 555 560

Ala Arg Val Glu Gly Val Gln Leu Phe Asn Ile Arg Gly Lys Asn Ala

565 570 575

Val Leu Pro Glu Gly Val Pro Val Leu Asp Phe Pro Gly Glu Leu Pro

580 585 590

Asn Leu Ala Ala Ser Glu Ala Val Val Val Lys Asn Ile Pro Glu Asp

595 600 605

Ile Thr Gln Leu Lys Thr Ile Phe Gln Glu Gln His Phe Ser Ala Val

610 615 620

Tyr Phe Lys Asn Asp Ile Asp Lys Ala Tyr Tyr Leu Thr Gly Tyr Gly

625 630 635 640

Thr Arg Asp Gln Phe Ala Lys Leu Tyr Lys Thr Ile Tyr Gln Phe Pro

645 650 655

Glu Phe Asp Ile Arg Tyr Lys Leu Lys Asp Leu Ala Ala Tyr Leu Asn

660 665 670

Ile Gln Gln Ile Leu Leu Val Lys Met Ile Gln Val Phe Glu Glu Leu

675 680 685

Gly Phe Val Thr Ile Lys Asp Gly Val Met Thr Val Asn Lys Glu Ala

690 695 700

Pro Lys Arg Glu Ile Gly Glu Ser Gln Ile Tyr Gln Asn Leu Lys Gln

705 710 715 720

Thr Val Lys Asp Gln Glu Met Met Ala Leu Gly Thr Val Gln Glu Ile

725 730 735

Tyr Asp Phe Leu Met Glu Lys Glu

740

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<211> 2235

<212> DNA

<213> Artificial Sequence

<220><223> Streptococcus pneumoniae R6 chromosome

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tttgagagag ggattcagga ccaagaaagt ctgaagaagt ttttagaacc ttccttggag 180

gacttacatg atgcttatct gctccatgat atggacaagg cagtggagcg gattcgtcag 240

gctattgaag aaggggaaaa tattctcgtt tatggagact atgatgcgga tggcatgact 300

tcggcttcta ttgtgaagga aagtttggaa caacttggtg ctgagtgccg agtttacctg 360

ccaaatcgtt ttaccgatgg ctatggccct aatgctagtg tttataaata ctttatcgag 420

caagaaggaa tttccttgat tgtgacggtg gacaatgggg ttgctggtca tgaggctatt 480

gcattggctc agtctatggg agtagatgtc attgtgacag accatcattc catgcctgaa 540

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caagtggaat tgcttgattt ggtcgctatt ggaactattg cagatatggt gagtctgacg 720

gatgaaaatc gtatcttagt tcaatatggt ctggaaatgt tgggtcatac ccagcgcatt 780

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<213> Artificial Sequence
<220><223> Lactococcus lactis subsp. lactis Il1403
<400> 5

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35 40 45
Gln Phe Leu Lys Pro Ser Leu Glu Asn Leu His Asp Pro Phe Leu Leu
50 55 60
His Asp Met Glu Lys Ala Thr Gly Arg Ile Leu Ser Ala Ile Glu Gln
65 70 75 80

Gly Glu Asn Ile Leu Ile Tyr Gly Asp Tyr Asp Ala Asp Gly Met Thr
85 90 95
Ala Ser Ser Val Met Lys Ser Ala Leu Asp Glu Leu Gly Ala Glu Ala

100 105 110

Gln Val Tyr Leu Pro Asn Arg Phe Thr Asp Gly Tyr Gly Pro Asn Leu

115 120 125

Asp Val Tyr Gln Tyr Tyr Ile Lys Asn Glu Asn Ile Asn Leu Ile Ile

130 135 140

Thr Val Asp Asn Gly Val Ala Gly Leu Glu Ala Ile Thr Trp Ala Gln

145 150 155 160

Glu Asn Gly Val Asp Val Ile Val Thr Asp His His Ser Ile Pro Asp

165 170 175

Gln Leu Pro Pro Ala Tyr Ala Ile Val His Pro Glu His Pro Asp Ser

180 185 190

Gln Tyr Pro Phe Lys Tyr Leu Ala Gly Val Gly Val Ala Phe Lys Val

195 200 205

Ala Cys Ala Leu Leu Glu Tyr Ala Pro Ser Glu Met Leu Asp Leu Val

210 215 220

Ala Ile Gly Thr Ile Ala Asp Met Val Ser Leu Thr Asp Glu Asn Arg

225 230 235 240

Ile Leu Val Ala His Gly Leu Lys Val Leu Ala Gln Thr Glu Arg Ala

245 250 255

Gly Leu Gln Glu Leu Met Lys Tyr Ala Gly Val Asp Phe Asp Lys Ile

260 265 270

Thr Glu Glu Thr Val Gly Phe Gln Ile Ala Pro Arg Leu Asn Ala Leu

275 280 285

Gly Arg Leu Asp Asp Pro Asn Pro Ala Ile Glu Leu Leu Thr Gly Trp

290 295 300

Asp Glu Asp Glu Ala His Glu Ile Ala Lys Met Ile Asp Gln Lys Asn

305 310 315 320

Ser Glu Arg Lys Glu Ile Val Glu Lys Ile His Asn Glu Ala Leu Ser

325 330 335

Met Leu Thr Asp Glu Pro Val Gln Ile Leu Tyr His Lys Asp Trp His

340 345 350

Lys Gly Val Leu Gly Ile Val Ala Gly Arg Leu Leu Glu Ala Ile His

355 360 365

Lys Pro Val Ile Met Leu Ala Gln Glu Asp Gly Ile Leu Arg Gly Ser
370 375 380
Ala Arg Ser Ile Glu Asn Phe Asp Ile Phe Lys Ala Leu Asn Ala His
385 390 395 400
Arg Glu Leu Phe Ile Ala Phe Gly Gly His Lys Gln Ala Ala Gly Met
405 410 415
Thr Leu Ser Leu Glu Asn Val Glu Ala Val Lys Lys Ala Met Ile Asp
420 425 430
Tyr Ile Val Asp Asn His Leu Asp Met Ser Lys Lys Ser Pro Leu Glu

435 440 445
Ile Ala Asp Arg Cys His Leu Asp Asp Ile Ser Leu Ser Thr Ile Thr
450 455 460
Asn Leu Ala Lys Leu Ala Pro Phe Gly Met Asp Asn Pro Lys Pro Arg
465 470 475 480
Phe Leu Ile Glu Asp Tyr Lys Val Ile Gln Ser Arg Ser Met Gly Lys
485 490 495
Asp Asn Ala His Leu Lys Leu Lys Ile Gln Glu Glu Lys Gln Gln Ile
500 505 510

Asp Ala Val Tyr Phe Gln His Gly Ser Glu Glu Leu Glu Phe Glu Gln
515 520 525
Ala Gln Thr Lys Leu Val Ala Thr Leu Ser Ser Asn Ser Trp Asn Gly
530 535 540
Asn Thr Ser Leu Gln Leu Met Ile Glu Asp Ala Asp Ser Val Gly Val
545 550 555 560
Glu Leu Leu Asp Ile Arg Ser Lys Gln Ile Pro Ile Pro Lys Glu Ala
565 570 575
Asn Ile Phe Ser Gln Asn Gln Leu Lys His Gly Ile Met Glu Asp Val

580 585 590
Leu Val Ile Glu Glu Ile Pro Gly Asp Leu Ala Gly Leu Ser Val Leu
595 600 605

Lys Glu Ala Val Ser Lys Ala Ala Ser Asp Asp Phe Lys Met Ile Tyr
610 615 620
Phe Lys Asn Lys Ile Thr Asp Ser Tyr Tyr Leu Thr Gly Ser Gly Thr
625 630 635 640
Arg Glu Glu Phe Ala Arg Leu Tyr Lys Ala Ile Tyr Gln Phe Pro Glu
645 650 655

Phe Asp Ile Arg Tyr Lys Leu Lys Ser Leu Ala Asp Tyr Leu Lys Ile
660 665 670
Pro His Leu Leu Leu Val Lys Met Ile Lys Ile Phe Glu Glu Leu Glu
675 680 685
Phe Val Ser Ile Asp Asn Gly Leu Met Thr Val Asn Lys Thr Ala Asp
690 695 700
Lys Arg Glu Ile Ser Glu Ser Thr Ile Tyr Gln Glu Leu Glu Lys Ile
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Val Lys Met Gln Glu Leu Phe Ala Leu Ala Pro Val Lys Glu Ile Tyr

725 730 735
Gln Asn Leu Leu Glu Lys
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<212> DNA
<213> Artificial Sequence
<220><223> Lactococcus lactis subsp. lactis Il1403
<400> 6

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ccttttctac ttcatgatat ggaaaaagca acagggcgta ttttatcagc aattgagcaa 240

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gaaaatggag tggatgtaat tgttaccgac catcactcaa ttcctgacca acttcccct 540

gcttatgcca ttgtccaccc cgaacatcct gacagtcaat atcctttcaa atatttggca 600

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cttgatttag ttgcaattgg tacaattgct gacatggtca gtttgactga tgaaaaccga 720

attttagtgg ctcatggtct aaaagttttg gcccaaacag agcgtgccgg cctacaagaa 780

ttaatgaagt atgccggggt tgattttgat aaaatcactg aggaaacggt tggttttcaa 840

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ctgacaggtt gggatgaaga tgaagcgcat gaaattgcca aaatgattga ccaaaaaaat 960

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gaacctgttc agattttata tcataaggac tggcataaag gtgtcttagg aattgttgcg 1080

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cgtgaacttt ttattgcttt tggtggtcat aaacaagccg cagggatgac tttaagtctg 1260

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cttaaattaa aaattcagga agaaaagcaa caaattgatg ccgtttattt ccaacatggt 1560

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cgagaagaat ttgcaagact ttataaagca atttatcaat ttccagaatt tgatattcgc 1980

tataaattaa aatcacttgc ggattatttg aaaatccctc atctcttgct tgtcaagatg 2040

ataaaaatct ttgaggaact tgaatttgtc agcattgaca atggcttaat gaccgttaat 2100

aagacggccg ataaacgcga aatttctgag agtacaattt atcaagaact agaaaaaatt 2160

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<211> 785

<212> PRT

<213> Unknown

<220><223> Bacillus clausii

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Arg Leu Leu Val Arg Arg Gly Ile Gln Thr Val Gln Ala Ala Lys Arg
35 40 45

Phe Leu His Tyr Glu Glu Pro Thr Phe Tyr Asp Pro Phe Leu Leu Lys
50 55 60

Gly Met Glu Glu Thr Ile Glu Arg Ile Ala Leu Ala Val Lys Arg Lys
65 70 75 80

Glu Arg Ile Leu Val Phe Gly Asp Tyr Asp Ala Asp Gly Val Ser Ser
85 90 95

Thr Ser Val Met Leu Thr Ala Leu Gln Thr Tyr Gly Ala Asp Cys Asp
100 105 110

Tyr Tyr Ile Pro Asn Arg Phe Thr Glu Gly Tyr Gly Pro Asn Cys Pro
115 120 125

Ala Leu Asp Phe Ala Lys Arg Gln Gly Tyr His Leu Val Ile Thr Val

130 135 140

Asp Thr Gly Ile Ser Ala Leu Asn Glu Ala Ala His Ala Lys Asp Ile
145 150 155 160

Gly Leu Asp Phe Ile Ile Thr Asp His His Glu Pro Pro Pro Val Leu
165 170 175

Pro Glu Ala Leu Ala Ile Ile Asn Pro Lys Gln Pro Gly Cys Pro Tyr
180 185 190

Pro Phe Lys Glu Leu Ala Gly Val Gly Val Ala Phe Lys Val Ala His
195 200 205

Ala Leu Leu Gly Arg Leu Pro Glu Glu Leu Leu Asp Tyr Ala Val Ile
210 215 220
Gly Thr Ile Ala Asp Leu Val Pro Leu Ile Asp Glu Asn Arg Leu Leu
225 230 235 240
Ala Lys Lys Gly Leu Arg Ala Ile Glu Ser Ser Gly Arg Pro Gly Ile
245 250 255
Arg Ala Leu Lys Glu Val Cys Gly Met Lys Gln Glu Ala Met Asp Ala
260 265 270
Asp His Ile Gly Phe Ala Ile Gly Pro Arg Leu Asn Ala Ala Gly Arg

275 280 285
Leu Asp Ser Ala Asn Pro Ala Val Glu Leu Leu Leu Ala Asp Asp Glu
290 295 300
Glu Glu Ala Lys Ala Leu Ala Thr Glu Ile Asp Ser Leu Asn Lys Glu
305 310 315 320
Arg Gln Ala Ile Val Ser Lys Met Thr Glu Glu Ala Ile Arg Leu Val
325 330 335
Glu Thr Thr Tyr Gly Thr Lys Ile Pro His Ala Ile Val Val Ala Lys
340 345 350

Glu Gly Trp Asn Pro Gly Val Ile Gly Ile Val Ala Ser Arg Leu Val
355 360 365
Glu Gln Phe Tyr Arg Pro Thr Ile Val Met Ser Ile Asp Glu Ser Ser
370 375 380
Gly Leu Ala Lys Gly Ser Ala Arg Ser Ile Glu Gly Phe Asp Met Tyr
385 390 395 400
Gln Glu Leu Ala Asn Asn Arg Asp Ile Leu Pro His Phe Gly Gly His
405 410 415
Pro Met Ala Ala Gly Met Thr Leu Lys Thr Glu Asp Ile Asp Asp Leu

420 425 430
Arg Ser Arg Leu Ile Lys Gln Ala Lys Glu Thr Leu Thr Asp Asp Met
435 440 445
Leu Thr Pro Ala Thr Asp Ile Asp Leu Val Ala Glu Val Glu Asp Val
450 455 460

Thr Val Gln Val Ile Gly Glu Leu Gln Ala Leu Ala Pro Phe Gly Val
465 470 475 480
Ala Asn Arg Lys Pro Ile Val Leu Val Glu Gly Ala His Ile Ser Asp
485 490 495

Met Arg Arg Ile Gly Ser Asn Gln Asn His Leu Lys Ile Gln Phe Thr
500 505 510
Gly Ala Gln Lys Pro Leu Asp Gly Ile Ala Phe Arg Met Gly His Leu
515 520 525
Phe Glu Glu Ile Thr Pro His Ala Lys Leu Ser Ala Ile Gly Thr Val
530 535 540
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545 550 555 560
Asp Leu Ala Val Leu Glu Trp Gln Leu Phe Asp Trp Arg Ser Ile Gln
565 570 575
Pro Asn Arg Leu Asn Ser Arg Leu Leu Asp Leu Pro Arg Glu Lys Leu
580 585 590
Val Ala Ile Ser Phe Gln Glu Gly Thr Lys Glu Arg Leu Gly Leu Asp
595 600 605
Val Pro Val Tyr Asp Tyr Arg Gln Ala Pro Ser Phe Phe Glu Ala Tyr
610 615 620
Val Val Leu Leu Asp Leu Pro Ser Asn Arg Val Glu Leu Glu Ser Leu
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Phe Ser Lys Lys Gly Glu Pro Ser Arg Val Tyr Val Val Phe Ser Glu
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Glu Glu Glu Ser Phe Phe Gln Thr Asn Pro Asn Arg Glu Gln Phe Lys
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Trp Tyr Tyr Gly Phe Ile Lys Lys Asn Gln Arg Phe Ser Leu Asn Gln
675 680 685
Leu Gly Ala Lys Leu Glu Lys His Lys Gly Trp Ser Ala Arg Thr Val
690 695 700
Glu Phe Met Thr Thr Val Phe Leu Glu Leu Gly Phe Ile Lys Leu Asp

705 710 715 720

Asp Ser Ile Val Glu Ala Val Glu Asn Pro Glu Lys Lys Ala Leu Thr

725 730 735

Ala Ser Pro Thr Tyr Gln Ala Lys Gln Glu Lys Ala Trp Leu Glu Asn

740 745 750

Glu Phe Val Phe Ala Ser Tyr Gln Gln Leu Lys Glu Trp Phe Gln Ala

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Leu

785

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<212> DNA

<213> Unknown

<220><223> Bacillus clausii

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gacagcatcg ttgaagcagt agaaaaccct gaaaaaaaag cgttgactgc atcgccgacc 2220

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<211> 590

<212> PRT

<213> Unknown

<220><223> Ehrlichia ruminantium strain Welgevonden

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Asp Ile Leu Thr Ile Lys Gln Lys Phe Tyr Leu Ser Glu Ile Val Ala
35 40 45
Arg Ile Leu Ser Ala Arg Lys Ile Asn Ile Glu Glu Ile Ser Asn Phe

50 55 60
Leu Tyr Pro Thr Leu Lys Ser Ser Leu Pro Asn Pro Phe His Met Leu
65 70 75 80
Asp Met Asp Lys Ala Val Tyr Arg Ile Cys His Ala Ile His Asn Arg
85 90 95
Glu Asn Ile Val Ile Phe Gly Asp Tyr Asp Val Asp Gly Ala Thr Ser
100 105 110
Ser Ala Leu Ile Lys Gln Tyr Leu Ser Gln Ile Gly Val Pro Thr Thr
115 120 125

Ile Tyr Ile Pro Asp Arg Ile Cys Glu Gly Tyr Gly Pro Asn Thr Gln
130 135 140
Ala Leu Leu Lys Leu Lys Glu Ile Gly Asn Ser Leu Cys Ile Thr Val
145 150 155 160
Asp Cys Gly Thr Ile Ala His Glu Pro Ile Ser Ala Ala Lys Ser Val
165 170 175
Asn Leu Asp Val Ile Val Ile Asp His His Ile Gly Ile Asn Thr Leu
180 185 190
Pro Asp Ala Val Ala Val Ile Asn Pro Asn Arg Leu Asp Glu Thr Ser

195 200 205
Pro Tyr Thr Tyr Leu Ala Gly Val Gly Val Ser Phe Leu Met Leu Val
210 215 220
Ala Leu His Lys Thr Leu Lys Glu Gln Gly Phe Phe Lys Asn Asn Gln
225 230 235 240
Glu Pro Asn Leu Ile Asn Tyr Leu Asp Leu Val Ala Leu Gly Thr Val
245 250 255

Cys Asp Val Met Pro Ile Ile Gly Leu Asn Arg Thr Phe Val Lys Gln
260 265 270

Gly Leu Lys Ile Met Thr Thr Arg Gln Asn Leu Gly Leu Lys Thr Leu
275 280 285
Ser Asp Val Ile Gly Leu Glu Glu Lys Pro Asn Ile Tyr Gln Leu Gly
290 295 300
Phe Asn Ile Gly Pro His Ile Asn Ala Gly Gly Arg Val Gly Asn Ala
305 310 315 320
Ser Leu Gly Ala Arg Leu Leu Ser Thr Asn Asn Glu Glu Glu Ala Leu
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Glu Ile Ser Glu Lys Leu Gln Asn Phe Asn Leu Glu Arg Lys Thr Leu

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Glu Asn Gln Ser Phe Asn Glu Ala Val Glu Gln Ile Glu Ala Thr Ile
355 360 365
Ile Ser Ser Asn Ile Ile Ile Ala Thr Gly Asn Trp His Pro Gly Ile
370 375 380
Ile Gly Ile Val Ala Gly Arg Leu Lys Asp Lys Phe Phe Leu Pro Ser
385 390 395 400
Ile Val Ile Ser Leu Glu Asn Gly Ile Gly Lys Ala Ser Ala Arg Ser
405 410 415

Ile Pro Asp Val Asp Leu Gly Ala Ala Ile Leu Glu Ala Lys Ala Leu
420 425 430
Gly Ile Ile Ile Glu Gly Gly Gly His Ala Met Ala Ala Gly Phe Ser
435 440 445
Ile Gln Glu Asn Lys Ile Lys Ile Leu His Glu Phe Leu Ser Gln Lys
450 455 460
Phe His Asn Ile Asn Thr Arg Lys Val Phe Lys Val Asp Gly Ile Val
465 470 475 480
Thr Ala Glu Ala Ile Asn Leu Lys Leu Trp Lys Glu Leu Gln Phe Leu

485 490 495
Glu Pro Phe Gly Ile Gly Asn Pro Glu Pro Arg Phe Ile Leu Thr Asn

500 505 510

Ile Arg Ile Lys Asn Ser Glu Val Ile Gly Glu Ser His Ile Arg Cys

515 520 525

Leu Ile Tyr Asp Asn Lys Thr Phe Ile Lys Gly Ile Cys Phe Arg Cys

530 535 540

Ile Gly Thr Glu Leu Gly Thr Thr Leu Leu Glu Cys Thr Thr Thr Thr

545 550 555 560

Leu Leu Gly Lys Ile Ser Ile Asn Tyr Trp Arg Gly Asn Glu Asn Ile

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Gln Phe Ile Ile Glu Asp Ala Leu Gln His Asn Gln Ile Arg

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<210> 10

<211> 1773

<212> DNA

<213> Unknown

<220><223> Ehrlichia ruminantium strain Welgevonden

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atctttggag actatgatgt agatggagca acatcatctg cattaataaa acaatatcta 360

tcacagatag gagtaccaac aacgatatat attccagatc gtatatgtga aggatatgga 420

ccaaatacac aggccttact aaaattaaaa gaaataggaa atagtctatg tataaccgta 480

gactgcggta ctatagcaca tgaaccaata tccgcagcaa agtcagtaaa tcttgatgtt 540

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cctaatcgct tagacgaaac atccccctac acatatctag caggagtagg cgtatctttt 660

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<210> 11

<211> 589

<212> PRT

<213> Unknown

<220><223> Chlamydia psittaci 6BC

<400> 11

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20 25 30

Leu His Pro Val Ala Ala Gln Thr Phe Ile Ser Arg Gly Phe Gln Thr
35 40 45

Val Asp Glu Val Arg Asp Phe Leu Tyr Val His Leu Ser Asn Leu His

50 55 60

Asp Pro Glu Leu Leu Leu Asp Met Ser Lys Ala Val Gln Arg Leu Leu
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Leu Ala Lys Glu Arg Gly Glu His Val Met Val Tyr Gly Asp Ser Asp
85 90 95
Val Asp Gly Ile Thr Gly Val Ala Leu Leu Val Glu Phe Leu Arg Ser
100 105 110
Ile Glu Met Lys Val Ser Tyr Cys Phe Leu Gly Ala Phe Leu Lys His
115 120 125

Tyr Gly Glu Pro Ser Leu Leu Ile Ala Lys Met Lys Glu Glu Gly Val
130 135 140
Thr Leu Leu Ile Thr Val Asp Cys Gly Ile Thr Ala Gly Lys Glu Val
145 150 155 160
Ser Asp Ile Asn Lys Gln Gly Ile Asp Val Ile Val Thr Asp His His
165 170 175
Met Pro Thr Gly Lys Ile Pro His Cys Ile Ala Thr Leu Asn Pro Lys
180 185 190
Leu Arg Asp His Thr Tyr Pro Asn Lys Asp Leu Thr Gly Val Gly Val

195 200 205
Ala Phe Lys Leu Ala Arg Gly Val Val Asn Ala Leu Gln Lys Asn Asn
210 215 220
Pro Lys Leu Lys Leu Asp Ile Lys His Leu Leu Asp Leu Val Thr Leu
225 230 235 240
Gly Thr Val Thr Asp Val Gly Thr Leu Leu Gly Glu Asn Arg Thr Met
245 250 255
Val Arg His Gly Ile Lys Glu Ile Ala Lys Gly Ser Arg Leu Gly Leu
260 265 270

Arg Lys Leu Cys Ile Phe Ser Gly Val Lys Pro Ser Glu Val Thr Ser
275 280 285
Thr Asp Ile Val Leu Lys Ile Ser Pro Lys Leu Asn Ser Leu Gly Arg
290 295 300
Leu Ala Asp Ala Ser Lys Gly Val Glu Leu Leu Leu Thr Lys Asp Pro
305 310 315 320
Glu Val Ala Asp Asp Leu Ile Gln Tyr Leu Asp Lys Ile Asn Arg Glu
325 330 335

Arg Gln Lys Ile Glu Ala Asp Val Phe His Asp Val Gln Lys Ile Leu

340 345 350

Lys Asn Gln Pro Asp Ile Val Lys Gln Ala Ala Ile Val Leu Ser Ser

355 360 365

Gln Asp Trp His Ser Arg Val Ile Pro Ile Ile Ser Ala Arg Leu Ala

370 375 380

Lys Ala Tyr Asn Lys Pro Val Ala Ile Ile Ser Asn Gln Gly Gly Ile

385 390 395 400

Gly Lys Gly Ser Leu Arg Thr Ile Gly Ser Phe Pro Leu Leu Gly Ile

405 410 415

Leu Gln Lys Cys Ser Pro Met Phe Ile Ser Tyr Gly Gly His Asp Phe

420 425 430

Ala Ala Gly Ile Ile Ile Asn Glu Asp Arg Ile Glu Ala Phe Arg Lys

435 440 445

Lys Phe Ile His Leu Val Asn Ser Ser Leu Lys Lys Glu Lys Ala Val

450 455 460

Val Thr Leu Pro Leu Asp Ala Arg Ala Asp Phe Asp Glu Ile Asp His

465 470 475 480

Asp Leu Leu Ser Ser Ile Asp Leu Phe Glu Pro Phe Gly Lys Gly Asn

485 490 495

Pro Val Pro Ile Phe Tyr Thr Ile Val His Gln Val Arg Tyr Pro Lys

500 505 510

Leu Leu Pro Gly Asn His Leu Lys Leu Tyr Leu Asn Tyr Gly Glu Arg

515 520 525

Asn Leu Glu Gly Ile Ala Phe Gly Leu Gly Asp Arg Ile Gly Ala Leu

530 535 540

Lys Ala Ser Trp Asn Gln Pro Leu Glu Leu Ala Tyr Thr Pro Arg Leu

545 550 555 560

Ser Gln Ser Ala Asn Gly Gly Val Ile His Leu Leu Val Arg Asp Phe

565 570 575

Arg Ile Leu Pro Leu Asn Tyr Lys Asp Thr Thr Ala Arg

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<211> 1773

<212> DNA

<213> Unknown

<220><223> Chlamydia psittaci 6BC

<400> 12

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cagaaaaaca atccaaaact caaattagac attaagcatt tattagattt agtaactcta 720

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<212> PRT

<213> Unknown

<220><223> Finegoldia magna ATCC 29328

<400> 13

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20 25 30

Arg Asn Ile Thr Asp Phe Glu Lys Phe Leu Asn Pro Asn Ala Glu Asn
35 40 45

Ser Tyr His Asn Pro Phe Leu Met Lys Asp Met Asp Lys Ala Val Asp

50 55 60

Ile Ile Ile Ser Thr Ile Asp His Asn Glu Lys Ile Arg Ile Val Gly
65 70 75 80

Asp Tyr Asp Gln Asp Gly Asn Ser Ser Thr Met Thr Leu Leu Asp Gly
85 90 95

Leu Gly Tyr Phe Thr Asp Lys Ile Ser Tyr Asp Ile Pro Asn Arg Met
100 105 110

Thr Asp Gly Tyr Gly Ile Ser Phe Ser Ile Ile Asp Lys Cys Ile Glu
115 120 125

Asp Asn Ile Asp Leu Ile Ile Thr Cys Asp Asn Gly Ile Ser Ala Ile
130 135 140

Glu Gln Cys Asp Tyr Ala Arg Lys Asn Gly Ile Lys Ile Ile Val Thr
145 150 155 160

Asp His His Gln Thr Ile Lys His Asp Gly Lys Glu Ile Ile Pro Asn
165 170 175
Ala Asp Ala Val Val Asn Pro Gln Gln Gln Ser Cys Lys Tyr Pro Phe
180 185 190
Lys Ser Leu Cys Gly Ala Gly Val Ala Tyr Lys Leu Ile Gln Ala Ile

195 200 205
Asn Ile Lys Lys Gly Tyr Gly Met Leu Gln Cys Glu Asn Leu Leu Gln
210 215 220
Tyr Val Ala Met Gly Thr Val Cys Asp Ile Val Asp Leu Lys Asp Glu
225 230 235 240
Asn Arg Tyr Phe Val Thr Lys Gly Leu Glu Glu Ile Asn Asn Thr Asp
245 250 255
Asn Tyr Gly Leu Lys Cys Leu Ile Asn Met Thr Gly Ile Lys Asn Ala
260 265 270

Val Asn Val Tyr Ser Leu Gly Phe Ile Ile Gly Pro Cys Ile Asn Ala
275 280 285
Ala Gly Arg Leu Asp Thr Ala Lys Leu Gly Val Glu Leu Phe Arg Asp
290 295 300
Glu Asn Met Asp Asn Val Glu Ala Tyr Ala Lys Ile Leu Val Asp Leu
305 310 315 320
Asn Glu Lys Arg Lys Lys Leu Thr Glu Asp Gly Phe Asn Lys Ala Val
325 330 335
Glu Ile Ile Glu Asn Thr Ala Leu Lys Asn Asp Asp Ile Leu Ile Cys

340 345 350
Asn Val Glu Gly Ile His Glu Ser Val Cys Gly Ile Ile Ala Gly Arg
355 360 365
Ile Lys Glu Lys Tyr Asn Lys Pro Thr Leu Ile Leu Thr Glu Ser Ala
370 375 380
Asn Lys Asn Leu Leu Lys Gly Ser Gly Arg Ser Ile Ser Glu Tyr Asn
385 390 395 400
Ile Phe Glu Glu Phe Asp Glu Phe Arg Glu Met Phe Val Ser Phe Gly

405 410 415

Gly His Pro Met Ala Cys Gly Leu Ser Ile Glu Lys Asn Lys Leu Asp
420 425 430
Glu Phe Arg Thr Lys Val Asn Lys Asn Ser Lys Leu Thr Glu Glu Asp
435 440 445
Phe Val Lys Lys Ile Leu Ile Asp Ser Ser Phe Tyr Ile Asp Lys Ile
450 455 460
Asp Phe Asp Leu Ile Glu Glu Ile Gly Asn Leu Arg Pro Phe Gly Lys
465 470 475 480
Asp Asn Pro Arg Pro Ile Leu Gly Asp Lys Asp Val Glu Ile Ile Phe

485 490 495
Ala Lys Met Ile Gly Lys Asn Gln Asn Val Leu Lys Met Lys Leu Leu
500 505 510
Lys Asn Asn Lys Ala Ile Asp Ala Ile Leu Phe Thr Asp Ala Ile Glu
515 520 525
Lys Tyr Thr Tyr Leu Leu Gly Lys Phe Gly Glu Asn Val Leu Lys Glu
530 535 540
Leu Glu Asn Ser Ile Ser Cys Asn Ala Tyr Ile Asp Ile Leu Tyr Tyr
545 550 555 560

Pro Glu Ile Asn Asp Phe Asn Asn Val Lys Asn Ile Gln Leu Asn Leu
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Ile Asp Leu Arg
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<211> 1743
<212> DNA
<213> Unknown
<220><223> Finegoldia magna ATCC 29328
<400> 14

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tttttaaatc ctaatgcaga aaattcatat cataatcctt ttttaatgaa agatatggat 180

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gattatgatc aagatggaaa ttcttctact atgactctac tagatggact tggatatttt 300

acagataaaa tttcctatga tattcctaat agaatgacag atggatatgg catttctttt 360

agtataatag acaaatgtat cgaagataat atagatttaa taataacttg tgacaatggt 420

attagtgcaa ttgaacagtg tgattatgca aggaaaaatg gaattaaaat aattgtaact 480

gatcatcacc aaacaataaa acatgatgga aaagaaataa ttccaaatgc tgatgcggtc 540

gttaacccac aacaacaatc ttgcaaatat ccttttaaaa gtttatgcgg tgcaggagtt 600

gcttataaat taatacaagc tattaatata aaaaaaggat atggaatgct acagtgtgaa 660

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taa 1743

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<211> 739

<212> PRT

<213> Unknown

<220><223> Streptococcus cristatus AS 1.3089

<400> 15

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35 40 45

Glu Phe Leu Glu Pro Ser Leu Asp Gln Leu His Asp Pro Tyr Asp Leu

50 55 60

His Asp Met Glu Arg Ala Val Glu Arg Ile Arg Ala Ala Ile Glu Asn
65 70 75 80

Tyr Glu Gln Ile Leu Ile Tyr Gly Asp Tyr Asp Ala Asp Gly Met Thr
85 90 95

Ser Ala Ser Ile Val Lys Glu Ala Leu Glu Gln Leu Gly Ala Glu Cys
100 105 110

Gln Val Tyr Leu Pro Asn Arg Phe Thr Asp Gly Tyr Gly Pro Asn Ser
115 120 125

Ser Val Tyr Lys Tyr Phe Ile Glu Asn Gln Gly Ile Ser Leu Ile Ile
130 135 140

Thr Val Asp Asn Gly Val Ala Gly Leu Glu Ala Ile Glu Leu Ala Gln
145 150 155 160

Ser Leu Gly Val Asp Val Ile Val Thr Asp His His Ser Met Pro Glu
165 170 175

Glu Leu Pro Asn Ala Tyr Ala Ile Val His Pro Glu His Ser Gly Ala
180 185 190

Asp Tyr Pro Phe Lys His Leu Ala Gly Cys Gly Val Ala Phe Lys Leu

195 200 205

Ala Thr Ala Leu Leu Glu Glu Val Gln Val Glu Leu Leu Asp Leu Val

210 215 220

Ala Ile Gly Thr Ile Ala Asp Met Val Ser Leu Thr Gly Glu Asn Arg

225 230 235 240

Ile Leu Val Lys Tyr Gly Leu Ser Val Leu Lys Asn Thr Gln Arg Val

245 250 255

Gly Leu Gln Glu Leu Phe Lys Ile Ala Gly Ile Gln Pro Asp Glu Leu

260 265 270

Asp Glu Glu Thr Val Gly Phe Gln Leu Ala Pro Arg Leu Asn Ala Leu

275 280 285

Gly Arg Leu Asp Asp Pro Asn Pro Ala Ile Glu Leu Leu Thr Gly Phe

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Asp Asp Glu Glu Ala Arg Asp Ile Ala Leu Met Ile Asn Gln Lys Asn

305 310 315 320

Asp Glu Arg Lys Glu Ile Val Gln Gln Ile Tyr Lys Glu Ala Gln Thr

325 330 335

Met Leu Asp Pro Lys Arg Pro Val Gln Val Leu Ala Lys Glu Gly Trp

340 345 350

Asn Pro Gly Val Leu Gly Ile Val Ala Gly Arg Leu Leu Glu Glu Leu

355 360 365

His Gln Pro Val Ile Val Leu Asn Ile Glu Asp Gly Ile Ala Lys Gly

370 375 380

Ser Ala Arg Ser Ile Glu Ala Val Asn Ile Phe Glu Ala Leu Asp Ser

385 390 395 400

His Arg Asp Leu Phe Leu Ala Phe Gly Gly His Ala Gly Ala Ala Gly

405 410 415

Met Thr Leu Glu Ala Asp Lys Leu Ala Glu Leu Ala Asp Ile Leu Thr

420 425 430

Glu Tyr Ile Leu Glu Asn Asp Leu Asp Leu Thr Gly Lys Thr Ala Leu

435 440 445

Tyr Leu Asp Glu Glu Leu His Leu Pro Glu Leu Thr Leu Asp Thr Leu

450 455 460

Lys Ser Phe Glu Lys Leu Ala Pro Phe Gly Met Asp Asn Lys Lys Pro

465 470 475 480

Leu Phe Tyr Leu Lys Asp Phe Lys Val Asp Asn Ala Arg Thr Met Gly

485 490 495

Ala Gly Asn Ser His Leu Lys Leu Lys Ile Ser Gln Ala Asp Ala Ser

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Phe Glu Val Val Ala Phe Gly Gln Gly Ser Leu Ala Thr Glu Phe Ala

515 520 525

Gln Thr Lys Asn Leu Glu Leu Ala Val Ser Leu Ser Val Asn Lys Trp

530 535 540

Asn Gly Gln Thr Ser Leu Gln Leu Met Leu Val Asp Ala Arg Val Asp

545 550 555 560

Gly Val Gln Leu Phe Asn Ile Arg Gly Lys Asn Ala Thr Leu Pro Asp

565 570 575

Lys Val Pro Val Leu Arg Phe Thr Glu Glu Leu Pro Asp Leu Thr Asn

580 585 590

Ser Arg Ala Val Val Val Tyr Asp Leu Pro Asp Asn Leu Gln Val Leu

595 600 605

Lys Lys Ile Leu Gln Tyr Gln Asp Phe Glu Ala Ile Tyr Phe Lys Asn

610 615 620

Glu Ile Ala Lys Pro Tyr Tyr Leu Thr Gly Tyr Gly Thr Arg Glu Gln

625 630 635 640

Phe Ala Lys Leu Tyr Lys Thr Ile Tyr Gln Phe Pro Glu Phe Asp Val

645 650 655

Arg Tyr Lys Leu Lys Glu Leu Ala Ala Tyr Leu Lys Ile Asp Pro Ile

660 665 670

Leu Leu Val Lys Met Ile Gln Ile Phe Glu Glu Leu Gly Phe Val Ser

675 680 685

Ile Thr Glu Gly Val Met Thr Val Asn Lys Glu Ala Glu Lys Lys Glu

690 695 700

Ile Asp Ser Ser His Ile Tyr Gln Asp Leu Lys Arg Leu Val Lys Glu

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Gln Glu Leu Met Ala Leu Gly Thr Val Gln Glu Ile Tyr Asp Tyr Leu
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Met Asp Cys

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<212> DNA

<213> Unknown

<220><223> Streptococcus cristatus AS 1.3089

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2220

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<213> Artificial Sequence

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1 5 10 15

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<211> 8

<212> PRT

<213> Artificial Sequence

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<400> 18

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1 5

<210

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<211> 6

<212> PRT

<213> Artificial Sequence

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<400> 19

Gly Gly Gly Gly Gly Gly

1 5

<210> 20

<211> 15

<212> PRT

<213> Artificial Sequence

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<210> 21

<211> 5

<212> PRT

<213> Artificial Sequence

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<212> PRT

<213> Artificial Sequence

<220><223> Rigid linker

<400> 22

Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys

1 5 10

<210> 23

<211> 46

<212> PRT

<213> Artificial Sequence

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<400> 23

Ala Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys

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Glu Ala Ala Ala Lys Ala Leu Glu Ala Glu Ala Ala Ala Lys Glu Ala

20 25 30

Ala Ala Lys Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Ala

35 40 45

<210> 24

<211> 12

<212> PRT

<213> Artificial Sequence

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<400> 24

Ala Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Ala

1 5 10

<210> 25

<211> 1442

<212> PRT

<213> Artificial Sequence

<220><223> SpyCas9

<400> 25

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Arg Gly Ser His Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg

20 25 30

Gly Ser Glu Phe Glu Leu Arg Arg Gln Ala Cys Gly Arg Met Asp Lys

35 40 45

Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val Gly Trp Ala

50 55 60

Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe Lys Val Leu
65 70 75 80

Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile Gly Ala Leu
85 90 95

Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu Lys Arg Thr
100 105 110

Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys Tyr Leu Gln
115 120 125

Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser Phe Phe His
130 135 140

Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys His Glu Arg

145 150 155 160

His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr His Glu Lys
165 170 175

Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp Ser Thr Asp
180 185 190

Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His Met Ile Lys
195 200 205

Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro Asp Asn Ser
210 215 220

Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr Asn Gln Leu
225 230 235 240

Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala Lys Ala Ile
245 250 255

Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn Leu Ile Ala
260 265 270

Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn Leu Ile Ala
275 280 285

Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe Asp Leu Ala

290 295 300

Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp Asp Asp Leu

305 310 315 320

Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp Leu Phe Leu

325 330 335

Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp Ile Leu Arg

340 345 350

Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser Met Ile Lys

355 360 365

Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys Ala Leu Val

370 375 380

Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe Asp Gln Ser

385 390 395 400

Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser Gln Glu Glu

405 410 415

Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp Gly Thr Glu

420 425 430

Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg Lys Gln Arg

435 440 445

Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu Gly Glu Leu

450 455 460

His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe Leu Lys Asp

465 470 475 480

Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile Pro Tyr Tyr

485 490 495

Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp Met Thr Arg

500 505 510

Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu Val Val Asp

515 520 525

Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr Asn Phe Asp

530 535 540

Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser Leu Leu Tyr

545 550 555 560

Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys Tyr Val Thr

565 570 575

Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln Lys Lys Ala

580 585 590

Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr Val Lys Gln

595 600 605

Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp Ser Val Glu

610 615 620

Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly Thr Tyr His

625 630 635 640

Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp Asn Glu Glu

645 650 655

Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr Leu Phe Glu

660 665 670

Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala His Leu Phe

675 680 685

Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr Thr Gly Trp

690 695 700

Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp Lys Gln Ser

705 710 715 720

Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe Ala Asn Arg

725 730 735

Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe Lys Glu Asp

740 745 750

Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu His Glu His

755 760 765

Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly Ile Leu Gln

770 775 780

Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly Arg His Lys

785 790 795 800

Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln Thr Thr Gln

805 810 815

Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile Glu Glu Gly
820 825 830
Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro Val Glu Asn
835 840 845
Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu Gln Asn Gly
850 855 860
Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg Leu Ser Asp

865 870 875 880
Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys Asp Asp Ser
885 890 895
Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg Gly Lys Ser
900 905 910
Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys Asn Tyr Trp
915 920 925
Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys Phe Asp Asn
930 935 940

Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp Lys Ala Gly
945 950 955 960
Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr Lys His Val
965 970 975
Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp Glu Asn Asp
980 985 990
Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser Lys Leu Val
995 1000 1005
Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg Glu Ile Asn

1010 1015 1020
Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val Val Gly Thr
1025 1030 1035 1040
Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe Val Tyr Gly
1045 1050 1055
Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala Lys Ser Glu Gln
1060 1065 1070

Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe Tyr Ser Asn Ile Met
1075 1080 1085

Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala Asn Gly Glu Ile Arg Lys
1090 1095 1100
Arg Pro Leu Ile Glu Thr Asn Gly Glu Thr Gly Glu Ile Val Trp Asp
1105 1110 1115 1120
Lys Gly Arg Asp Phe Ala Thr Val Arg Lys Val Leu Ser Met Pro Gln
1125 1130 1135
Val Asn Ile Val Lys Lys Thr Glu Val Gln Thr Gly Gly Phe Ser Lys
1140 1145 1150
Glu Ser Ile Leu Pro Lys Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys

1155 1160 1165
Lys Asp Trp Asp Pro Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val
1170 1175 1180
Ala Tyr Ser Val Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys
1185 1190 1195 1200
Lys Leu Lys Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg
1205 1210 1215
Ser Ser Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr
1220 1225 1230

Lys Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu
1235 1240 1245
Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly Glu
1250 1255 1260
Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val Asn Phe
1265 1270 1275 1280
Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser Pro Glu Asp
1285 1290 1295
Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys His Tyr Leu Asp

1300 1305 1310
Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys Arg Val Ile Leu Ala

1315 1320 1325

Asp Ala Asn Leu Asp Lys Val Leu Ser Ala Tyr Asn Lys His Arg Asp

1330 1335 1340

Lys Pro Ile Arg Glu Gln Ala Glu Asn Ile Ile His Leu Phe Thr Leu

1345 1350 1355 1360

Thr Asn Leu Gly Ala Pro Ala Ala Phe Lys Tyr Phe Asp Thr Thr Ile

1365 1370 1375

Asp Arg Lys Arg Tyr Thr Ser Thr Lys Glu Val Leu Asp Ala Thr Leu

1380 1385 1390

Ile His Gln Ser Ile Thr Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser

1395 1400 1405

Gln Leu Gly Gly Asp Ala Ala Ala Leu Asp Leu Glu Lys Arg Pro Ala

1410 1415 1420

Ala Thr Lys Lys Ala Gly Gln Ala Lys Lys Lys Glu His His His His

1425 1430 1435 1440

His His

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210> 26

<211> 4329

<212> DNA

<213> Artificial Sequence

<220><223> SpyCas9

<400> 26

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caagcttgcg gccgcatgga caagaagtac agcatcggcc tggacatcgg taccaacagc 180

gtgggctggg ccgtgatcac cgacgagtac aaggtgccca gcaagaagtt caaggtgctg 240

ggcaacaccg accgccacag catcaagaag aacctgatcg gcgccctgct gttcgacagc 300

ggcgagaccg ccgaggccac ccgcctgaag cgcaccgccc gccgccgcta cacccgccgc 360

aagaaccgca tctgctacct gcaggagatc ttcagcaacg agatggccaa ggtggacgac 420

agcttcttcc accgcctgga ggagagcttc ctggtggagg aggacaagaa gcacgagcgc 480

caccccatct tcggcaacat cgtggacgag gtggcctacc acgagaagta ccccaccatc 540

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<210> 27

<211> 1682

<212> PRT

<213> Artificial Sequence

<220><223> SpyCas9-GFP

<400> 27

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Gly Ser Glu Phe Glu Leu Arg Arg Gln Ala Cys Gly Arg Met Asp Lys
35 40 45

Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val Gly Trp Ala
50 55 60

Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe Lys Val Leu
65 70 75 80

Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile Gly Ala Leu

85 90 95

Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu Lys Arg Thr
100 105 110

Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys Tyr Leu Gln
115 120 125

Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser Phe Phe His
130 135 140

Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys His Glu Arg
145 150 155 160

His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr His Glu Lys

165 170 175

Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp Ser Thr Asp
180 185 190
Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His Met Ile Lys
195 200 205
Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro Asp Asn Ser
210 215 220
Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr Asn Gln Leu

225 230 235 240
Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala Lys Ala Ile
245 250 255
Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn Leu Ile Ala
260 265 270
Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn Leu Ile Ala
275 280 285
Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe Asp Leu Ala
290 295 300

Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp Asp Asp Leu
305 310 315 320
Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp Leu Phe Leu
325 330 335
Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp Ile Leu Arg
340 345 350
Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser Met Ile Lys
355 360 365
Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys Ala Leu Val

370 375 380
Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe Asp Gln Ser
385 390 395 400
Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser Gln Glu Glu
405 410 415
Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp Gly Thr Glu
420 425 430

Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg Lys Gln Arg
435 440 445

Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu Gly Glu Leu
450 455 460
His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe Leu Lys Asp
465 470 475 480
Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile Pro Tyr Tyr
485 490 495
Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp Met Thr Arg
500 505 510
Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu Val Val Asp

515 520 525
Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr Asn Phe Asp
530 535 540
Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser Leu Leu Tyr
545 550 555 560
Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys Tyr Val Thr
565 570 575
Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln Lys Lys Ala
580 585 590

Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr Val Lys Gln
595 600 605
Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp Ser Val Glu
610 615 620
Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly Thr Tyr His
625 630 635 640
Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp Asn Glu Glu
645 650 655
Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr Leu Phe Glu

660 665 670
Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala His Leu Phe

675 680 685

Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr Thr Gly Trp

690 695 700

Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp Lys Gln Ser

705 710 715 720

Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe Ala Asn Arg

725 730 735

Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe Lys Glu Asp

740 745 750

Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu His Glu His

755 760 765

Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly Ile Leu Gln

770 775 780

Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly Arg His Lys

785 790 795 800

Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln Thr Thr Gln

805 810 815

Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile Glu Glu Gly

820 825 830

Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro Val Glu Asn

835 840 845

Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu Gln Asn Gly

850 855 860

Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg Leu Ser Asp

865 870 875 880

Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys Asp Asp Ser

885 890 895

Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg Gly Lys Ser

900 905 910

Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys Asn Tyr Trp

915 920 925

Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys Phe Asp Asn

930 935 940

Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp Lys Ala Gly

945 950 955 960

Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr Lys His Val

965 970 975

Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp Glu Asn Asp

980 985 990

Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser Lys Leu Val

995 1000 1005

Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg Glu Ile Asn

1010 1015 1020

Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val Val Gly Thr

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Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe Val Tyr Gly

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Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala Lys Ser Glu Gln

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Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe Tyr Ser Asn Ile Met

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Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala Asn Gly Glu Ile Arg Lys

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Lys Gly Arg Asp Phe Ala Thr Val Arg Lys Val Leu Ser Met Pro Gln

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1140 1145 1150

Glu Ser Ile Leu Pro Lys Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys

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Lys Asp Trp Asp Pro Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val

1170 1175 1180

Ala Tyr Ser Val Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys
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Lys Leu Lys Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg
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Lys Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu

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Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val Asn Phe
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Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser Pro Glu Asp
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Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys His Tyr Leu Asp
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Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys Arg Val Ile Leu Ala
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Lys Pro Ile Arg Glu Gln Ala Glu Asn Ile Ile His Leu Phe Thr Leu
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Thr Asn Leu Gly Ala Pro Ala Ala Phe Lys Tyr Phe Asp Thr Thr Ile
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1380 1385 1390
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His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro
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Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly
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<213> Artificial Sequence

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<400> 28

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<212> PRT

<213> Artificial Sequence

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<400> 29

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Gly Ser Glu Phe Glu Leu Arg Arg Gln Ala Cys Gly Arg Met Asp Lys
35 40 45

Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val Gly Trp Ala
50 55 60

Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe Lys Val Leu
65 70 75 80

Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile Gly Ala Leu

85 90 95

Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu Lys Arg Thr
100 105 110

Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys Tyr Leu Gln
115 120 125

Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser Phe Phe His
130 135 140

Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys His Glu Arg
145 150 155 160

His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr His Glu Lys

165 170 175

Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp Ser Thr Asp
180 185 190

Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His Met Ile Lys

195 200 205

Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro Asp Asn Ser

210 215 220

Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr Asn Gln Leu

225 230 235 240

Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala Lys Ala Ile

245 250 255

Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn Leu Ile Ala

260 265 270

Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn Leu Ile Ala

275 280 285

Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe Asp Leu Ala

290 295 300

Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp Asp Asp Leu

305 310 315 320

Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp Leu Phe Leu

325 330 335

Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp Ile Leu Arg

340 345 350

Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser Met Ile Lys

355 360 365

Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys Ala Leu Val

370 375 380

Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe Asp Gln Ser

385 390 395 400

Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser Gln Glu Glu

405 410 415

Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp Gly Thr Glu

420 425 430

Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg Lys Gln Arg

435 440 445

Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu Gly Glu Leu
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His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe Leu Lys Asp
465 470 475 480
Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile Pro Tyr Tyr
485 490 495
Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp Met Thr Arg
500 505 510
Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu Val Val Asp

515 520 525
Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr Asn Phe Asp
530 535 540
Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser Leu Leu Tyr
545 550 555 560
Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys Tyr Val Thr
565 570 575
Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln Lys Lys Ala
580 585 590

Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr Val Lys Gln
595 600 605
Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp Ser Val Glu
610 615 620
Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly Thr Tyr His
625 630 635 640
Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp Asn Glu Glu
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Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr Leu Phe Glu

660 665 670
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675 680 685
Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr Thr Gly Trp
690 695 700

Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp Lys Gln Ser
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Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe Ala Asn Arg
725 730 735

Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe Lys Glu Asp
740 745 750
Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu His Glu His
755 760 765
Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly Ile Leu Gln
770 775 780
Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly Arg His Lys
785 790 795 800
Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln Thr Thr Gln

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Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile Glu Glu Gly
820 825 830
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835 840 845
Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu Gln Asn Gly
850 855 860
Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg Leu Ser Asp
865 870 875 880

Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys Asp Asp Ser
885 890 895
Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg Gly Lys Ser
900 905 910
Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys Asn Tyr Trp
915 920 925
Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys Phe Asp Asn
930 935 940
Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp Lys Ala Gly

945 950 955 960

Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr Lys His Val

965 970 975

Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp Glu Asn Asp

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Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser Lys Leu Val

995 1000 1005

Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg Glu Ile Asn

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1090 1095 1100

Arg Pro Leu Ile Glu Thr Asn Gly Glu Thr Gly Glu Ile Val Trp Asp

1105 1110 1115 1120

Lys Gly Arg Asp Phe Ala Thr Val Arg Lys Val Leu Ser Met Pro Gln

1125 1130 1135

Val Asn Ile Val Lys Lys Thr Glu Val Gln Thr Gly Gly Phe Ser Lys

1140 1145 1150

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Lys Asp Trp Asp Pro Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val

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1315 1320 1325

Asp Ala Asn Leu Asp Lys Val Leu Ser Ala Tyr Asn Lys His Arg Asp

1330 1335 1340

Lys Pro Ile Arg Glu Gln Ala Glu Asn Ile Ile His Leu Phe Thr Leu

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Thr Asn Leu Gly Ala Pro Ala Ala Phe Lys Tyr Phe Asp Thr Thr Ile

1365 1370 1375

Asp Arg Lys Arg Tyr Thr Ser Thr Lys Glu Val Leu Asp Ala Thr Leu

1380 1385 1390

Ile His Gln Ser Ile Thr Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser

1395 1400 1405

Gln Leu Gly Gly Asp Ala Ala Ala Leu Asp Leu Gln Met Asp Pro Pro

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Arg Ala Ser His Leu Ser Pro Arg Lys Lys Arg Pro Arg Gln Thr Gly

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Ala Leu Met Ala Ser Ser Pro Gln Asp Ile Lys Phe Gln Asp Leu Val

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Phe Arg Glu Asn Glu Asp Ser Cys Val Thr Phe Met Arg Ala Ala Ser
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Asp Ala Phe Val Thr Met Thr Gly Gly Phe Arg Arg Gly Lys Lys Met
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Leu Ile Phe Lys Leu Pro Arg Gln Arg Val Asp Ser Asp Gln Ser Ser
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Trp Gln Glu Gly Lys Thr Trp Lys Ala Ile Arg Val Asp Leu Val Leu
1845 1850 1855
Cys Pro Tyr Glu Arg Arg Ala Phe Ala Leu Leu Gly Trp Thr Gly Ser
1860 1865 1870
Arg Gln Phe Glu Arg Asp Leu Arg Arg Tyr Ala Thr His Glu Arg Lys
1875 1880 1885

Met Ile Leu Asp Asn His Ala Leu Tyr Asp Lys Thr Lys Arg Ile Phe
1890 1895 1900
Leu Lys Ala Glu Ser Glu Glu Glu Ile Phe Ala His Leu Gly Leu Asp
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35 40 45

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50 55 60

Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe Lys Val Leu
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Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile Gly Ala Leu
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Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu Lys Arg Thr

100 105 110

Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys Tyr Leu Gln

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Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser Phe Phe His

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Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys His Glu Arg

145 150 155 160

His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr His Glu Lys

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Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp Ser Thr Asp

180 185 190

Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His Met Ile Lys

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Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro Asp Asn Ser

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Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr Asn Gln Leu

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Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala Lys Ala Ile

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260 265 270

Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn Leu Ile Ala

275 280 285

Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe Asp Leu Ala

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Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp Asp Asp Leu

305 310 315 320

Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp Leu Phe Leu

325 330 335

Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp Ile Leu Arg

340 345 350

Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser Met Ile Lys

355 360 365

Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys Ala Leu Val

370 375 380

Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe Asp Gln Ser

385 390 395 400

Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser Gln Glu Glu

405 410 415

Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp Gly Thr Glu

420 425 430

Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg Lys Gln Arg

435 440 445

Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu Gly Glu Leu

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His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe Leu Lys Asp

465 470 475 480

Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile Pro Tyr Tyr

485 490 495

Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp Met Thr Arg

500 505 510

Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu Val Val Asp

515 520 525

Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr Asn Phe Asp

530 535 540

Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser Leu Leu Tyr

545 550 555 560

Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys Tyr Val Thr

565 570 575

Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln Lys Lys Ala

580 585 590

Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr Val Lys Gln

595 600 605

Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp Ser Val Glu
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625 630 635 640
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675 680 685
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725 730 735
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755 760 765
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770 775 780
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785 790 795 800
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Lys Asp Trp Asp Pro Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val

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Asp Ala Asn Leu Asp Lys Val Leu Ser Ala Tyr Asn Lys His Arg Asp

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1780 1785 1790

Leu Ala Ser Arg Ile Lys Glu Arg Phe His Arg Pro Val Ile Ala Phe

1795 1800 1805

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1810 1815 1820

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1825 1830 1835 1840

Gly Met Ile Leu Lys Phe Gly Gly His Ala Met Ala Ala Gly Leu Ser

1845 1850 1855

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Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe Lys Val Leu
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Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile Gly Ala Leu
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Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu Lys Arg Thr
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Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys Tyr Leu Gln
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Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys His Glu Arg

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His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr His Glu Lys

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Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp Ser Thr Asp

180 185 190

Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His Met Ile Lys

195 200 205

Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro Asp Asn Ser

210 215 220

Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr Asn Gln Leu

225 230 235 240

Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala Lys Ala Ile

245 250 255

Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn Leu Ile Ala

260 265 270

Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn Leu Ile Ala

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Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe Asp Leu Ala

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Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp Asp Asp Leu

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Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp Leu Phe Leu

325 330 335

Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp Ile Leu Arg

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Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser Met Ile Lys

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Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys Ala Leu Val

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Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys Tyr Val Thr
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595 600 605
Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp Ser Val Glu
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Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly Thr Tyr His
625 630 635 640
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Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr Leu Phe Glu
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675 680 685
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690 695 700
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Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe Ala Asn Arg
725 730 735
Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe Lys Glu Asp

740 745 750
Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu His Glu His
755 760 765
Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly Ile Leu Gln
770 775 780
Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly Arg His Lys
785 790 795 800
Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln Thr Thr Gln
805 810 815

Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile Glu Glu Gly
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835 840 845
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850 855 860
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865 870 875 880
Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys Asp Asp Ser

885 890 895
Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg Gly Lys Ser

900 905 910

Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys Asn Tyr Trp

915 920 925

Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys Phe Asp Asn

930 935 940

Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp Lys Ala Gly

945 950 955 960

Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr Lys His Val

965 970 975

Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp Glu Asn Asp

980 985 990

Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser Lys Leu Val

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Ala Gln His Gly Ser Glu Lys Pro Val Thr His Val Ser Arg Asp Gly
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Arg Glu Leu His Lys Leu Val Arg Asp Thr Asp Lys Val Phe Pro Asn

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1925 1930 1935

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Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe Lys Val Leu
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35 40 45

Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val Gly Trp Ala
50 55 60

Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe Lys Val Leu
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Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile Gly Ala Leu
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Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu Lys Arg Thr

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Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys Tyr Leu Gln
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Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser Phe Phe His
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Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys His Glu Arg
145 150 155 160
His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr His Glu Lys
165 170 175

Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp Ser Thr Asp
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Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His Met Ile Lys
195 200 205
Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro Asp Asn Ser
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Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr Asn Gln Leu
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Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala Lys Ala Ile

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Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn Leu Ile Ala
260 265 270
Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn Leu Ile Ala
275 280 285
Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe Asp Leu Ala
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Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp Asp Asp Leu
305 310 315 320

Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp Leu Phe Leu

325 330 335

Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp Ile Leu Arg

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Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser Met Ile Lys

355 360 365

Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys Ala Leu Val

370 375 380

Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe Asp Gln Ser

385 390 395 400

Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser Gln Glu Glu

405 410 415

Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp Gly Thr Glu

420 425 430

Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg Lys Gln Arg

435 440 445

Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu Gly Glu Leu

450 455 460

His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe Leu Lys Asp

465 470 475 480

Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile Pro Tyr Tyr

485 490 495

Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp Met Thr Arg

500 505 510

Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu Val Val Asp

515 520 525

Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr Asn Phe Asp

530 535 540

Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser Leu Leu Tyr

545 550 555 560

Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys Tyr Val Thr

565 570 575

Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln Lys Lys Ala

580 585 590

Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr Val Lys Gln

595 600 605

Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp Ser Val Glu

610 615 620

Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly Thr Tyr His

625 630 635 640

Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp Asn Glu Glu

645 650 655

Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr Leu Phe Glu

660 665 670

Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala His Leu Phe

675 680 685

Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr Thr Gly Trp

690 695 700

Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp Lys Gln Ser

705 710 715 720

Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe Ala Asn Arg

725 730 735

Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe Lys Glu Asp

740 745 750

Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu His Glu His

755 760 765

Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly Ile Leu Gln

770 775 780

Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly Arg His Lys

785 790 795 800

Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln Thr Thr Gln

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Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile Glu Glu Gly

820 825 830

Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro Val Glu Asn
835 840 845

Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu Gln Asn Gly
850 855 860

Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg Leu Ser Asp
865 870 875 880

Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys Asp Asp Ser
885 890 895

Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg Gly Lys Ser
900 905 910

Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys Asn Tyr Trp
915 920 925

Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys Phe Asp Asn
930 935 940

Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp Lys Ala Gly
945 950 955 960

Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr Lys His Val
965 970 975

Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp Glu Asn Asp
980 985 990

Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser Lys Leu Val
995 1000 1005

Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg Glu Ile Asn
1010 1015 1020

Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val Val Gly Thr
1025 1030 1035 1040

Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe Val Tyr Gly
1045 1050 1055

Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala Lys Ser Glu Gln
1060 1065 1070

Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe Tyr Ser Asn Ile Met
1075 1080 1085

Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala Asn Gly Glu Ile Arg Lys

1090 1095 1100

Arg Pro Leu Ile Glu Thr Asn Gly Glu Thr Gly Glu Ile Val Trp Asp

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Lys Gly Arg Asp Phe Ala Thr Val Arg Lys Val Leu Ser Met Pro Gln

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Val Asn Ile Val Lys Lys Thr Glu Val Gln Thr Gly Gly Phe Ser Lys

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Glu Ser Ile Leu Pro Lys Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys

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Lys Asp Trp Asp Pro Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val

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Ala Tyr Ser Val Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys

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Lys Leu Lys Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg

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Ser Ser Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr

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Lys Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu

1235 1240 1245

Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly Glu

1250 1255 1260

Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val Asn Phe

1265 1270 1275 1280

Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser Pro Glu Asp

1285 1290 1295

Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys His Tyr Leu Asp

1300 1305 1310

Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys Arg Val Ile Leu Ala

1315 1320 1325

Asp Ala Asn Leu Asp Lys Val Leu Ser Ala Tyr Asn Lys His Arg Asp

1330 1335 1340

Lys Pro Ile Arg Glu Gln Ala Glu Asn Ile Ile His Leu Phe Thr Leu

1345 1350 1355 1360

Thr Asn Leu Gly Ala Pro Ala Ala Phe Lys Tyr Phe Asp Thr Thr Ile

1365 1370 1375

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1380 1385 1390

Ile His Gln Ser Ile Thr Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser

1395 1400 1405

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1410 1415 1420

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1425 1430 1435 1440

Lys Arg Tyr Pro Thr Phe Ser Ala Ser Cys Arg Thr Gln Lys Thr Ala

1445 1450 1455

Ile Thr Lys Thr Glu Lys Val Phe Phe Ser Glu Ser Phe Asp Gln Thr

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Phe Gly Arg Trp Leu Ser Leu Phe Leu His Gln Val Ser Leu Thr Asp

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Pro Val Ile Ala Val Ile Asp Gly Glu Gly Gly Ser Glu His Arg Arg

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Lys Leu Leu Pro Ser Tyr Lys Ala His Arg Lys Lys Phe Met Arg His

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Met Ser Ser Gly His Val Gly Arg Ser His Gln Val Ile Asn Asp Val

1555 1560 1565

Leu Gly Lys Cys Asn Val Pro Val Ile Lys Val Ala Gly His Glu Ala

1570 1575 1580

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Ser Asp Leu Ser Phe Arg Cys Ile Val Gly Asp Glu Val Asp Gly Val

1650 1655 1660

Pro Gly Ile Gln His Leu Val Pro Ser Phe Gly Arg Lys Thr Ala Met

1665 1670 1675 1680

Lys Leu Ile Lys Lys His Gly Ser Leu Glu Thr Leu Leu Asn Ala Ala

1685 1690 1695

Ala Ile Arg Thr Val Gly Arg Pro Tyr Ala Gln Asp Ala Leu Lys Asn

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His Ala Asp Tyr Leu Arg Arg Asn Tyr Glu Val Leu Ala Leu Lys Arg

1715 1720 1725

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1730 1735 1740

His Asn Asp Lys Thr Ala Leu Ser Ser Phe Phe Lys Tyr Leu Gly Glu

1745 1750 1755 1760

Ser Lys Glu Leu Ser Tyr Asn Gly Arg Pro Ile Ser Tyr Asn Gly Leu

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<213> Artificial Sequence

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Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val Gly Trp Ala
50 55 60

Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe Lys Val Leu
65 70 75 80

Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile Gly Ala Leu
85 90 95

Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu Lys Arg Thr
100 105 110

Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys Tyr Leu Gln
115 120 125

Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser Phe Phe His

130 135 140

Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys His Glu Arg
145 150 155 160

His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr His Glu Lys
165 170 175

Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp Ser Thr Asp

180 185 190

Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His Met Ile Lys

195 200 205

Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro Asp Asn Ser

210 215 220

Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr Asn Gln Leu

225 230 235 240

Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala Lys Ala Ile

245 250 255

Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn Leu Ile Ala

260 265 270

Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn Leu Ile Ala

275 280 285

Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe Asp Leu Ala

290 295 300

Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp Asp Asp Leu

305 310 315 320

Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp Leu Phe Leu

325 330 335

Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp Ile Leu Arg

340 345 350

Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser Met Ile Lys

355 360 365

Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys Ala Leu Val

370 375 380

Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe Asp Gln Ser

385 390 395 400

Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser Gln Glu Glu

405 410 415

Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp Gly Thr Glu

420 425 430

Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg Lys Gln Arg
435 440 445
Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu Gly Glu Leu
450 455 460
His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe Leu Lys Asp
465 470 475 480
Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile Pro Tyr Tyr
485 490 495

Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp Met Thr Arg
500 505 510
Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu Val Val Asp
515 520 525
Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr Asn Phe Asp
530 535 540
Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser Leu Leu Tyr
545 550 555 560
Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys Tyr Val Thr
565 570 575
Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln Lys Lys Ala
580 585 590
Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr Val Lys Gln
595 600 605
Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp Ser Val Glu
610 615 620
Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly Thr Tyr His
625 630 635 640

Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp Asn Glu Glu
645 650 655
Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr Leu Phe Glu
660 665 670
Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala His Leu Phe
675 680 685

Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr Thr Gly Trp
690 695 700
Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp Lys Gln Ser

705 710 715 720
Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe Ala Asn Arg
725 730 735
Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe Lys Glu Asp
740 745 750
Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu His Glu His
755 760 765
Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly Ile Leu Gln
770 775 780

Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly Arg His Lys
785 790 795 800
Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln Thr Thr Gln
805 810 815
Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile Glu Glu Gly
820 825 830
Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro Val Glu Asn
835 840 845
Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu Gln Asn Gly

850 855 860
Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg Leu Ser Asp
865 870 875 880
Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys Asp Asp Ser
885 890 895
Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg Gly Lys Ser
900 905 910
Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys Asn Tyr Trp
915 920 925

Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys Phe Asp Asn

930 935 940

Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp Lys Ala Gly

945 950 955 960

Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr Lys His Val

965 970 975

Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp Glu Asn Asp

980 985 990

Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser Lys Leu Val

995 1000 1005

Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg Glu Ile Asn

1010 1015 1020

Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val Val Gly Thr

1025 1030 1035 1040

Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe Val Tyr Gly

1045 1050 1055

Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala Lys Ser Glu Gln

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Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe Tyr Ser Asn Ile Met

1075 1080 1085

Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala Asn Gly Glu Ile Arg Lys

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Arg Pro Leu Ile Glu Thr Asn Gly Glu Thr Gly Glu Ile Val Trp Asp

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Lys Gly Arg Asp Phe Ala Thr Val Arg Lys Val Leu Ser Met Pro Gln

1125 1130 1135

Val Asn Ile Val Lys Lys Thr Glu Val Gln Thr Gly Gly Phe Ser Lys

1140 1145 1150

Glu Ser Ile Leu Pro Lys Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys

1155 1160 1165

Lys Asp Trp Asp Pro Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val

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Ala Tyr Ser Val Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys

1185 1190 1195 1200

Lys Leu Lys Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg

1205 1210 1215

Ser Ser Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr

1220 1225 1230

Lys Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu

1235 1240 1245

Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly Glu

1250 1255 1260

Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val Asn Phe

1265 1270 1275 1280

Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser Pro Glu Asp

1285 1290 1295

Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys His Tyr Leu Asp

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Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys Arg Val Ile Leu Ala

1315 1320 1325

Asp Ala Asn Leu Asp Lys Val Leu Ser Ala Tyr Asn Lys His Arg Asp

1330 1335 1340

Lys Pro Ile Arg Glu Gln Ala Glu Asn Ile Ile His Leu Phe Thr Leu

1345 1350 1355 1360

Thr Asn Leu Gly Ala Pro Ala Ala Phe Lys Tyr Phe Asp Thr Thr Ile

1365 1370 1375

Asp Arg Lys Arg Tyr Thr Ser Thr Lys Glu Val Leu Asp Ala Thr Leu

1380 1385 1390

Ile His Gln Ser Ile Thr Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser

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1410 1415 1420

Arg Asn Leu Met Ile Val Asp Gly Thr Asn Leu Gly Phe Arg Phe Lys

1425 1430 1435 1440

His Asn Asn Ser Lys Lys Pro Phe Ala Ser Ser Tyr Val Ser Thr Ile
1445 1450 1455
Gln Ser Leu Ala Lys Ser Tyr Ser Ala Arg Thr Thr Ile Val Leu Gly
1460 1465 1470
Asp Lys Gly Lys Ser Val Phe Arg Leu Glu His Leu Pro Glu Tyr Lys
1475 1480 1485
Gly Asn Arg Asp Glu Lys Tyr Ala Gln Arg Thr Glu Glu Glu Lys Ala
1490 1495 1500

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1505 1510 1515 1520
Lys Thr Thr Phe Pro Thr Phe Thr Ile Arg Gly Val Glu Ala Asp Asp
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1540 1545 1550
Trp Leu Ile Ser Thr Asp Gly Asp Trp Asp Thr Leu Leu Thr Asp Lys
1555 1560 1565
Val Ser Arg Phe Ser Phe Thr Thr Arg Arg Glu Tyr His Leu Arg Asp

1570 1575 1580
Met Tyr Glu His His Asn Val Asp Asp Val Glu Gln Phe Ile Ser Leu
1585 1590 1595 1600
Lys Ala Ile Met Gly Asp Leu Gly Asp Asn Ile Arg Gly Val Glu Gly
1605 1610 1615
Ile Gly Ala Lys Arg Gly Tyr Asn Ile Ile Arg Glu Phe Gly Asn Val
1620 1625 1630
Leu Asp Ile Ile Asp Gln Leu Pro Leu Pro Gly Lys Gln Lys Tyr Ile
1635 1640 1645

Gln Asn Leu Asn Ala Ser Glu Glu Leu Leu Phe Arg Asn Leu Ile Leu
1650 1655 1660
Val Asp Leu Pro Thr Tyr Cys Val Asp Ala Ile Ala Ala Val Gly Gln
1665 1670 1675 1680
Asp Val Leu Asp Lys Phe Thr Lys Asp Ile Leu Glu Ile Ala Glu Gln
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Leu Gln Lys Arg Pro Ala Ala Thr Lys Lys Ala Gly Gln Ala Lys Lys

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Lys Lys His His His His His His

1715 1720

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aagaaccgca tctgctacct gcaggagatc ttcagcaacg agatggccaa ggtggacgac 420

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35 40 45
Thr Ala Asp Leu Pro Ala Glu Leu Pro Pro Leu Leu Arg Arg Leu Tyr
50 55 60
Ala Ser Arg Gly Val Arg Ser Ala Gln Glu Leu Glu Arg Ser Val Lys

65 70 75 80
Gly Met Leu Pro Trp Gln Gln Leu Ser Gly Val Glu Lys Ala Val Glu
85 90 95
Ile Leu Tyr Asn Ala Phe Arg Glu Gly Thr Arg Ile Ile Val Val Gly
100 105 110
Asp Phe Asp Ala Asp Gly Ala Thr Ser Thr Ala Leu Ser Val Leu Ala
115 120 125
Met Arg Ser Leu Gly Cys Ser Asn Ile Asp Tyr Leu Val Pro Asn Arg
130 135 140

Phe Glu Asp Gly Tyr Gly Leu Ser Pro Glu Val Val Asp Gln Ala His

145 150 155 160

Ala Arg Gly Ala Gln Leu Ile Val Thr Val Asp Asn Gly Ile Ser Ser

165 170 175

His Ala Gly Val Glu His Ala Arg Ser Leu Gly Ile Pro Val Ile Val

180 185 190

Thr Asp His His Leu Pro Gly Glu Thr Leu Pro Ala Ala Glu Ala Ile

195 200 205

Ile Asn Pro Asn Leu Arg Asp Cys Asn Phe Pro Ser Lys Ser Leu Ala

210 215 220

Gly Val Gly Val Ala Phe Tyr Leu Met Leu Ala Leu Arg Thr Phe Leu

225 230 235 240

Arg Asp Gln Gly Trp Phe Asp Glu Arg Gly Ile Ala Ile Pro Asn Leu

245 250 255

Ala Glu Leu Leu Asp Leu Val Ala Leu Gly Thr Val Ala Asp Val Val

260 265 270

Pro Leu Asp Ala Asn Asn Arg Ile Leu Thr Trp Gln Gly Met Ser Arg

275 280 285

Ile Arg Ala Gly Lys Cys Arg Pro Gly Ile Lys Ala Leu Leu Glu Val

290 295 300

Ala Asn Arg Asp Ala Gln Lys Leu Ala Ala Ser Asp Leu Gly Phe Ala

305 310 315 320

Leu Gly Pro Arg Leu Asn Ala Ala Gly Arg Leu Asp Asp Met Ser Val

325 330 335

Gly Val Ala Leu Leu Leu Cys Asp Asn Ile Gly Glu Ala Arg Val Leu

340 345 350

Ala Asn Glu Leu Asp Ala Leu Asn Gln Thr Arg Lys Glu Ile Glu Gln

355 360 365

Gly Met Gln Val Glu Ala Leu Thr Leu Cys Glu Lys Leu Glu Arg Ser

370 375 380

Arg Asp Thr Leu Pro Gly Gly Leu Ala Met Tyr His Pro Glu Trp His

385 390 395 400

Gln Gly Val Val Gly Ile Leu Ala Ser Arg Ile Lys Glu Arg Phe His

405 410 415

Arg Pro Val Ile Ala Phe Ala Pro Ala Gly Asp Gly Thr Leu Lys Gly

420 425 430

Ser Gly Arg Ser Ile Gln Gly Leu His Met Arg Asp Ala Leu Glu Arg

435 440 445

Leu Asp Thr Leu Tyr Pro Gly Met Ile Leu Lys Phe Gly Gly His Ala

450 455 460

Met Ala Ala Gly Leu Ser Leu Glu Glu Asp Lys Phe Glu Leu Phe Gln

465 470 475 480

Gln Arg Phe Gly Glu Leu Val Thr Glu Trp Leu Asp Pro Ser Leu Leu

485 490 495

Gln Gly Glu Val Val Ser Asp Gly Pro Leu Ser Pro Ala Glu Met Thr

500 505 510

Met Glu Val Ala Gln Leu Leu Arg Asp Ala Gly Pro Trp Gly Gln Met

515 520 525

Phe Pro Glu Pro Leu Phe Asp Gly His Phe Arg Leu Leu Gln Gln Arg

530 535 540

Leu Val Gly Glu Arg His Leu Lys Val Met Val Glu Pro Val Gly Gly

545 550 555 560

Gly Pro Leu Leu Asp Gly Ile Ala Phe Asn Val Asp Thr Ala Leu Trp

565 570 575

Pro Asp Asn Gly Val Arg Glu Val Gln Leu Ala Tyr Lys Leu Asp Ile

580 585 590

Asn Glu Phe Arg Gly Asn Arg Ser Leu Gln Ile Ile Ile Asp Asn Ile

595 600 605

Trp Pro Ile Gly Ser Glu Phe Glu Leu Arg Arg Gln Ala Cys Gly Arg

610 615 620

Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val

625 630 635 640

Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe

645 650 655

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
660 665 670
Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
675 680 685
Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
690 695 700
Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
705 710 715 720

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
725 730 735
His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
740 745 750
His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
755 760 765
Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
770 775 780
Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro

785 790 795 800
Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
805 810 815
Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
820 825 830
Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
835 840 845
Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
850 855 860

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Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
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Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
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Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
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Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser

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Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
945 950 955 960
Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
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980 985 990
Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
995 1000 1005

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
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Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
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Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
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Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
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Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln

1155 1160 1165

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr

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Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp

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Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly

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Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp

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His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr

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Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp

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Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe

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Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe

1315 1320 1325

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu

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His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly

1345 1350 1355 1360

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly

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Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln

1380 1385 1390

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile

1395 1400 1405

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro

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Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu

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Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg

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Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys

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Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg

1475 1480 1485

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys

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Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr

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Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp

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Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser

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1635 1640 1645

Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe Tyr Ser

1650 1655 1660

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Met Pro Gln Val Asn Ile Val Lys Lys Thr Glu Val Gln Thr Gly Gly
1715 1720 1725

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Ala Arg Lys Lys Asp Trp Asp Pro Lys Lys Tyr Gly Gly Phe Asp Ser
1745 1750 1755 1760
Pro Thr Val Ala Tyr Ser Val Leu Val Val Ala Lys Val Glu Lys Gly
1765 1770 1775
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Met Glu Arg Ser Ser Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala

1795 1800 1805
Lys Gly Tyr Lys Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys
1810 1815 1820
Tyr Ser Leu Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser
1825 1830 1835 1840
Ala Gly Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr
1845 1850 1855
Val Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser
1860 1865 1870

Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys His
1875 1880 1885
Tyr Leu Asp Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys Arg Val
1890 1895 1900
Ile Leu Ala Asp Ala Asn Leu Asp Lys Val Leu Ser Ala Tyr Asn Lys
1905 1910 1915 1920

His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu Asn Ile Ile His Leu
1925 1930 1935
Phe Thr Leu Thr Asn Leu Gly Ala Pro Ala Ala Phe Lys Tyr Phe Asp

1940 1945 1950
Thr Thr Ile Asp Arg Lys Arg Tyr Thr Ser Thr Lys Glu Val Leu Asp
1955 1960 1965
Ala Thr Leu Ile His Gln Ser Ile Thr Gly Leu Tyr Glu Thr Arg Ile
1970 1975 1980
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His His His His His
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Gly Ser Met Ser Thr Lys Pro Leu Phe Leu Leu Arg Lys Ala Lys Lys
35 40 45

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50 55 60

Ser Thr Cys Ala Thr Leu Asp Tyr Leu Ile Val Lys Ser Gly Lys Lys
65 70 75 80

Leu Ser Ser Tyr Phe Lys Ala Val Ala Thr Asn Phe Pro Val Val Asn
85 90 95

Asp Leu Pro Ala Glu Gly Glu Ile Asp Phe Thr Trp Ser Glu Arg Tyr
100 105 110

Gln Leu Ser Lys Asp Ser Met Thr Trp Glu Leu Lys Pro Gly Ala Ala
115 120 125

Pro Asp Asn Ala His Tyr Gln Gly Asn Thr Asn Val Asn Gly Glu Asp

130 135 140

Met Thr Glu Ile Glu Glu Asn Met Leu Leu Pro Ile Ser Gly Gln Glu
145 150 155 160

Leu Pro Ile Arg Trp Leu Ala Gln His Gly Ser Glu Lys Pro Val Thr
165 170 175

His Val Ser Arg Asp Gly Leu Gln Ala Leu His Ile Ala Arg Ala Glu
180 185 190

Glu Leu Pro Ala Val Thr Ala Leu Ala Val Ser His Lys Thr Ser Leu

195 200 205

Leu Asp Pro Leu Glu Ile Arg Glu Leu His Lys Leu Val Arg Asp Thr
210 215 220
Asp Lys Val Phe Pro Asn Pro Gly Asn Ser Asn Leu Gly Leu Ile Thr
225 230 235 240
Ala Phe Phe Glu Ala Tyr Leu Asn Ala Asp Tyr Thr Asp Arg Gly Leu
245 250 255
Leu Thr Lys Glu Trp Met Lys Gly Asn Arg Val Ser His Ile Thr Arg
260 265 270
Thr Ala Ser Gly Ala Asn Ala Gly Gly Gly Asn Leu Thr Asp Arg Gly

275 280 285
Glu Gly Phe Val His Asp Leu Thr Ser Leu Ala Arg Asp Val Ala Thr
290 295 300
Gly Val Leu Ala Arg Ser Met Asp Leu Asp Ile Tyr Asn Leu His Pro
305 310 315 320
Ala His Ala Lys Arg Ile Glu Glu Ile Ile Ala Glu Asn Lys Pro Pro
325 330 335
Phe Ser Val Phe Arg Asp Lys Phe Ile Thr Met Pro Gly Gly Leu Asp
340 345 350

Tyr Ser Arg Ala Ile Val Val Ala Ser Val Lys Glu Ala Pro Ile Gly
355 360 365
Ile Glu Val Ile Pro Ala His Val Thr Glu Tyr Leu Asn Lys Val Leu
370 375 380
Thr Glu Thr Asp His Ala Asn Pro Asp Pro Glu Ile Val Asp Ile Ala
385 390 395 400
Cys Gly Arg Ser Ser Ala Pro Met Pro Gln Arg Val Thr Glu Glu Gly
405 410 415
Lys Gln Asp Asp Glu Glu Lys Pro Gln Pro Ser Gly Thr Thr Ala Val

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Glu Gln Gly Glu Ala Glu Thr Met Glu Pro Asp Ala Thr Glu His His
435 440 445

Gln Asp Thr Gln Pro Leu Asp Ala Gln Ser Gln Val Asn Ser Val Asp
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Ile Pro Ser Lys Asn Pro Val Asp Asp Asp Lys Leu Leu Ala Ala Ser
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Thr Glu Lys Thr Ser Pro Asp Met Asn Gln Pro Glu Pro Val Val Gln
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565 570 575
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610 615 620
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625 630 635 640

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645 650 655
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660 665 670
Thr Thr Lys Thr Lys Thr Leu Asp Leu Gly Thr Ala Phe His Cys Arg
675 680 685
Val Leu Glu Pro Glu Glu Phe Ser Asn Arg Phe Ile Val Ala Pro Glu
690 695 700

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705 710 715 720

Met Glu Cys Ala Ser Thr Gly Lys Thr Val Ile Thr Ala Glu Glu Gly

725 730 735

Arg Lys Ile Glu Leu Met Tyr Gln Ser Val Met Ala Leu Pro Leu Gly

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Gln Trp Leu Val Glu Ser Ala Gly His Ala Glu Ser Ser Ile Tyr Trp

755 760 765

Glu Asp Pro Glu Thr Gly Ile Leu Cys Arg Cys Arg Pro Asp Lys Ile

770 775 780

Ile Pro Glu Phe His Trp Ile Met Asp Val Lys Thr Thr Ala Asp Ile

785 790 795 800

Gln Arg Phe Lys Thr Ala Tyr Tyr Asp Tyr Arg Tyr His Val Gln Asp

805 810 815

Ala Phe Tyr Ser Asp Gly Tyr Glu Ala Gln Phe Gly Val Gln Pro Thr

820 825 830

Phe Val Phe Leu Val Ala Ser Thr Thr Ile Glu Cys Gly Arg Tyr Pro

835 840 845

Val Glu Ile Phe Met Met Gly Glu Glu Ala Lys Leu Ala Gly Gln Gln

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Glu Trp Pro Ala Ile Lys Thr Leu Ser Leu Pro Arg Trp Ala Lys Glu

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Tyr Ala Asn Asp Gly Ser Glu Phe Glu Leu Arg Arg Gln Ala Cys Gly

900 905 910

Arg Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser

915 920 925

Val Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys

930 935 940

Phe Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu

945 950 955 960

Ile Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg

965 970 975

Leu Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile

980 985 990

Cys Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp

995 1000 1005

Ser Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys

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Lys His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala

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Tyr His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val

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Asp Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala

1060 1065 1070

His Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn

1075 1080 1085

Pro Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr

1090 1095 1100

Tyr Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp

1105 1110 1115 1120

Ala Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu

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1140 1145 1150

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Phe Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr

1170 1175 1180

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1185 1190 1195 1200

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Arg Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met

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Thr Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr

1845 1850 1855

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1860 1865 1870

Ser Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val

1875 1880 1885

Arg Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala

1890 1895 1900

Val Val Gly Thr Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu

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Phe Val Tyr Gly Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala

1925 1930 1935

Lys Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe Tyr

1940 1945 1950

Ser Asn Ile Met Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala Asn Gly

1955 1960 1965

Glu Ile Arg Lys Arg Pro Leu Ile Glu Thr Asn Gly Glu Thr Gly Glu

1970 1975 1980

Ile Val Trp Asp Lys Gly Arg Asp Phe Ala Thr Val Arg Lys Val Leu

1985 1990 1995 2000

Ser Met Pro Gln Val Asn Ile Val Lys Lys Thr Glu Val Gln Thr Gly

2005 2010 2015

Gly Phe Ser Lys Glu Ser Ile Leu Pro Lys Arg Asn Ser Asp Lys Leu

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Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn Leu Ile Ala

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Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp Ile Leu Arg

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Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser Met Ile Lys

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Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys Ala Leu Val

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Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe Ala Asn Arg
965 970 975
Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe Lys Glu Asp
980 985 990

Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu His Glu His
995 1000 1005
Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly Ile Leu Gln
1010 1015 1020
Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly Arg His Lys
1025 1030 1035 1040
Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln Thr Thr Gln
1045 1050 1055
Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile Glu Glu Gly

1060 1065 1070
Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro Val Glu Asn
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Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu Gln Asn Gly
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Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg Leu Ser Asp
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Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys Asp Asp Ser
1125 1130 1135

Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg Gly Lys Ser
1140 1145 1150
Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys Asn Tyr Trp
1155 1160 1165
Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys Phe Asp Asn
1170 1175 1180
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Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr Lys His Val

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Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser Lys Leu Val
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Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg Glu Ile Asn
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Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe Val Tyr Gly
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Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala Lys Ser Glu Gln
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Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe Tyr Ser Asn Ile Met
1315 1320 1325
Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala Asn Gly Glu Ile Arg Lys
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1345 1350 1355 1360

Lys Gly Arg Asp Phe Ala Thr Val Arg Lys Val Leu Ser Met Pro Gln

1365 1370 1375

Val Asn Ile Val Lys Lys Thr Glu Val Gln Thr Gly Gly Phe Ser Lys

1380 1385 1390

Glu Ser Ile Leu Pro Lys Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys

1395 1400 1405

Lys Asp Trp Asp Pro Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val

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Ala Tyr Ser Val Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys

1425 1430 1435 1440

Lys Leu Lys Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg

1445 1450 1455

Ser Ser Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr

1460 1465 1470

Lys Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu

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Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly Glu

1490 1495 1500

Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val Asn Phe

1505 1510 1515 1520

Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser Pro Glu Asp

1525 1530 1535

Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys His Tyr Leu Asp

1540 1545 1550

Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys Arg Val Ile Leu Ala

1555 1560 1565

Asp Ala Asn Leu Asp Lys Val Leu Ser Ala Tyr Asn Lys His Arg Asp

1570 1575 1580

Lys Pro Ile Arg Glu Gln Ala Glu Asn Ile Ile His Leu Phe Thr Leu

1585 1590 1595 1600

Thr Asn Leu Gly Ala Pro Ala Ala Phe Lys Tyr Phe Asp Thr Thr Ile

1605 1610 1615

Asp Arg Lys Arg Tyr Thr Ser Thr Lys Glu Val Leu Asp Ala Thr Leu

1620 1625 1630

Ile His Gln Ser Ile Thr Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser

1635 1640 1645

Gln Leu Gly Gly Asp Ala Ala Ala Leu Asp Leu Glu Lys Arg Pro Ala

1650 1655 1660

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1665 1670 1675 1680

His His

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<210> 47

<211> 1560

<212> PRT

<213> Artificial Sequence

<220><223> SSB-SpyCas9

<400> 47

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Gly Ser Met Ala Ser Arg Gly Val Asn Lys Val Ile Leu Val Gly Asn
35 40 45

Leu Gly Gln Asp Pro Glu Val Arg Tyr Met Pro Asn Gly Gly Ala Val
50 55 60

Ala Asn Ile Thr Leu Ala Thr Ser Glu Ser Trp Arg Asp Lys Ala Thr
65 70 75 80

Gly Glu Met Lys Glu Gln Thr Glu Trp His Arg Val Val Leu Phe Gly
85 90 95

Lys Leu Ala Glu Val Ala Ser Glu Tyr Leu Arg Lys Gly Ser Gln Val
100 105 110

Tyr Ile Glu Gly Gln Leu Arg Thr Arg Lys Trp Thr Asp Gln Ser Gly

115 120 125

Gln Asp Arg Tyr Thr Thr Glu Val Val Val Asn Val Gly Gly Thr Met
130 135 140
Gln Met Leu Gly Gly Arg Gly Ser Glu Phe Glu Leu Arg Arg Gln Ala
145 150 155 160
Cys Gly Arg Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr
165 170 175
Asn Ser Val Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser
180 185 190
Lys Lys Phe Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys

195 200 205
Asn Leu Ile Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala
210 215 220
Thr Arg Leu Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn
225 230 235 240
Arg Ile Cys Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val
245 250 255
Asp Asp Ser Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu
260 265 270

Asp Lys Lys His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu
275 280 285
Val Ala Tyr His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys
290 295 300
Leu Val Asp Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala
305 310 315 320
Leu Ala His Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp
325 330 335
Leu Asn Pro Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val

340 345 350
Gln Thr Tyr Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly
355 360 365

Val Asp Ala Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg
370 375 380
Leu Glu Asn Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu
385 390 395 400
Phe Gly Asn Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys
405 410 415

Ser Asn Phe Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp
420 425 430
Thr Tyr Asp Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln
435 440 445
Tyr Ala Asp Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu
450 455 460
Leu Ser Asp Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu
465 470 475 480
Ser Ala Ser Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr
485 490 495
Leu Leu Lys Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu
500 505 510
Ile Phe Phe Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly
515 520 525
Gly Ala Ser Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu
530 535 540
Lys Met Asp Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp
545 550 555 560

Leu Leu Arg Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln
565 570 575
Ile His Leu Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe
580 585 590
Tyr Pro Phe Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr
595 600 605
Phe Arg Ile Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg
610 615 620

Phe Ala Trp Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn

625 630 635 640

Phe Glu Glu Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu

645 650 655

Arg Met Thr Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro

660 665 670

Lys His Ser Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr

675 680 685

Lys Val Lys Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser

690 695 700

Gly Glu Gln Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg

705 710 715 720

Lys Val Thr Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu

725 730 735

Cys Phe Asp Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala

740 745 750

Ser Leu Gly Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp

755 760 765

Phe Leu Asp Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu

770 775 780

Thr Leu Thr Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys

785 790 795 800

Thr Tyr Ala His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg

805 810 815

Arg Arg Tyr Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly

820 825 830

Ile Arg Asp Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser

835 840 845

Asp Gly Phe Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser

850 855 860

Leu Thr Phe Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly

865 870 875 880

Asp Ser Leu His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile

885 890 895

Lys Lys Gly Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys

900 905 910

Val Met Gly Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg

915 920 925

Glu Asn Gln Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met

930 935 940

Lys Arg Ile Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys

945 950 955 960

Glu His Pro Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu

965 970 975

Tyr Tyr Leu Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp

980 985 990

Ile Asn Arg Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser

995 1000 1005

Phe Leu Lys Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp

1010 1015 1020

Lys Asn Arg Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys

1025 1030 1035 1040

Lys Met Lys Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr

1045 1050 1055

Gln Arg Lys Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser

1060 1065 1070

Glu Leu Asp Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg

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Gln Ile Thr Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr

1090 1095 1100

Lys Tyr Asp Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr

1105 1110 1115 1120

Leu Lys Ser Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr

1125 1130 1135

Lys Val Arg Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu
1140 1145 1150
Asn Ala Val Val Gly Thr Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu
1155 1160 1165
Ser Glu Phe Val Tyr Gly Asp Tyr Lys Val Tyr Asp Val Arg Lys Met
1170 1175 1180
Ile Ala Lys Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe
1185 1190 1195 1200
Phe Tyr Ser Asn Ile Met Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala

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Lys Leu Ile Ala Arg Lys Lys Asp Trp Asp Pro Lys Lys Tyr Gly Gly
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Phe Asp Ser Pro Thr Val Ala Tyr Ser Val Leu Val Val Ala Lys Val
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1315 1320 1325
Ile Thr Ile Met Glu Arg Ser Ser Phe Glu Lys Asn Pro Ile Asp Phe
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Leu Glu Ala Lys Gly Tyr Lys Glu Val Lys Lys Asp Leu Ile Ile Lys

1345 1350 1355 1360
Leu Pro Lys Tyr Ser Leu Phe Glu Leu Glu Asn Gly Arg Lys Arg Met
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Leu Ala Ser Ala Gly Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro

1380 1385 1390

Ser Lys Tyr Val Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu

1395 1400 1405

Lys Gly Ser Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln

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Lys Arg Val Ile Leu Ala Asp Ala Asn Leu Asp Lys Val Leu Ser Ala

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Tyr Asn Lys His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu Asn Ile

1460 1465 1470

Ile His Leu Phe Thr Leu Thr Asn Leu Gly Ala Pro Ala Ala Phe Lys

1475 1480 1485

Tyr Phe Asp Thr Thr Ile Asp Arg Lys Arg Tyr Thr Ser Thr Lys Glu

1490 1495 1500

Val Leu Asp Ala Thr Leu Ile His Gln Ser Ile Thr Gly Leu Tyr Glu

1505 1510 1515 1520

Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly Asp Ala Ala Ala Leu Asp

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Leu Glu Lys Arg Pro Ala Ala Thr Lys Lys Ala Gly Gln Ala Lys Lys

1540 1545 1550

Lys Lys His His His His His His

1555 1560

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Tyr Ile Glu Gly Gln Leu Arg Thr Arg Lys Trp Thr Asp Gln Ser Gly

115 120 125

Gln Asp Arg Tyr Thr Thr Glu Val Val Val Asn Val Gly Gly Thr Met

130 135 140

Gln Met Leu Gly Gly Arg Gly Ser Glu Phe Glu Leu Arg Arg Gln Ala

145 150 155 160

Cys Gly Arg Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr

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Asn Ser Val Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser

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Lys Lys Phe Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys

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260 265 270

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275 280 285

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Tyr Pro Phe Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr

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Phe Glu Glu Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu
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Arg Met Thr Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro
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Lys Val Lys Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser
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755 760 765
Phe Leu Asp Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu
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Lys Lys Gly Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys

900 905 910

Val Met Gly Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg

915 920 925

Glu Asn Gln Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met

930 935 940

Lys Arg Ile Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys

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Glu His Pro Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu

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<210> 51

<211> 1533

<212> PRT

<213> Artificial Sequence

<220><223> DSB-SpyCas9

<400> 51

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Gly Ser Met Ala Lys Lys Glu Met Val Glu Phe Asp Glu Ala Ile His
35 40 45

Gly Glu Asp Leu Ala Lys Phe Ile Lys Glu Ala Ser Asp His Lys Leu
50 55 60

Lys Ile Ser Gly Tyr Asn Glu Leu Ile Lys Asp Ile Arg Ile Arg Ala
65 70 75 80

Lys Asp Glu Leu Gly Val Asp Gly Lys Met Phe Asn Arg Leu Leu Ala
85 90 95

Leu Tyr His Lys Asp Asn Arg Asp Val Phe Glu Ala Glu Thr Glu Glu
100 105 110

Val Val Glu Leu Tyr Asp Thr Val Phe Ser Lys Gly Ser Glu Phe Glu
115 120 125

Leu Arg Arg Gln Ala Cys Gly Arg Met Asp Lys Lys Tyr Ser Ile Gly
130 135 140

Leu Asp Ile Gly Thr Asn Ser Val Gly Trp Ala Val Ile Thr Asp Glu
145 150 155 160

Tyr Lys Val Pro Ser Lys Lys Phe Lys Val Leu Gly Asn Thr Asp Arg
165 170 175

His Ser Ile Lys Lys Asn Leu Ile Gly Ala Leu Leu Phe Asp Ser Gly
180 185 190

Glu Thr Ala Glu Ala Thr Arg Leu Lys Arg Thr Ala Arg Arg Arg Tyr
195 200 205

Thr Arg Arg Lys Asn Arg Ile Cys Tyr Leu Gln Glu Ile Phe Ser Asn
210 215 220
Glu Met Ala Lys Val Asp Asp Ser Phe Phe His Arg Leu Glu Glu Ser
225 230 235 240
Phe Leu Val Glu Glu Asp Lys Lys His Glu Arg His Pro Ile Phe Gly
245 250 255
Asn Ile Val Asp Glu Val Ala Tyr His Glu Lys Tyr Pro Thr Ile Tyr
260 265 270

His Leu Arg Lys Lys Leu Val Asp Ser Thr Asp Lys Ala Asp Leu Arg
275 280 285
Leu Ile Tyr Leu Ala Leu Ala His Met Ile Lys Phe Arg Gly His Phe
290 295 300
Leu Ile Glu Gly Asp Leu Asn Pro Asp Asn Ser Asp Val Asp Lys Leu
305 310 315 320
Phe Ile Gln Leu Val Gln Thr Tyr Asn Gln Leu Phe Glu Glu Asn Pro
325 330 335
Ile Asn Ala Ser Gly Val Asp Ala Lys Ala Ile Leu Ser Ala Arg Leu

340 345 350
Ser Lys Ser Arg Arg Leu Glu Asn Leu Ile Ala Gln Leu Pro Gly Glu
355 360 365
Lys Lys Asn Gly Leu Phe Gly Asn Leu Ile Ala Leu Ser Leu Gly Leu
370 375 380
Thr Pro Asn Phe Lys Ser Asn Phe Asp Leu Ala Glu Asp Ala Lys Leu
385 390 395 400
Gln Leu Ser Lys Asp Thr Tyr Asp Asp Asp Leu Asp Asn Leu Leu Ala
405 410 415

Gln Ile Gly Asp Gln Tyr Ala Asp Leu Phe Leu Ala Ala Lys Asn Leu
420 425 430
Ser Asp Ala Ile Leu Leu Ser Asp Ile Leu Arg Val Asn Thr Glu Ile
435 440 445
Thr Lys Ala Pro Leu Ser Ala Ser Met Ile Lys Arg Tyr Asp Glu His
450 455 460

His Gln Asp Leu Thr Leu Leu Lys Ala Leu Val Arg Gln Gln Leu Pro
465 470 475 480
Glu Lys Tyr Lys Glu Ile Phe Phe Asp Gln Ser Lys Asn Gly Tyr Ala

485 490 495
Gly Tyr Ile Asp Gly Gly Ala Ser Gln Glu Glu Phe Tyr Lys Phe Ile
500 505 510
Lys Pro Ile Leu Glu Lys Met Asp Gly Thr Glu Glu Leu Leu Val Lys
515 520 525
Leu Asn Arg Glu Asp Leu Leu Arg Lys Gln Arg Thr Phe Asp Asn Gly
530 535 540
Ser Ile Pro His Gln Ile His Leu Gly Glu Leu His Ala Ile Leu Arg
545 550 555 560

Arg Gln Glu Asp Phe Tyr Pro Phe Leu Lys Asp Asn Arg Glu Lys Ile
565 570 575
Glu Lys Ile Leu Thr Phe Arg Ile Pro Tyr Tyr Val Gly Pro Leu Ala
580 585 590
Arg Gly Asn Ser Arg Phe Ala Trp Met Thr Arg Lys Ser Glu Glu Thr
595 600 605
Ile Thr Pro Trp Asn Phe Glu Glu Val Val Asp Lys Gly Ala Ser Ala
610 615 620
Gln Ser Phe Ile Glu Arg Met Thr Asn Phe Asp Lys Asn Leu Pro Asn

625 630 635 640
Glu Lys Val Leu Pro Lys His Ser Leu Leu Tyr Glu Tyr Phe Thr Val
645 650 655
Tyr Asn Glu Leu Thr Lys Val Lys Tyr Val Thr Glu Gly Met Arg Lys
660 665 670
Pro Ala Phe Leu Ser Gly Glu Gln Lys Lys Ala Ile Val Asp Leu Leu
675 680 685
Phe Lys Thr Asn Arg Lys Val Thr Val Lys Gln Leu Lys Glu Asp Tyr
690 695 700

Phe Lys Lys Ile Glu Cys Phe Asp Ser Val Glu Ile Ser Gly Val Glu

705 710 715 720

Asp Arg Phe Asn Ala Ser Leu Gly Thr Tyr His Asp Leu Leu Lys Ile

725 730 735

Ile Lys Asp Lys Asp Phe Leu Asp Asn Glu Glu Asn Glu Asp Ile Leu

740 745 750

Glu Asp Ile Val Leu Thr Leu Thr Leu Phe Glu Asp Arg Glu Met Ile

755 760 765

Glu Glu Arg Leu Lys Thr Tyr Ala His Leu Phe Asp Asp Lys Val Met

770 775 780

Lys Gln Leu Lys Arg Arg Arg Tyr Thr Gly Trp Gly Arg Leu Ser Arg

785 790 795 800

Lys Leu Ile Asn Gly Ile Arg Asp Lys Gln Ser Gly Lys Thr Ile Leu

805 810 815

Asp Phe Leu Lys Ser Asp Gly Phe Ala Asn Arg Asn Phe Met Gln Leu

820 825 830

Ile His Asp Asp Ser Leu Thr Phe Lys Glu Asp Ile Gln Lys Ala Gln

835 840 845

Val Ser Gly Gln Gly Asp Ser Leu His Glu His Ile Ala Asn Leu Ala

850 855 860

Gly Ser Pro Ala Ile Lys Lys Gly Ile Leu Gln Thr Val Lys Val Val

865 870 875 880

Asp Glu Leu Val Lys Val Met Gly Arg His Lys Pro Glu Asn Ile Val

885 890 895

Ile Glu Met Ala Arg Glu Asn Gln Thr Thr Gln Lys Gly Gln Lys Asn

900 905 910

Ser Arg Glu Arg Met Lys Arg Ile Glu Glu Gly Ile Lys Glu Leu Gly

915 920 925

Ser Gln Ile Leu Lys Glu His Pro Val Glu Asn Thr Gln Leu Gln Asn

930 935 940

Glu Lys Leu Tyr Leu Tyr Tyr Leu Gln Asn Gly Arg Asp Met Tyr Val

945 950 955 960

Asp Gln Glu Leu Asp Ile Asn Arg Leu Ser Asp Tyr Asp Val Asp His

965 970 975

Ile Val Pro Gln Ser Phe Leu Lys Asp Asp Ser Ile Asp Asn Lys Val

980 985 990

Leu Thr Arg Ser Asp Lys Asn Arg Gly Lys Ser Asp Asn Val Pro Ser

995 1000 1005

Glu Glu Val Val Lys Lys Met Lys Asn Tyr Trp Arg Gln Leu Leu Asn

1010 1015 1020

Ala Lys Leu Ile Thr Gln Arg Lys Phe Asp Asn Leu Thr Lys Ala Glu

1025 1030 1035 1040

Arg Gly Gly Leu Ser Glu Leu Asp Lys Ala Gly Phe Ile Lys Arg Gln

1045 1050 1055

Leu Val Glu Thr Arg Gln Ile Thr Lys His Val Ala Gln Ile Leu Asp

1060 1065 1070

Ser Arg Met Asn Thr Lys Tyr Asp Glu Asn Asp Lys Leu Ile Arg Glu

1075 1080 1085

Val Lys Val Ile Thr Leu Lys Ser Lys Leu Val Ser Asp Phe Arg Lys

1090 1095 1100

Asp Phe Gln Phe Tyr Lys Val Arg Glu Ile Asn Asn Tyr His His Ala

1105 1110 1115 1120

His Asp Ala Tyr Leu Asn Ala Val Val Gly Thr Ala Leu Ile Lys Lys

1125 1130 1135

Tyr Pro Lys Leu Glu Ser Glu Phe Val Tyr Gly Asp Tyr Lys Val Tyr

1140 1145 1150

Asp Val Arg Lys Met Ile Ala Lys Ser Glu Gln Glu Ile Gly Lys Ala

1155 1160 1165

Thr Ala Lys Tyr Phe Phe Tyr Ser Asn Ile Met Asn Phe Phe Lys Thr

1170 1175 1180

Glu Ile Thr Leu Ala Asn Gly Glu Ile Arg Lys Arg Pro Leu Ile Glu

1185 1190 1195 1200

Thr Asn Gly Glu Thr Gly Glu Ile Val Trp Asp Lys Gly Arg Asp Phe

1205 1210 1215

Ala Thr Val Arg Lys Val Leu Ser Met Pro Gln Val Asn Ile Val Lys
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Lys Thr Glu Val Gln Thr Gly Gly Phe Ser Lys Glu Ser Ile Leu Pro
1235 1240 1245
Lys Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys Lys Asp Trp Asp Pro
1250 1255 1260
Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val Ala Tyr Ser Val Leu
1265 1270 1275 1280

Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys Lys Leu Lys Ser Val
1285 1290 1295
Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg Ser Ser Phe Glu Lys
1300 1305 1310
Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr Lys Glu Val Lys Lys
1315 1320 1325
Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu Phe Glu Leu Glu Asn
1330 1335 1340
Gly Arg Lys Arg Met Leu Ala Ser Ala Gly Glu Leu Gln Lys Gly Asn

1345 1350 1355 1360
Glu Leu Ala Leu Pro Ser Lys Tyr Val Asn Phe Leu Tyr Leu Ala Ser
1365 1370 1375
His Tyr Glu Lys Leu Lys Gly Ser Pro Glu Asp Asn Glu Gln Lys Gln
1380 1385 1390
Leu Phe Val Glu Gln His Lys His Tyr Leu Asp Glu Ile Ile Glu Gln
1395 1400 1405
Ile Ser Glu Phe Ser Lys Arg Val Ile Leu Ala Asp Ala Asn Leu Asp
1410 1415 1420

Lys Val Leu Ser Ala Tyr Asn Lys His Arg Asp Lys Pro Ile Arg Glu
1425 1430 1435 1440
Gln Ala Glu Asn Ile Ile His Leu Phe Thr Leu Thr Asn Leu Gly Ala
1445 1450 1455
Pro Ala Ala Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg Lys Arg Tyr
1460 1465 1470

Thr Ser Thr Lys Glu Val Leu Asp Ala Thr Leu Ile His Gln Ser Ile

1475 1480 1485

Thr Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly Asp

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Gly Gln Ala Lys Lys Lys Lys His His His His His His

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<211> 4602

<212> DNA

<213> Artificial Sequence

<220><223> DSB-SpyCas9

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<211> 2112

<212> PRT

<213> Artificial Sequence

<220><223> DSB-SpyCas9-RecJ

<400> 53

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Gly Ser Met Ala Lys Lys Glu Met Val Glu Phe Asp Glu Ala Ile His
35 40 45

Gly Glu Asp Leu Ala Lys Phe Ile Lys Glu Ala Ser Asp His Lys Leu
50 55 60

Lys Ile Ser Gly Tyr Asn Glu Leu Ile Lys Asp Ile Arg Ile Arg Ala

65 70 75 80

Lys Asp Glu Leu Gly Val Asp Gly Lys Met Phe Asn Arg Leu Leu Ala
85 90 95

Leu Tyr His Lys Asp Asn Arg Asp Val Phe Glu Ala Glu Thr Glu Glu
100 105 110

Val Val Glu Leu Tyr Asp Thr Val Phe Ser Lys Gly Ser Glu Phe Glu
115 120 125

Leu Arg Arg Gln Ala Cys Gly Arg Met Asp Lys Lys Tyr Ser Ile Gly
130 135 140

Leu Asp Ile Gly Thr Asn Ser Val Gly Trp Ala Val Ile Thr Asp Glu
145 150 155 160

Tyr Lys Val Pro Ser Lys Lys Phe Lys Val Leu Gly Asn Thr Asp Arg
165 170 175

His Ser Ile Lys Lys Asn Leu Ile Gly Ala Leu Leu Phe Asp Ser Gly
180 185 190

Glu Thr Ala Glu Ala Thr Arg Leu Lys Arg Thr Ala Arg Arg Arg Tyr
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Arg Gln Glu Asp Phe Tyr Pro Phe Leu Lys Asp Asn Arg Glu Lys Ile

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Glu Lys Ile Leu Thr Phe Arg Ile Pro Tyr Tyr Val Gly Pro Leu Ala

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Arg Gly Asn Ser Arg Phe Ala Trp Met Thr Arg Lys Ser Glu Glu Thr

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Ile Thr Pro Trp Asn Phe Glu Glu Val Val Asp Lys Gly Ala Ser Ala

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Gln Ser Phe Ile Glu Arg Met Thr Asn Phe Asp Lys Asn Leu Pro Asn

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Tyr Asn Glu Leu Thr Lys Val Lys Tyr Val Thr Glu Gly Met Arg Lys

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Lys Glu Arg Phe His Arg Pro Val Ile Ala Phe Ala Pro Ala Gly Asp
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340 345 350

Ser Gln Gln Ile Asn Asp Lys Thr Leu Lys Lys Tyr Lys Met Ser Val

355 360 365

Leu Phe Lys Gln Ile Leu Ser Asp Thr Glu Ser Lys Ser Phe Val Ile

370 375 380

Asp Lys Leu Glu Asp Asp Ser Asp Val Val Thr Thr Met Gln Ser Phe

385 390 395 400

Tyr Glu Gln Ile Ala Ala Phe Lys Thr Val Glu Glu Lys Ser Ile Lys

405 410 415

Glu Thr Leu Ser Leu Leu Phe Asp Asp Leu Lys Ala Gln Lys Leu Asp

420 425 430

Leu Ser Lys Ile Tyr Phe Lys Asn Asp Lys Ser Leu Thr Asp Leu Ser

435 440 445

Gln Gln Val Phe Asp Asp Tyr Ser Val Ile Gly Thr Ala Val Leu Glu

450 455 460

Tyr Ile Thr Gln Gln Ile Ala Pro Lys Asn Leu Asp Asn Pro Ser Lys

465 470 475 480

Lys Glu Gln Glu Leu Ile Ala Lys Lys Thr Glu Lys Ala Lys Tyr Leu

485 490 495

Ser Leu Glu Thr Ile Lys Leu Ala Leu Glu Glu Phe Asn Lys His Arg

500 505 510

Asp Ile Asp Lys Gln Cys Arg Phe Glu Glu Ile Leu Ala Asn Phe Ala

515 520 525

Ala Ile Pro Met Ile Phe Asp Glu Ile Ala Gln Asn Lys Asp Asn Leu

530 535 540

Ala Gln Ile Ser Ile Lys Tyr Gln Asn Gln Gly Lys Lys Asp Leu Leu

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Gln Ala Ser Ala Glu Asp Asp Val Lys Ala Ile Lys Asp Leu Leu Asp

565 570 575

Gln Thr Asn Asn Leu Leu His Lys Leu Lys Ile Phe His Ile Ser Gln

580 585 590

Ser Glu Asp Lys Ala Asn Ile Leu Asp Lys Asp Glu His Phe Tyr Leu

595 600 605

Val Phe Glu Glu Cys Tyr Phe Glu Leu Ala Asn Ile Val Pro Leu Tyr

610 615 620

Asn Lys Ile Arg Asn Tyr Ile Thr Gln Lys Pro Tyr Ser Asp Glu Lys

625 630 635 640

Phe Lys Leu Asn Phe Glu Asn Ser Thr Leu Ala Asn Gly Trp Asp Lys

645 650 655

Asn Lys Glu Pro Asp Asn Thr Ala Ile Leu Phe Ile Lys Asp Asp Lys
660 665 670

Tyr Tyr Leu Gly Val Met Asn Lys Lys Asn Asn Lys Ile Phe Asp Asp
675 680 685

Lys Ala Ile Lys Glu Asn Lys Gly Glu Gly Tyr Lys Lys Ile Val Tyr
690 695 700

Lys Leu Leu Pro Gly Ala Asn Lys Met Leu Pro Lys Val Phe Phe Ser
705 710 715 720

Ala Lys Ser Ile Lys Phe Tyr Asn Pro Ser Glu Asp Ile Leu Arg Ile

725 730 735

Arg Asn His Ser Thr His Thr Lys Asn Gly Ser Pro Gln Lys Gly Tyr
740 745 750

Glu Lys Phe Glu Phe Asn Ile Glu Asp Cys Arg Lys Phe Ile Asp Phe
755 760 765

Tyr Lys Gln Ser Ile Ser Lys His Pro Glu Trp Lys Asp Phe Gly Phe
770 775 780

Arg Phe Ser Asp Thr Gln Arg Tyr Asn Ser Ile Asp Glu Phe Tyr Arg
785 790 795 800

Glu Val Glu Asn Gln Gly Tyr Lys Leu Thr Phe Glu Asn Ile Ser Glu
805 810 815

Ser Tyr Ile Asp Ser Val Val Asn Gln Gly Lys Leu Tyr Leu Phe Gln
820 825 830

Ile Tyr Asn Lys Asp Phe Ser Ala Tyr Ser Lys Gly Arg Pro Asn Leu
835 840 845

His Thr Leu Tyr Trp Lys Ala Leu Phe Asp Glu Arg Asn Leu Gln Asp
850 855 860

Val Val Tyr Lys Leu Asn Gly Glu Ala Glu Leu Phe Tyr Arg Lys Gln

865 870 875 880

Ser Ile Pro Lys Lys Ile Thr His Pro Ala Lys Glu Ala Ile Ala Asn
885 890 895

Lys Asn Lys Asp Asn Pro Lys Lys Glu Ser Val Phe Glu Tyr Asp Leu
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Ile Lys Asp Lys Arg Phe Thr Glu Asp Lys Phe Phe Phe His Cys Pro
915 920 925
Ile Thr Ile Asn Phe Lys Ser Ser Gly Ala Asn Lys Phe Asn Asp Glu
930 935 940

Ile Asn Leu Leu Leu Lys Glu Lys Ala Asn Asp Val His Ile Leu Ser
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Ile Asp Arg Gly Glu Arg His Leu Ala Tyr Tyr Thr Leu Val Asp Gly
965 970 975
Lys Gly Asn Ile Ile Lys Gln Asp Thr Phe Asn Ile Ile Gly Asn Asp
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995 1000 1005
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Val Ile Glu Tyr Asn Ala Ile Val Val Phe Glu Asp Leu Asn Phe Gly
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Tyr Val Pro Ala Gly Phe Thr Ser Lys Ile Cys Pro Val Thr Gly Phe
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Val Asn Gln Leu Tyr Pro Lys Tyr Glu Ser Val Ser Lys Ser Gln Glu
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Phe Phe Ser Lys Phe Asp Lys Ile Cys Tyr Asn Leu Asp Lys Gly Tyr

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Phe Glu Phe Ser Phe Asp Tyr Lys Asn Phe Gly Asp Lys Ala Ala Lys

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Gly Lys Trp Thr Ile Ala Ser Phe Gly Ser Arg Leu Ile Asn Phe Arg

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Asn Ser Asp Lys Asn His Asn Trp Asp Thr Arg Glu Val Tyr Pro Thr

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Lys Glu Leu Glu Lys Leu Leu Lys Asp Tyr Ser Ile Glu Tyr Gly His

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Gly Glu Cys Ile Lys Ala Ala Ile Cys Gly Glu Ser Asp Lys Lys Phe

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Phe Ala Lys Leu Thr Ser Val Leu Asn Thr Ile Leu Gln Met Arg Asn

1250 1255 1260

Ser Lys Thr Gly Thr Glu Leu Asp Tyr Leu Ile Ser Pro Val Ala Asp

1265 1270 1275 1280

Val Asn Gly Asn Phe Phe Asp Ser Arg Gln Ala Pro Lys Asn Met Pro

1285 1290 1295

Gln Asp Ala Asp Ala Asn Gly Ala Tyr His Ile Gly Leu Lys Gly Leu

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Met Leu Leu Gly Arg Ile Lys Asn Asn Gln Glu Gly Lys Lys Leu Asn

1315 1320 1325

Leu Val Ile Lys Asn Glu Glu Tyr Phe Glu Phe Val Gln Asn Arg Asn

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caagcttgcg gccgcatgtc aatttatcaa gaatttgtta ataaatatag tttaagtaaa 180

actctaagat ttgagttaat cccacagggt aaaacacttg aaaacataaa agcaagaggt 240

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aatagtgata tcacagatat agatgaggcg ttagaaataa tcaaatcttt taaaggttgg 660

acaacttatt ttaagggttt tcatgaaaat agaaaaaatg tttatagtag caatgatatt 720

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agagtttttt cacttgatga agtttttgag atagcaaact ttaataatta tctaaatcaa 960

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35 40 45

Tyr Gln Glu Phe Val Asn Lys Tyr Ser Leu Ser Lys Thr Leu Arg Phe
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Glu Leu Ile Pro Gln Gly Lys Thr Leu Glu Asn Ile Lys Ala Arg Gly
65 70 75 80

Leu Ile Leu Asp Asp Glu Lys Arg Ala Lys Asp Tyr Lys Lys Ala Lys
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Gln Ile Ile Asp Lys Tyr His Gln Phe Phe Ile Glu Glu Ile Leu Ser

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Ser Val Cys Ile Ser Glu Asp Leu Leu Gln Asn Tyr Ser Asp Val Tyr
115 120 125
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Ser Ala Lys Asp Thr Ile Lys Lys Gln Ile Ser Glu Tyr Ile Lys Asp
145 150 155 160
Ser Glu Lys Phe Lys Asn Leu Phe Asn Gln Asn Leu Ile Asp Ala Lys
165 170 175

Lys Gly Gln Glu Ser Asp Leu Ile Leu Trp Leu Lys Gln Ser Lys Asp
180 185 190
Asn Gly Ile Glu Leu Phe Lys Ala Asn Ser Asp Ile Thr Asp Ile Asp
195 200 205
Glu Ala Leu Glu Ile Ile Lys Ser Phe Lys Gly Trp Thr Thr Tyr Phe
210 215 220
Lys Gly Phe His Glu Asn Arg Lys Asn Val Tyr Ser Ser Asn Asp Ile
225 230 235 240
Pro Thr Ser Ile Ile Tyr Arg Ile Val Asp Asp Asn Leu Pro Lys Phe

245 250 255
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260 265 270
Ala Ile Asn Tyr Glu Gln Ile Lys Lys Asp Leu Ala Glu Glu Leu Thr
275 280 285
Phe Asp Ile Asp Tyr Lys Thr Ser Glu Val Asn Gln Arg Val Phe Ser
290 295 300
Leu Asp Glu Val Phe Glu Ile Ala Asn Phe Asn Asn Tyr Leu Asn Gln
305 310 315 320

Ser Gly Ile Thr Lys Phe Asn Thr Ile Ile Gly Gly Lys Phe Val Asn
325 330 335
Gly Glu Asn Thr Lys Arg Lys Gly Ile Asn Glu Tyr Ile Asn Leu Tyr
340 345 350
Ser Gln Gln Ile Asn Asp Lys Thr Leu Lys Lys Tyr Lys Met Ser Val
355 360 365

Leu Phe Lys Gln Ile Leu Ser Asp Thr Glu Ser Lys Ser Phe Val Ile
370 375 380
Asp Lys Leu Glu Asp Asp Ser Asp Val Val Thr Thr Met Gln Ser Phe

385 390 395 400
Tyr Glu Gln Ile Ala Ala Phe Lys Thr Val Glu Glu Lys Ser Ile Lys
405 410 415
Glu Thr Leu Ser Leu Leu Phe Asp Asp Leu Lys Ala Gln Lys Leu Asp
420 425 430
Leu Ser Lys Ile Tyr Phe Lys Asn Asp Lys Ser Leu Thr Asp Leu Ser
435 440 445
Gln Gln Val Phe Asp Asp Tyr Ser Val Ile Gly Thr Ala Val Leu Glu
450 455 460

Tyr Ile Thr Gln Gln Ile Ala Pro Lys Asn Leu Asp Asn Pro Ser Lys
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Lys Glu Gln Glu Leu Ile Ala Lys Lys Thr Glu Lys Ala Lys Tyr Leu
485 490 495
Ser Leu Glu Thr Ile Lys Leu Ala Leu Glu Glu Phe Asn Lys His Arg
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Asp Ile Asp Lys Gln Cys Arg Phe Glu Glu Ile Leu Ala Asn Phe Ala
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565 570 575
Gln Thr Asn Asn Leu Leu His Lys Leu Lys Ile Phe His Ile Ser Gln
580 585 590
Ser Glu Asp Lys Ala Asn Ile Leu Asp Lys Asp Glu His Phe Tyr Leu
595 600 605

Val Phe Glu Glu Cys Tyr Phe Glu Leu Ala Asn Ile Val Pro Leu Tyr

610 615 620

Asn Lys Ile Arg Asn Tyr Ile Thr Gln Lys Pro Tyr Ser Asp Glu Lys

625 630 635 640

Phe Lys Leu Asn Phe Glu Asn Ser Thr Leu Ala Asn Gly Trp Asp Lys

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Asn Lys Glu Pro Asp Asn Thr Ala Ile Leu Phe Ile Lys Asp Asp Lys

660 665 670

Tyr Tyr Leu Gly Val Met Asn Lys Lys Asn Asn Lys Ile Phe Asp Asp

675 680 685

Lys Ala Ile Lys Glu Asn Lys Gly Glu Gly Tyr Lys Lys Ile Val Tyr

690 695 700

Lys Leu Leu Pro Gly Ala Asn Lys Met Leu Pro Lys Val Phe Phe Ser

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Ala Lys Ser Ile Lys Phe Tyr Asn Pro Ser Glu Asp Ile Leu Arg Ile

725 730 735

Arg Asn His Ser Thr His Thr Lys Asn Gly Ser Pro Gln Lys Gly Tyr

740 745 750

Glu Lys Phe Glu Phe Asn Ile Glu Asp Cys Arg Lys Phe Ile Asp Phe

755 760 765

Tyr Lys Gln Ser Ile Ser Lys His Pro Glu Trp Lys Asp Phe Gly Phe

770 775 780

Arg Phe Ser Asp Thr Gln Arg Tyr Asn Ser Ile Asp Glu Phe Tyr Arg

785 790 795 800

Glu Val Glu Asn Gln Gly Tyr Lys Leu Thr Phe Glu Asn Ile Ser Glu

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Ser Ile Pro Lys Lys Ile Thr His Pro Ala Lys Glu Ala Ile Ala Asn

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Lys Asn Lys Asp Asn Pro Lys Lys Glu Ser Val Phe Glu Tyr Asp Leu

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945 950 955 960

Ile Asp Arg Gly Glu Arg His Leu Ala Tyr Tyr Thr Leu Val Asp Gly

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Lys Gly Asn Ile Ile Lys Gln Asp Thr Phe Asn Ile Ile Gly Asn Asp

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995 1000 1005

Arg Asp Ser Ala Arg Lys Asp Trp Lys Lys Ile Asn Asn Ile Lys Glu

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Val Ile Glu Tyr Asn Ala Ile Val Val Phe Glu Asp Leu Asn Phe Gly

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Phe Lys Arg Gly Arg Phe Lys Val Glu Lys Gln Val Tyr Gln Lys Leu

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Glu Phe Asp Lys Thr Gly Gly Val Leu Arg Ala Tyr Gln Leu Thr Ala

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1475 1480 1485
Val Asp Asn Gly Ile Ser Ser His Ala Gly Val Glu His Ala Arg Ser
1490 1495 1500
Leu Gly Ile Pro Val Ile Val Thr Asp His His Leu Pro Gly Glu Thr
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1635 1640 1645

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1665 1670 1675 1680

Thr Arg Lys Glu Ile Glu Gln Gly Met Gln Val Glu Ala Leu Thr Leu

1685 1690 1695

Cys Glu Lys Leu Glu Arg Ser Arg Asp Thr Leu Pro Gly Gly Leu Ala

1700 1705 1710

Met Tyr His Pro Glu Trp His Gln Gly Val Val Gly Ile Leu Ala Ser

1715 1720 1725

Arg Ile Lys Glu Arg Phe His Arg Pro Val Ile Ala Phe Ala Pro Ala

1730 1735 1740

Gly Asp Gly Thr Leu Lys Gly Ser Gly Arg Ser Ile Gln Gly Leu His

1745 1750 1755 1760

Met Arg Asp Ala Leu Glu Arg Leu Asp Thr Leu Tyr Pro Gly Met Ile

1765 1770 1775

Leu Lys Phe Gly Gly His Ala Met Ala Ala Gly Leu Ser Leu Glu Glu

1780 1785 1790

Asp Lys Phe Glu Leu Phe Gln Gln Arg Phe Gly Glu Leu Val Thr Glu

1795 1800 1805

Trp Leu Asp Pro Ser Leu Leu Gln Gly Glu Val Val Ser Asp Gly Pro

1810 1815 1820

Leu Ser Pro Ala Glu Met Thr Met Glu Val Ala Gln Leu Leu Arg Asp

1825 1830 1835 1840

Ala Gly Pro Trp Gly Gln Met Phe Pro Glu Pro Leu Phe Asp Gly His

1845 1850 1855

Phe Arg Leu Leu Gln Gln Arg Leu Val Gly Glu Arg His Leu Lys Val

1860 1865 1870

Met Val Glu Pro Val Gly Gly Gly Pro Leu Leu Asp Gly Ile Ala Phe

1875 1880 1885

Asn Val Asp Thr Ala Leu Trp Pro Asp Asn Gly Val Arg Glu Val Gln

1890 1895 1900

Leu Ala Tyr Lys Leu Asp Ile Asn Glu Phe Arg Gly Asn Arg Ser Leu

1905 1910 1915 1920

Gln Ile Ile Ile Asp Asn Ile Trp Pro Ile Leu Gln Lys Arg Pro Ala

1925 1930 1935

Ala Thr Lys Lys Ala Gly Gln Ala Lys Lys Lys Lys His His His His

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Gln Ile Ile Asp Lys Tyr His Gln Phe Phe Ile Glu Glu Ile Leu Ser

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Ser Glu Asp Lys Ala Asn Ile Leu Asp Lys Asp Glu His Phe Tyr Leu
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1845 1850 1855

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1860 1865 1870

Cys Gly Gln Thr Gly Gly Asp Asn Cys Pro Asp Cys Gly Ala Val Met

1875 1880 1885

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1890 1895 1900

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1925 1930 1935

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1940 1945 1950

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1955 1960 1965

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2005 2010 2015

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Glu Ala Leu Glu Ile Ile Lys Ser Phe Lys Gly Trp Thr Thr Tyr Phe

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<211> 1602

<212> PRT

<213> Artificial Sequence

<220><223> FnCpf1-lambda

<400> 65

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Gly Ser Glu Phe Glu Leu Arg Arg Gln Ala Cys Gly Arg Met Ser Ile
35 40 45

Tyr Gln Glu Phe Val Asn Lys Tyr Ser Leu Ser Lys Thr Leu Arg Phe
50 55 60

Glu Leu Ile Pro Gln Gly Lys Thr Leu Glu Asn Ile Lys Ala Arg Gly
65 70 75 80

Leu Ile Leu Asp Asp Glu Lys Arg Ala Lys Asp Tyr Lys Lys Ala Lys
85 90 95

Gln Ile Ile Asp Lys Tyr His Gln Phe Phe Ile Glu Glu Ile Leu Ser

100 105 110

Ser Val Cys Ile Ser Glu Asp Leu Leu Gln Asn Tyr Ser Asp Val Tyr

115 120 125

Phe Lys Leu Lys Lys Ser Asp Asp Asp Asn Leu Gln Lys Asp Phe Lys

130 135 140

Ser Ala Lys Asp Thr Ile Lys Lys Gln Ile Ser Glu Tyr Ile Lys Asp

145 150 155 160

Ser Glu Lys Phe Lys Asn Leu Phe Asn Gln Asn Leu Ile Asp Ala Lys

165 170 175

Lys Gly Gln Glu Ser Asp Leu Ile Leu Trp Leu Lys Gln Ser Lys Asp

180 185 190

Asn Gly Ile Glu Leu Phe Lys Ala Asn Ser Asp Ile Thr Asp Ile Asp

195 200 205

Glu Ala Leu Glu Ile Ile Lys Ser Phe Lys Gly Trp Thr Thr Tyr Phe

210 215 220

Lys Gly Phe His Glu Asn Arg Lys Asn Val Tyr Ser Ser Asn Asp Ile

225 230 235 240

Pro Thr Ser Ile Ile Tyr Arg Ile Val Asp Asp Asn Leu Pro Lys Phe

245 250 255

Leu Glu Asn Lys Ala Lys Tyr Glu Ser Leu Lys Asp Lys Ala Pro Glu

260 265 270

Ala Ile Asn Tyr Glu Gln Ile Lys Lys Asp Leu Ala Glu Glu Leu Thr

275 280 285

Phe Asp Ile Asp Tyr Lys Thr Ser Glu Val Asn Gln Arg Val Phe Ser

290 295 300

Leu Asp Glu Val Phe Glu Ile Ala Asn Phe Asn Asn Tyr Leu Asn Gln

305 310 315 320

Ser Gly Ile Thr Lys Phe Asn Thr Ile Ile Gly Gly Lys Phe Val Asn

325 330 335

Gly Glu Asn Thr Lys Arg Lys Gly Ile Asn Glu Tyr Ile Asn Leu Tyr

340 345 350

Ser Gln Gln Ile Asn Asp Lys Thr Leu Lys Lys Tyr Lys Met Ser Val

355 360 365

Leu Phe Lys Gln Ile Leu Ser Asp Thr Glu Ser Lys Ser Phe Val Ile

370 375 380

Asp Lys Leu Glu Asp Asp Ser Asp Val Val Thr Thr Met Gln Ser Phe

385 390 395 400

Tyr Glu Gln Ile Ala Ala Phe Lys Thr Val Glu Glu Lys Ser Ile Lys

405 410 415

Glu Thr Leu Ser Leu Leu Phe Asp Asp Leu Lys Ala Gln Lys Leu Asp

420 425 430

Leu Ser Lys Ile Tyr Phe Lys Asn Asp Lys Ser Leu Thr Asp Leu Ser

435 440 445

Gln Gln Val Phe Asp Asp Tyr Ser Val Ile Gly Thr Ala Val Leu Glu

450 455 460

Tyr Ile Thr Gln Gln Ile Ala Pro Lys Asn Leu Asp Asn Pro Ser Lys

465 470 475 480

Lys Glu Gln Glu Leu Ile Ala Lys Lys Thr Glu Lys Ala Lys Tyr Leu

485 490 495

Ser Leu Glu Thr Ile Lys Leu Ala Leu Glu Glu Phe Asn Lys His Arg

500 505 510

Asp Ile Asp Lys Gln Cys Arg Phe Glu Glu Ile Leu Ala Asn Phe Ala

515 520 525

Ala Ile Pro Met Ile Phe Asp Glu Ile Ala Gln Asn Lys Asp Asn Leu

530 535 540

Ala Gln Ile Ser Ile Lys Tyr Gln Asn Gln Gly Lys Lys Asp Leu Leu

545 550 555 560

Gln Ala Ser Ala Glu Asp Asp Val Lys Ala Ile Lys Asp Leu Leu Asp

565 570 575

Gln Thr Asn Asn Leu Leu His Lys Leu Lys Ile Phe His Ile Ser Gln

580 585 590

Ser Glu Asp Lys Ala Asn Ile Leu Asp Lys Asp Glu His Phe Tyr Leu

595 600 605

Val Phe Glu Glu Cys Tyr Phe Glu Leu Ala Asn Ile Val Pro Leu Tyr
610 615 620
Asn Lys Ile Arg Asn Tyr Ile Thr Gln Lys Pro Tyr Ser Asp Glu Lys
625 630 635 640
Phe Lys Leu Asn Phe Glu Asn Ser Thr Leu Ala Asn Gly Trp Asp Lys
645 650 655
Asn Lys Glu Pro Asp Asn Thr Ala Ile Leu Phe Ile Lys Asp Asp Lys
660 665 670
Tyr Tyr Leu Gly Val Met Asn Lys Lys Asn Asn Lys Ile Phe Asp Asp

675 680 685
Lys Ala Ile Lys Glu Asn Lys Gly Glu Gly Tyr Lys Lys Ile Val Tyr
690 695 700
Lys Leu Leu Pro Gly Ala Asn Lys Met Leu Pro Lys Val Phe Phe Ser
705 710 715 720
Ala Lys Ser Ile Lys Phe Tyr Asn Pro Ser Glu Asp Ile Leu Arg Ile
725 730 735
Arg Asn His Ser Thr His Thr Lys Asn Gly Ser Pro Gln Lys Gly Tyr
740 745 750

Glu Lys Phe Glu Phe Asn Ile Glu Asp Cys Arg Lys Phe Ile Asp Phe
755 760 765
Tyr Lys Gln Ser Ile Ser Lys His Pro Glu Trp Lys Asp Phe Gly Phe
770 775 780
Arg Phe Ser Asp Thr Gln Arg Tyr Asn Ser Ile Asp Glu Phe Tyr Arg
785 790 795 800
Glu Val Glu Asn Gln Gly Tyr Lys Leu Thr Phe Glu Asn Ile Ser Glu
805 810 815
Ser Tyr Ile Asp Ser Val Val Asn Gln Gly Lys Leu Tyr Leu Phe Gln

820 825 830
Ile Tyr Asn Lys Asp Phe Ser Ala Tyr Ser Lys Gly Arg Pro Asn Leu
835 840 845

His Thr Leu Tyr Trp Lys Ala Leu Phe Asp Glu Arg Asn Leu Gln Asp
850 855 860
Val Val Tyr Lys Leu Asn Gly Glu Ala Glu Leu Phe Tyr Arg Lys Gln
865 870 875 880
Ser Ile Pro Lys Lys Ile Thr His Pro Ala Lys Glu Ala Ile Ala Asn
885 890 895

Lys Asn Lys Asp Asn Pro Lys Lys Glu Ser Val Phe Glu Tyr Asp Leu
900 905 910
Ile Lys Asp Lys Arg Phe Thr Glu Asp Lys Phe Phe Phe His Cys Pro
915 920 925
Ile Thr Ile Asn Phe Lys Ser Ser Gly Ala Asn Lys Phe Asn Asp Glu
930 935 940
Ile Asn Leu Leu Leu Lys Glu Lys Ala Asn Asp Val His Ile Leu Ser
945 950 955 960
Ile Asp Arg Gly Glu Arg His Leu Ala Tyr Tyr Thr Leu Val Asp Gly

965 970 975
Lys Gly Asn Ile Ile Lys Gln Asp Thr Phe Asn Ile Ile Gly Asn Asp
980 985 990
Arg Met Lys Thr Asn Tyr His Asp Lys Leu Ala Ala Ile Glu Lys Asp
995 1000 1005
Arg Asp Ser Ala Arg Lys Asp Trp Lys Lys Ile Asn Asn Ile Lys Glu
1010 1015 1020
Met Lys Glu Gly Tyr Leu Ser Gln Val Val His Glu Ile Ala Lys Leu
1025 1030 1035 1040

Val Ile Glu Tyr Asn Ala Ile Val Val Phe Glu Asp Leu Asn Phe Gly
1045 1050 1055
Phe Lys Arg Gly Arg Phe Lys Val Glu Lys Gln Val Tyr Gln Lys Leu
1060 1065 1070
Glu Lys Met Leu Ile Glu Lys Leu Asn Tyr Leu Val Phe Lys Asp Asn
1075 1080 1085
Glu Phe Asp Lys Thr Gly Gly Val Leu Arg Ala Tyr Gln Leu Thr Ala
1090 1095 1100

Pro Phe Glu Thr Phe Lys Lys Met Gly Lys Gln Thr Gly Ile Ile Tyr

1105 1110 1115 1120

Tyr Val Pro Ala Gly Phe Thr Ser Lys Ile Cys Pro Val Thr Gly Phe

1125 1130 1135

Val Asn Gln Leu Tyr Pro Lys Tyr Glu Ser Val Ser Lys Ser Gln Glu

1140 1145 1150

Phe Phe Ser Lys Phe Asp Lys Ile Cys Tyr Asn Leu Asp Lys Gly Tyr

1155 1160 1165

Phe Glu Phe Ser Phe Asp Tyr Lys Asn Phe Gly Asp Lys Ala Ala Lys

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1185 1190 1195 1200

Asn Ser Asp Lys Asn His Asn Trp Asp Thr Arg Glu Val Tyr Pro Thr

1205 1210 1215

Lys Glu Leu Glu Lys Leu Leu Lys Asp Tyr Ser Ile Glu Tyr Gly His

1220 1225 1230

Gly Glu Cys Ile Lys Ala Ala Ile Cys Gly Glu Ser Asp Lys Lys Phe

1235 1240 1245

Phe Ala Lys Leu Thr Ser Val Leu Asn Thr Ile Leu Gln Met Arg Asn

1250 1255 1260

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1265 1270 1275 1280

Val Asn Gly Asn Phe Phe Asp Ser Arg Gln Ala Pro Lys Asn Met Pro

1285 1290 1295

Gln Asp Ala Asp Ala Asn Gly Ala Tyr His Ile Gly Leu Lys Gly Leu

1300 1305 1310

Met Leu Leu Gly Arg Ile Lys Asn Asn Gln Glu Gly Lys Lys Leu Asn

1315 1320 1325

Leu Val Ile Lys Asn Glu Glu Tyr Phe Glu Phe Val Gln Asn Arg Asn

1330 1335 1340

Asn Gln Ala Ala Ala Leu Asp Leu Glu Met Thr Pro Asp Ile Ile Leu

1345 1350 1355 1360

Gln Arg Thr Gly Ile Asp Val Arg Ala Val Glu Gln Gly Asp Asp Ala

1365 1370 1375

Trp His Lys Leu Arg Leu Gly Val Ile Thr Ala Ser Glu Val His Asn

1380 1385 1390

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1395 1400 1405

Ser Tyr Phe His Thr Leu Leu Ala Glu Val Cys Thr Gly Val Ala Pro

1410 1415 1420

Glu Val Asn Ala Lys Ala Leu Ala Trp Gly Lys Gln Tyr Glu Asn Asp

1425 1430 1435 1440

Ala Arg Thr Leu Phe Glu Phe Thr Ser Gly Val Asn Val Thr Glu Ser

1445 1450 1455

Pro Ile Ile Tyr Arg Asp Glu Ser Met Arg Thr Ala Cys Ser Pro Asp

1460 1465 1470

Gly Leu Cys Ser Asp Gly Asn Gly Leu Glu Leu Lys Cys Pro Phe Thr

1475 1480 1485

Ser Arg Asp Phe Met Lys Phe Arg Leu Gly Gly Phe Glu Ala Ile Lys

1490 1495 1500

Ser Ala Tyr Met Ala Gln Val Gln Tyr Ser Met Trp Val Thr Arg Lys

1505 1510 1515 1520

Asn Ala Trp Tyr Phe Ala Asn Tyr Asp Pro Arg Met Lys Arg Glu Gly

1525 1530 1535

Leu His Tyr Val Val Ile Glu Arg Asp Glu Lys Tyr Met Ala Ser Phe

1540 1545 1550

Asp Glu Ile Val Pro Glu Phe Ile Glu Lys Met Asp Glu Ala Leu Ala

1555 1560 1565

Glu Ile Gly Phe Val Phe Gly Glu Gln Trp Arg Leu Glu Lys Arg Pro

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Ala Ala Thr Lys Lys Ala Gly Gln Ala Lys Lys Lys Lys His His His

1585 1590 1595 1600

His His

<210> 66

<211> 4809

<212> DNA

<213> Artificial Sequence

<220><223> FnCpf1-lambda

<400> 66

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caagcttgcg gccgcatgtc aatttatcaa gaatttgtta ataaatatag tttaagtaaa 180

actctaagat ttgagttaat cccacagggt aaaacacttg aaaacataaa agcaagaggt 240

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tcagagaaat ttaagaattt gtttaatcaa aaccttatcg atgctaaaaa agggcaagag 540

tcagatttaa ttctatggct aaagcaatct aaggataatg gtatagaact atttaaagcc 600

aatagtgata tcacagatat agatgaggcg ttagaaataa tcaaatcttt taaaggttgg 660

acaacttatt ttaagggttt tcatgaaaat agaaaaaatg tttatagtag caatgatatt 720

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gctaagtatg agagtttaaa agacaaagct ccagaagcta taaactatga acaaattaaa 840

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<211> 1731

<212> PRT

<213> Artificial Sequence

<220><223> FnCpf1-mungbean

<400> 67

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35 40 45
Tyr Gln Glu Phe Val Asn Lys Tyr Ser Leu Ser Lys Thr Leu Arg Phe
50 55 60
Glu Leu Ile Pro Gln Gly Lys Thr Leu Glu Asn Ile Lys Ala Arg Gly

65 70 75 80
Leu Ile Leu Asp Asp Glu Lys Arg Ala Lys Asp Tyr Lys Lys Ala Lys
85 90 95
Gln Ile Ile Asp Lys Tyr His Gln Phe Phe Ile Glu Glu Ile Leu Ser
100 105 110
Ser Val Cys Ile Ser Glu Asp Leu Leu Gln Asn Tyr Ser Asp Val Tyr
115 120 125
Phe Lys Leu Lys Lys Ser Asp Asp Asp Asn Leu Gln Lys Asp Phe Lys
130 135 140

Ser Ala Lys Asp Thr Ile Lys Lys Gln Ile Ser Glu Tyr Ile Lys Asp
145 150 155 160
Ser Glu Lys Phe Lys Asn Leu Phe Asn Gln Asn Leu Ile Asp Ala Lys
165 170 175
Lys Gly Gln Glu Ser Asp Leu Ile Leu Trp Leu Lys Gln Ser Lys Asp
180 185 190
Asn Gly Ile Glu Leu Phe Lys Ala Asn Ser Asp Ile Thr Asp Ile Asp
195 200 205
Glu Ala Leu Glu Ile Ile Lys Ser Phe Lys Gly Trp Thr Thr Tyr Phe

210 215 220
Lys Gly Phe His Glu Asn Arg Lys Asn Val Tyr Ser Ser Asn Asp Ile

225 230 235 240

Pro Thr Ser Ile Ile Tyr Arg Ile Val Asp Asp Asn Leu Pro Lys Phe

245 250 255

Leu Glu Asn Lys Ala Lys Tyr Glu Ser Leu Lys Asp Lys Ala Pro Glu

260 265 270

Ala Ile Asn Tyr Glu Gln Ile Lys Lys Asp Leu Ala Glu Glu Leu Thr

275 280 285

Phe Asp Ile Asp Tyr Lys Thr Ser Glu Val Asn Gln Arg Val Phe Ser

290 295 300

Leu Asp Glu Val Phe Glu Ile Ala Asn Phe Asn Asn Tyr Leu Asn Gln

305 310 315 320

Ser Gly Ile Thr Lys Phe Asn Thr Ile Ile Gly Gly Lys Phe Val Asn

325 330 335

Gly Glu Asn Thr Lys Arg Lys Gly Ile Asn Glu Tyr Ile Asn Leu Tyr

340 345 350

Ser Gln Gln Ile Asn Asp Lys Thr Leu Lys Lys Tyr Lys Met Ser Val

355 360 365

Leu Phe Lys Gln Ile Leu Ser Asp Thr Glu Ser Lys Ser Phe Val Ile

370 375 380

Asp Lys Leu Glu Asp Asp Ser Asp Val Val Thr Thr Met Gln Ser Phe

385 390 395 400

Tyr Glu Gln Ile Ala Ala Phe Lys Thr Val Glu Glu Lys Ser Ile Lys

405 410 415

Glu Thr Leu Ser Leu Leu Phe Asp Asp Leu Lys Ala Gln Lys Leu Asp

420 425 430

Leu Ser Lys Ile Tyr Phe Lys Asn Asp Lys Ser Leu Thr Asp Leu Ser

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Gln Gln Val Phe Asp Asp Tyr Ser Val Ile Gly Thr Ala Val Leu Glu

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Tyr Ile Thr Gln Gln Ile Ala Pro Lys Asn Leu Asp Asn Pro Ser Lys

465 470 475 480

Lys Glu Gln Glu Leu Ile Ala Lys Lys Thr Glu Lys Ala Lys Tyr Leu

485 490 495

Ser Leu Glu Thr Ile Lys Leu Ala Leu Glu Glu Phe Asn Lys His Arg

500 505 510

Asp Ile Asp Lys Gln Cys Arg Phe Glu Glu Ile Leu Ala Asn Phe Ala

515 520 525

Ala Ile Pro Met Ile Phe Asp Glu Ile Ala Gln Asn Lys Asp Asn Leu

530 535 540

Ala Gln Ile Ser Ile Lys Tyr Gln Asn Gln Gly Lys Lys Asp Leu Leu

545 550 555 560

Gln Ala Ser Ala Glu Asp Asp Val Lys Ala Ile Lys Asp Leu Leu Asp

565 570 575

Gln Thr Asn Asn Leu Leu His Lys Leu Lys Ile Phe His Ile Ser Gln

580 585 590

Ser Glu Asp Lys Ala Asn Ile Leu Asp Lys Asp Glu His Phe Tyr Leu

595 600 605

Val Phe Glu Glu Cys Tyr Phe Glu Leu Ala Asn Ile Val Pro Leu Tyr

610 615 620

Asn Lys Ile Arg Asn Tyr Ile Thr Gln Lys Pro Tyr Ser Asp Glu Lys

625 630 635 640

Phe Lys Leu Asn Phe Glu Asn Ser Thr Leu Ala Asn Gly Trp Asp Lys

645 650 655

Asn Lys Glu Pro Asp Asn Thr Ala Ile Leu Phe Ile Lys Asp Asp Lys

660 665 670

Tyr Tyr Leu Gly Val Met Asn Lys Lys Asn Asn Lys Ile Phe Asp Asp

675 680 685

Lys Ala Ile Lys Glu Asn Lys Gly Glu Gly Tyr Lys Lys Ile Val Tyr

690 695 700

Lys Leu Leu Pro Gly Ala Asn Lys Met Leu Pro Lys Val Phe Phe Ser

705 710 715 720

Ala Lys Ser Ile Lys Phe Tyr Asn Pro Ser Glu Asp Ile Leu Arg Ile

725 730 735

Arg Asn His Ser Thr His Thr Lys Asn Gly Ser Pro Gln Lys Gly Tyr
740 745 750
Glu Lys Phe Glu Phe Asn Ile Glu Asp Cys Arg Lys Phe Ile Asp Phe
755 760 765
Tyr Lys Gln Ser Ile Ser Lys His Pro Glu Trp Lys Asp Phe Gly Phe
770 775 780
Arg Phe Ser Asp Thr Gln Arg Tyr Asn Ser Ile Asp Glu Phe Tyr Arg

785 790 795 800
Glu Val Glu Asn Gln Gly Tyr Lys Leu Thr Phe Glu Asn Ile Ser Glu
805 810 815
Ser Tyr Ile Asp Ser Val Val Asn Gln Gly Lys Leu Tyr Leu Phe Gln
820 825 830
Ile Tyr Asn Lys Asp Phe Ser Ala Tyr Ser Lys Gly Arg Pro Asn Leu
835 840 845
His Thr Leu Tyr Trp Lys Ala Leu Phe Asp Glu Arg Asn Leu Gln Asp
850 855 860

Val Val Tyr Lys Leu Asn Gly Glu Ala Glu Leu Phe Tyr Arg Lys Gln
865 870 875 880
Ser Ile Pro Lys Lys Ile Thr His Pro Ala Lys Glu Ala Ile Ala Asn
885 890 895
Lys Asn Lys Asp Asn Pro Lys Lys Glu Ser Val Phe Glu Tyr Asp Leu
900 905 910
Ile Lys Asp Lys Arg Phe Thr Glu Asp Lys Phe Phe Phe His Cys Pro
915 920 925
Ile Thr Ile Asn Phe Lys Ser Ser Gly Ala Asn Lys Phe Asn Asp Glu

930 935 940
Ile Asn Leu Leu Leu Lys Glu Lys Ala Asn Asp Val His Ile Leu Ser
945 950 955 960
Ile Asp Arg Gly Glu Arg His Leu Ala Tyr Tyr Thr Leu Val Asp Gly
965 970 975
Lys Gly Asn Ile Ile Lys Gln Asp Thr Phe Asn Ile Ile Gly Asn Asp
980 985 990

Arg Met Lys Thr Asn Tyr His Asp Lys Leu Ala Ala Ile Glu Lys Asp
995 1000 1005

Arg Asp Ser Ala Arg Lys Asp Trp Lys Lys Ile Asn Asn Ile Lys Glu
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Met Lys Glu Gly Tyr Leu Ser Gln Val Val His Glu Ile Ala Lys Leu
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Val Ile Glu Tyr Asn Ala Ile Val Val Phe Glu Asp Leu Asn Phe Gly
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Phe Lys Arg Gly Arg Phe Lys Val Glu Lys Gln Val Tyr Gln Lys Leu
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Glu Lys Met Leu Ile Glu Lys Leu Asn Tyr Leu Val Phe Lys Asp Asn

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1140 1145 1150

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Gly Lys Trp Thr Ile Ala Ser Phe Gly Ser Arg Leu Ile Asn Phe Arg
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Asn Ser Asp Lys Asn His Asn Trp Asp Thr Arg Glu Val Tyr Pro Thr
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Ser Lys Thr Gly Thr Glu Leu Asp Tyr Leu Ile Ser Pro Val Ala Asp

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Thr Glu Lys Val Phe Phe Ser Glu Ser Phe Asp Gln Thr Arg Cys Thr

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Gln Pro Leu Ser Glu Lys Lys Lys Arg Val Phe Phe Leu Asp Val Asn

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Trp Leu Ser Leu Phe Leu His Gln Val Ser Leu Thr Asp Pro Val Ile

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Ala Val Ile Asp Gly Glu Gly Gly Ser Glu His Arg Arg Lys Leu Leu

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Pro Ser Tyr Lys Ala His Arg Lys Lys Phe Met Arg His Met Ser Ser

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Gly His Val Gly Arg Ser His Gln Val Ile Asn Asp Val Leu Gly Lys

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Gln His Leu Val Pro Ser Phe Gly Arg Lys Thr Ala Met Lys Leu Ile

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Lys Lys His Gly Ser Leu Glu Thr Leu Leu Asn Ala Ala Ala Ile Arg

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Thr Val Gly Arg Pro Tyr Ala Gln Asp Ala Leu Lys Asn His Ala Asp

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Tyr Leu Arg Arg Asn Tyr Glu Val Leu Ala Leu Lys Arg Asp Val Asn

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Ile Gln Leu Tyr Asp Glu Trp Leu Val Lys Arg Asp Asn His Asn Asp

1665 1670 1675 1680

Lys Thr Ala Leu Ser Ser Phe Phe Lys Tyr Leu Gly Glu Ser Lys Glu

1685 1690 1695

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His His His

1730

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<211> 5196

<212> DNA

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Tyr Gln Glu Phe Val Asn Lys Tyr Ser Leu Ser Lys Thr Leu Arg Phe

50 55 60

Glu Leu Ile Pro Gln Gly Lys Thr Leu Glu Asn Ile Lys Ala Arg Gly

65 70 75 80

Leu Ile Leu Asp Asp Glu Lys Arg Ala Lys Asp Tyr Lys Lys Ala Lys

85 90 95

Gln Ile Ile Asp Lys Tyr His Gln Phe Phe Ile Glu Glu Ile Leu Ser

100 105 110

Ser Val Cys Ile Ser Glu Asp Leu Leu Gln Asn Tyr Ser Asp Val Tyr

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Phe Lys Leu Lys Lys Ser Asp Asp Asp Asn Leu Gln Lys Asp Phe Lys

130 135 140

Ser Ala Lys Asp Thr Ile Lys Lys Gln Ile Ser Glu Tyr Ile Lys Asp

145 150 155 160

Ser Glu Lys Phe Lys Asn Leu Phe Asn Gln Asn Leu Ile Asp Ala Lys

165 170 175

Lys Gly Gln Glu Ser Asp Leu Ile Leu Trp Leu Lys Gln Ser Lys Asp

180 185 190

Asn Gly Ile Glu Leu Phe Lys Ala Asn Ser Asp Ile Thr Asp Ile Asp
195 200 205
Glu Ala Leu Glu Ile Ile Lys Ser Phe Lys Gly Trp Thr Thr Tyr Phe
210 215 220

Lys Gly Phe His Glu Asn Arg Lys Asn Val Tyr Ser Ser Asn Asp Ile
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Pro Thr Ser Ile Ile Tyr Arg Ile Val Asp Asp Asn Leu Pro Lys Phe
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Leu Glu Asn Lys Ala Lys Tyr Glu Ser Leu Lys Asp Lys Ala Pro Glu
260 265 270
Ala Ile Asn Tyr Glu Gln Ile Lys Lys Asp Leu Ala Glu Glu Leu Thr
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Phe Asp Ile Asp Tyr Lys Thr Ser Glu Val Asn Gln Arg Val Phe Ser

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Ser Gly Ile Thr Lys Phe Asn Thr Ile Ile Gly Gly Lys Phe Val Asn
325 330 335
Gly Glu Asn Thr Lys Arg Lys Gly Ile Asn Glu Tyr Ile Asn Leu Tyr
340 345 350
Ser Gln Gln Ile Asn Asp Lys Thr Leu Lys Lys Tyr Lys Met Ser Val
355 360 365

Leu Phe Lys Gln Ile Leu Ser Asp Thr Glu Ser Lys Ser Phe Val Ile
370 375 380
Asp Lys Leu Glu Asp Asp Ser Asp Val Val Thr Thr Met Gln Ser Phe
385 390 395 400
Tyr Glu Gln Ile Ala Ala Phe Lys Thr Val Glu Glu Lys Ser Ile Lys
405 410 415
Glu Thr Leu Ser Leu Leu Phe Asp Asp Leu Lys Ala Gln Lys Leu Asp
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Leu Ser Lys Ile Tyr Phe Lys Asn Asp Lys Ser Leu Thr Asp Leu Ser

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Gln Gln Val Phe Asp Asp Tyr Ser Val Ile Gly Thr Ala Val Leu Glu

450 455 460

Tyr Ile Thr Gln Gln Ile Ala Pro Lys Asn Leu Asp Asn Pro Ser Lys

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Lys Glu Gln Glu Leu Ile Ala Lys Lys Thr Glu Lys Ala Lys Tyr Leu

485 490 495

Ser Leu Glu Thr Ile Lys Leu Ala Leu Glu Glu Phe Asn Lys His Arg

500 505 510

Asp Ile Asp Lys Gln Cys Arg Phe Glu Glu Ile Leu Ala Asn Phe Ala

515 520 525

Ala Ile Pro Met Ile Phe Asp Glu Ile Ala Gln Asn Lys Asp Asn Leu

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Ala Gln Ile Ser Ile Lys Tyr Gln Asn Gln Gly Lys Lys Asp Leu Leu

545 550 555 560

Gln Ala Ser Ala Glu Asp Asp Val Lys Ala Ile Lys Asp Leu Leu Asp

565 570 575

Gln Thr Asn Asn Leu Leu His Lys Leu Lys Ile Phe His Ile Ser Gln

580 585 590

Ser Glu Asp Lys Ala Asn Ile Leu Asp Lys Asp Glu His Phe Tyr Leu

595 600 605

Val Phe Glu Glu Cys Tyr Phe Glu Leu Ala Asn Ile Val Pro Leu Tyr

610 615 620

Asn Lys Ile Arg Asn Tyr Ile Thr Gln Lys Pro Tyr Ser Asp Glu Lys

625 630 635 640

Phe Lys Leu Asn Phe Glu Asn Ser Thr Leu Ala Asn Gly Trp Asp Lys

645 650 655

Asn Lys Glu Pro Asp Asn Thr Ala Ile Leu Phe Ile Lys Asp Asp Lys

660 665 670

Tyr Tyr Leu Gly Val Met Asn Lys Lys Asn Asn Lys Ile Phe Asp Asp

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Lys Ala Ile Lys Glu Asn Lys Gly Glu Gly Tyr Lys Lys Ile Val Tyr

690 695 700

Lys Leu Leu Pro Gly Ala Asn Lys Met Leu Pro Lys Val Phe Phe Ser

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725 730 735

Arg Asn His Ser Thr His Thr Lys Asn Gly Ser Pro Gln Lys Gly Tyr

740 745 750

Glu Lys Phe Glu Phe Asn Ile Glu Asp Cys Arg Lys Phe Ile Asp Phe

755 760 765

Tyr Lys Gln Ser Ile Ser Lys His Pro Glu Trp Lys Asp Phe Gly Phe

770 775 780

Arg Phe Ser Asp Thr Gln Arg Tyr Asn Ser Ile Asp Glu Phe Tyr Arg

785 790 795 800

Glu Val Glu Asn Gln Gly Tyr Lys Leu Thr Phe Glu Asn Ile Ser Glu

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Ser Tyr Ile Asp Ser Val Val Asn Gln Gly Lys Leu Tyr Leu Phe Gln

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Ile Tyr Asn Lys Asp Phe Ser Ala Tyr Ser Lys Gly Arg Pro Asn Leu

835 840 845

His Thr Leu Tyr Trp Lys Ala Leu Phe Asp Glu Arg Asn Leu Gln Asp

850 855 860

Val Val Tyr Lys Leu Asn Gly Glu Ala Glu Leu Phe Tyr Arg Lys Gln

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Lys Asn Lys Asp Asn Pro Lys Lys Glu Ser Val Phe Glu Tyr Asp Leu

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Ile Lys Asp Lys Arg Phe Thr Glu Asp Lys Phe Phe Phe His Cys Pro

915 920 925

Ile Thr Ile Asn Phe Lys Ser Ser Gly Ala Asn Lys Phe Asn Asp Glu

930 935 940

Ile Asn Leu Leu Leu Lys Glu Lys Ala Asn Asp Val His Ile Leu Ser
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1460 1465 1470

Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn

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Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg

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Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His

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Gly Met Asp Glu Leu Tyr Lys Leu Glu Lys Arg Pro Ala Ala Thr Lys

1585 1590 1595 1600

Lys Ala Gly Gln Ala Lys Lys Lys Lys His His His His His His

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Gly Met Gln Val Glu Ala Leu Thr Leu Cys Glu Lys Leu Glu Arg Ser

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<212> PRT

<213> Artificial Sequence

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<400> 73

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Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser
50 55 60

Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe
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Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr
85 90 95

Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met
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Lys Arg His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln
115 120 125

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130 135 140

Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys
145 150 155 160

Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu
165 170 175

Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys
180 185 190

Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly
195 200 205

Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp

210 215 220

Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala

225 230 235 240

Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu

245 250 255

Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys

260 265 270

Gly Ser Glu Phe Glu Leu Arg Arg Gln Ala Cys Gly Arg Met Ser Ile

275 280 285

Tyr Gln Glu Phe Val Asn Lys Tyr Ser Leu Ser Lys Thr Leu Arg Phe

290 295 300

Glu Leu Ile Pro Gln Gly Lys Thr Leu Glu Asn Ile Lys Ala Arg Gly

305 310 315 320

Leu Ile Leu Asp Asp Glu Lys Arg Ala Lys Asp Tyr Lys Lys Ala Lys

325 330 335

Gln Ile Ile Asp Lys Tyr His Gln Phe Phe Ile Glu Glu Ile Leu Ser

340 345 350

Ser Val Cys Ile Ser Glu Asp Leu Leu Gln Asn Tyr Ser Asp Val Tyr

355 360 365

Phe Lys Leu Lys Lys Ser Asp Asp Asp Asn Leu Gln Lys Asp Phe Lys

370 375 380

Ser Ala Lys Asp Thr Ile Lys Lys Gln Ile Ser Glu Tyr Ile Lys Asp

385 390 395 400

Ser Glu Lys Phe Lys Asn Leu Phe Asn Gln Asn Leu Ile Asp Ala Lys

405 410 415

Lys Gly Gln Glu Ser Asp Leu Ile Leu Trp Leu Lys Gln Ser Lys Asp

420 425 430

Asn Gly Ile Glu Leu Phe Lys Ala Asn Ser Asp Ile Thr Asp Ile Asp

435 440 445

Glu Ala Leu Glu Ile Ile Lys Ser Phe Lys Gly Trp Thr Thr Tyr Phe

450 455 460

Lys Gly Phe His Glu Asn Arg Lys Asn Val Tyr Ser Ser Asn Asp Ile

465 470 475 480

Pro Thr Ser Ile Ile Tyr Arg Ile Val Asp Asp Asn Leu Pro Lys Phe

485 490 495

Leu Glu Asn Lys Ala Lys Tyr Glu Ser Leu Lys Asp Lys Ala Pro Glu

500 505 510

Ala Ile Asn Tyr Glu Gln Ile Lys Lys Asp Leu Ala Glu Glu Leu Thr

515 520 525

Phe Asp Ile Asp Tyr Lys Thr Ser Glu Val Asn Gln Arg Val Phe Ser

530 535 540

Leu Asp Glu Val Phe Glu Ile Ala Asn Phe Asn Asn Tyr Leu Asn Gln

545 550 555 560

Ser Gly Ile Thr Lys Phe Asn Thr Ile Ile Gly Gly Lys Phe Val Asn

565 570 575

Gly Glu Asn Thr Lys Arg Lys Gly Ile Asn Glu Tyr Ile Asn Leu Tyr

580 585 590

Ser Gln Gln Ile Asn Asp Lys Thr Leu Lys Lys Tyr Lys Met Ser Val

595 600 605

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Asp Lys Leu Glu Asp Asp Ser Asp Val Val Thr Thr Met Gln Ser Phe

625 630 635 640

Tyr Glu Gln Ile Ala Ala Phe Lys Thr Val Glu Glu Lys Ser Ile Lys

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Glu Thr Leu Ser Leu Leu Phe Asp Asp Leu Lys Ala Gln Lys Leu Asp

660 665 670

Leu Ser Lys Ile Tyr Phe Lys Asn Asp Lys Ser Leu Thr Asp Leu Ser

675 680 685

Gln Gln Val Phe Asp Asp Tyr Ser Val Ile Gly Thr Ala Val Leu Glu

690 695 700

Tyr Ile Thr Gln Gln Ile Ala Pro Lys Asn Leu Asp Asn Pro Ser Lys

705 710 715 720

Lys Glu Gln Glu Leu Ile Ala Lys Lys Thr Glu Lys Ala Lys Tyr Leu
725 730 735
Ser Leu Glu Thr Ile Lys Leu Ala Leu Glu Glu Phe Asn Lys His Arg
740 745 750
Asp Ile Asp Lys Gln Cys Arg Phe Glu Glu Ile Leu Ala Asn Phe Ala
755 760 765
Ala Ile Pro Met Ile Phe Asp Glu Ile Ala Gln Asn Lys Asp Asn Leu
770 775 780

Ala Gln Ile Ser Ile Lys Tyr Gln Asn Gln Gly Lys Lys Asp Leu Leu
785 790 795 800
Gln Ala Ser Ala Glu Asp Asp Val Lys Ala Ile Lys Asp Leu Leu Asp
805 810 815
Gln Thr Asn Asn Leu Leu His Lys Leu Lys Ile Phe His Ile Ser Gln
820 825 830
Ser Glu Asp Lys Ala Asn Ile Leu Asp Lys Asp Glu His Phe Tyr Leu
835 840 845
Val Phe Glu Glu Cys Tyr Phe Glu Leu Ala Asn Ile Val Pro Leu Tyr

850 855 860
Asn Lys Ile Arg Asn Tyr Ile Thr Gln Lys Pro Tyr Ser Asp Glu Lys
865 870 875 880
Phe Lys Leu Asn Phe Glu Asn Ser Thr Leu Ala Asn Gly Trp Asp Lys
885 890 895
Asn Lys Glu Pro Asp Asn Thr Ala Ile Leu Phe Ile Lys Asp Asp Lys
900 905 910
Tyr Tyr Leu Gly Val Met Asn Lys Lys Asn Asn Lys Ile Phe Asp Asp
915 920 925

Lys Ala Ile Lys Glu Asn Lys Gly Glu Gly Tyr Lys Lys Ile Val Tyr
930 935 940
Lys Leu Leu Pro Gly Ala Asn Lys Met Leu Pro Lys Val Phe Phe Ser
945 950 955 960
Ala Lys Ser Ile Lys Phe Tyr Asn Pro Ser Glu Asp Ile Leu Arg Ile
965 970 975

Arg Asn His Ser Thr His Thr Lys Asn Gly Ser Pro Gln Lys Gly Tyr
980 985 990
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995 1000 1005
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Ser Ile Pro Lys Lys Ile Thr His Pro Ala Lys Glu Ala Ile Ala Asn
1125 1130 1135
Lys Asn Lys Asp Asn Pro Lys Lys Glu Ser Val Phe Glu Tyr Asp Leu

1140 1145 1150
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Phe Ala Lys Leu Thr Ser Val Leu Asn Thr Ile Leu Gln Met Arg Asn

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Gln Asp Ala Asp Ala Asn Gly Ala Tyr His Ile Gly Leu Lys Gly Leu

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<211> 1497

<212> PRT

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<400> 75

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35 40 45

Leu Gly Gln Asp Pro Glu Val Arg Tyr Met Pro Asn Gly Gly Ala Val
50 55 60

Ala Asn Ile Thr Leu Ala Thr Ser Glu Ser Trp Arg Asp Lys Ala Thr
65 70 75 80

Gly Glu Met Lys Glu Gln Thr Glu Trp His Arg Val Val Leu Phe Gly

85 90 95

Lys Leu Ala Glu Val Ala Ser Glu Tyr Leu Arg Lys Gly Ser Gln Val

100 105 110

Tyr Ile Glu Gly Gln Leu Arg Thr Arg Lys Trp Thr Asp Gln Ser Gly

115 120 125

Gln Asp Arg Tyr Thr Thr Glu Val Val Val Asn Val Gly Gly Thr Met

130 135 140

Gln Met Leu Gly Gly Arg Gly Ser Glu Phe Glu Leu Arg Arg Gln Ala

145 150 155 160

Cys Gly Arg Met Ser Ile Tyr Gln Glu Phe Val Asn Lys Tyr Ser Leu

165 170 175

Ser Lys Thr Leu Arg Phe Glu Leu Ile Pro Gln Gly Lys Thr Leu Glu

180 185 190

Asn Ile Lys Ala Arg Gly Leu Ile Leu Asp Asp Glu Lys Arg Ala Lys

195 200 205

Asp Tyr Lys Lys Ala Lys Gln Ile Ile Asp Lys Tyr His Gln Phe Phe

210 215 220

Ile Glu Glu Ile Leu Ser Ser Val Cys Ile Ser Glu Asp Leu Leu Gln

225 230 235 240

Asn Tyr Ser Asp Val Tyr Phe Lys Leu Lys Lys Ser Asp Asp Asp Asn

245 250 255

Leu Gln Lys Asp Phe Lys Ser Ala Lys Asp Thr Ile Lys Lys Gln Ile

260 265 270

Ser Glu Tyr Ile Lys Asp Ser Glu Lys Phe Lys Asn Leu Phe Asn Gln

275 280 285

Asn Leu Ile Asp Ala Lys Lys Gly Gln Glu Ser Asp Leu Ile Leu Trp

290 295 300

Leu Lys Gln Ser Lys Asp Asn Gly Ile Glu Leu Phe Lys Ala Asn Ser

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Asp Ile Thr Asp Ile Asp Glu Ala Leu Glu Ile Ile Lys Ser Phe Lys

325 330 335

Gly Trp Thr Thr Tyr Phe Lys Gly Phe His Glu Asn Arg Lys Asn Val

340 345 350

Tyr Ser Ser Asn Asp Ile Pro Thr Ser Ile Ile Tyr Arg Ile Val Asp

355 360 365

Asp Asn Leu Pro Lys Phe Leu Glu Asn Lys Ala Lys Tyr Glu Ser Leu

370 375 380

Lys Asp Lys Ala Pro Glu Ala Ile Asn Tyr Glu Gln Ile Lys Lys Asp

385 390 395 400

Leu Ala Glu Glu Leu Thr Phe Asp Ile Asp Tyr Lys Thr Ser Glu Val

405 410 415

Asn Gln Arg Val Phe Ser Leu Asp Glu Val Phe Glu Ile Ala Asn Phe

420 425 430

Asn Asn Tyr Leu Asn Gln Ser Gly Ile Thr Lys Phe Asn Thr Ile Ile

435 440 445

Gly Gly Lys Phe Val Asn Gly Glu Asn Thr Lys Arg Lys Gly Ile Asn

450 455 460

Glu Tyr Ile Asn Leu Tyr Ser Gln Gln Ile Asn Asp Lys Thr Leu Lys

465 470 475 480

Lys Tyr Lys Met Ser Val Leu Phe Lys Gln Ile Leu Ser Asp Thr Glu

485 490 495

Ser Lys Ser Phe Val Ile Asp Lys Leu Glu Asp Asp Ser Asp Val Val

500 505 510

Thr Thr Met Gln Ser Phe Tyr Glu Gln Ile Ala Ala Phe Lys Thr Val

515 520 525

Glu Glu Lys Ser Ile Lys Glu Thr Leu Ser Leu Leu Phe Asp Asp Leu

530 535 540

Lys Ala Gln Lys Leu Asp Leu Ser Lys Ile Tyr Phe Lys Asn Asp Lys

545 550 555 560

Ser Leu Thr Asp Leu Ser Gln Gln Val Phe Asp Asp Tyr Ser Val Ile

565 570 575

Gly Thr Ala Val Leu Glu Tyr Ile Thr Gln Gln Ile Ala Pro Lys Asn

580 585 590

Leu Asp Asn Pro Ser Lys Lys Glu Gln Glu Leu Ile Ala Lys Lys Thr
595 600 605
Glu Lys Ala Lys Tyr Leu Ser Leu Glu Thr Ile Lys Leu Ala Leu Glu
610 615 620
Glu Phe Asn Lys His Arg Asp Ile Asp Lys Gln Cys Arg Phe Glu Glu
625 630 635 640
Ile Leu Ala Asn Phe Ala Ala Ile Pro Met Ile Phe Asp Glu Ile Ala
645 650 655
Gln Asn Lys Asp Asn Leu Ala Gln Ile Ser Ile Lys Tyr Gln Asn Gln

660 665 670
Gly Lys Lys Asp Leu Leu Gln Ala Ser Ala Glu Asp Asp Val Lys Ala
675 680 685
Ile Lys Asp Leu Leu Asp Gln Thr Asn Asn Leu Leu His Lys Leu Lys
690 695 700
Ile Phe His Ile Ser Gln Ser Glu Asp Lys Ala Asn Ile Leu Asp Lys
705 710 715 720
Asp Glu His Phe Tyr Leu Val Phe Glu Glu Cys Tyr Phe Glu Leu Ala
725 730 735

Asn Ile Val Pro Leu Tyr Asn Lys Ile Arg Asn Tyr Ile Thr Gln Lys
740 745 750
Pro Tyr Ser Asp Glu Lys Phe Lys Leu Asn Phe Glu Asn Ser Thr Leu
755 760 765
Ala Asn Gly Trp Asp Lys Asn Lys Glu Pro Asp Asn Thr Ala Ile Leu
770 775 780
Phe Ile Lys Asp Asp Lys Tyr Tyr Leu Gly Val Met Asn Lys Lys Asn
785 790 795 800
Asn Lys Ile Phe Asp Asp Lys Ala Ile Lys Glu Asn Lys Gly Glu Gly

805 810 815
Tyr Lys Lys Ile Val Tyr Lys Leu Leu Pro Gly Ala Asn Lys Met Leu
820 825 830

Pro Lys Val Phe Phe Ser Ala Lys Ser Ile Lys Phe Tyr Asn Pro Ser
835 840 845

Glu Asp Ile Leu Arg Ile Arg Asn His Ser Thr His Thr Lys Asn Gly
850 855 860

Ser Pro Gln Lys Gly Tyr Glu Lys Phe Glu Phe Asn Ile Glu Asp Cys
865 870 875 880

Arg Lys Phe Ile Asp Phe Tyr Lys Gln Ser Ile Ser Lys His Pro Glu
885 890 895

Trp Lys Asp Phe Gly Phe Arg Phe Ser Asp Thr Gln Arg Tyr Asn Ser
900 905 910

Ile Asp Glu Phe Tyr Arg Glu Val Glu Asn Gln Gly Tyr Lys Leu Thr
915 920 925

Phe Glu Asn Ile Ser Glu Ser Tyr Ile Asp Ser Val Val Asn Gln Gly
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Lys Leu Tyr Leu Phe Gln Ile Tyr Asn Lys Asp Phe Ser Ala Tyr Ser

945 950 955 960

Lys Gly Arg Pro Asn Leu His Thr Leu Tyr Trp Lys Ala Leu Phe Asp
965 970 975

Glu Arg Asn Leu Gln Asp Val Val Tyr Lys Leu Asn Gly Glu Ala Glu
980 985 990

Leu Phe Tyr Arg Lys Gln Ser Ile Pro Lys Lys Ile Thr His Pro Ala
995 1000 1005

Lys Glu Ala Ile Ala Asn Lys Asn Lys Asp Asn Pro Lys Lys Glu Ser
1010 1015 1020

Val Phe Glu Tyr Asp Leu Ile Lys Asp Lys Arg Phe Thr Glu Asp Lys
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Phe Phe Phe His Cys Pro Ile Thr Ile Asn Phe Lys Ser Ser Gly Ala
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Asn Lys Phe Asn Asp Glu Ile Asn Leu Leu Leu Lys Glu Lys Ala Asn
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Asp Val His Ile Leu Ser Ile Asp Arg Gly Glu Arg His Leu Ala Tyr
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Tyr Thr Leu Val Asp Gly Lys Gly Asn Ile Ile Lys Gln Asp Thr Phe

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Ala Ala Ile Glu Lys Asp Arg Asp Ser Ala Arg Lys Asp Trp Lys Lys
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Ile Asn Asn Ile Lys Glu Met Lys Glu Gly Tyr Leu Ser Gln Val Val
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His Glu Ile Ala Lys Leu Val Ile Glu Tyr Asn Ala Ile Val Val Phe
1155 1160 1165

Glu Asp Leu Asn Phe Gly Phe Lys Arg Gly Arg Phe Lys Val Glu Lys
1170 1175 1180

Gln Val Tyr Gln Lys Leu Glu Lys Met Leu Ile Glu Lys Leu Asn Tyr
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Leu Val Phe Lys Asp Asn Glu Phe Asp Lys Thr Gly Gly Val Leu Arg
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Ala Tyr Gln Leu Thr Ala Pro Phe Glu Thr Phe Lys Lys Met Gly Lys
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Gln Thr Gly Ile Ile Tyr Tyr Val Pro Ala Gly Phe Thr Ser Lys Ile

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Cys Pro Val Thr Gly Phe Val Asn Gln Leu Tyr Pro Lys Tyr Glu Ser
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Val Ser Lys Ser Gln Glu Phe Phe Ser Lys Phe Asp Lys Ile Cys Tyr
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Asn Leu Asp Lys Gly Tyr Phe Glu Phe Ser Phe Asp Tyr Lys Asn Phe
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Gly Asp Lys Ala Ala Lys Gly Lys Trp Thr Ile Ala Ser Phe Gly Ser
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Arg Leu Ile Asn Phe Arg Asn Ser Asp Lys Asn His Asn Trp Asp Thr
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Arg Glu Val Tyr Pro Thr Lys Glu Leu Glu Lys Leu Leu Lys Asp Tyr

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Ser Ile Glu Tyr Gly His Gly Glu Cys Ile Lys Ala Ala Ile Cys Gly

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Glu Ser Asp Lys Lys Phe Phe Ala Lys Leu Thr Ser Val Leu Asn Thr

1365 1370 1375

Ile Leu Gln Met Arg Asn Ser Lys Thr Gly Thr Glu Leu Asp Tyr Leu

1380 1385 1390

Ile Ser Pro Val Ala Asp Val Asn Gly Asn Phe Phe Asp Ser Arg Gln

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Ala Pro Lys Asn Met Pro Gln Asp Ala Asp Ala Asn Gly Ala Tyr His

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<211> 4494

<212> DNA

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catcagtttt ttatagagga gatattaagt tcggtttgta ttagcgaaga tttattacaa 720

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Leu Gly Gln Asp Pro Glu Val Arg Tyr Met Pro Asn Gly Gly Ala Val
50 55 60

Ala Asn Ile Thr Leu Ala Thr Ser Glu Ser Trp Arg Asp Lys Ala Thr
65 70 75 80

Gly Glu Met Lys Glu Gln Thr Glu Trp His Arg Val Val Leu Phe Gly

85 90 95

Lys Leu Ala Glu Val Ala Ser Glu Tyr Leu Arg Lys Gly Ser Gln Val
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Tyr Ile Glu Gly Gln Leu Arg Thr Arg Lys Trp Thr Asp Gln Ser Gly

115 120 125

Gln Asp Arg Tyr Thr Thr Glu Val Val Val Asn Val Gly Gly Thr Met

130 135 140

Gln Met Leu Gly Gly Arg Gly Ser Glu Phe Glu Leu Arg Arg Gln Ala

145 150 155 160

Cys Gly Arg Met Ser Ile Tyr Gln Glu Phe Val Asn Lys Tyr Ser Leu

165 170 175

Ser Lys Thr Leu Arg Phe Glu Leu Ile Pro Gln Gly Lys Thr Leu Glu

180 185 190

Asn Ile Lys Ala Arg Gly Leu Ile Leu Asp Asp Glu Lys Arg Ala Lys

195 200 205

Asp Tyr Lys Lys Ala Lys Gln Ile Ile Asp Lys Tyr His Gln Phe Phe

210 215 220

Ile Glu Glu Ile Leu Ser Ser Val Cys Ile Ser Glu Asp Leu Leu Gln

225 230 235 240

Asn Tyr Ser Asp Val Tyr Phe Lys Leu Lys Lys Ser Asp Asp Asp Asn

245 250 255

Leu Gln Lys Asp Phe Lys Ser Ala Lys Asp Thr Ile Lys Lys Gln Ile

260 265 270

Ser Glu Tyr Ile Lys Asp Ser Glu Lys Phe Lys Asn Leu Phe Asn Gln

275 280 285

Asn Leu Ile Asp Ala Lys Lys Gly Gln Glu Ser Asp Leu Ile Leu Trp

290 295 300

Leu Lys Gln Ser Lys Asp Asn Gly Ile Glu Leu Phe Lys Ala Asn Ser

305 310 315 320

Asp Ile Thr Asp Ile Asp Glu Ala Leu Glu Ile Ile Lys Ser Phe Lys

325 330 335

Gly Trp Thr Thr Tyr Phe Lys Gly Phe His Glu Asn Arg Lys Asn Val

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Tyr Ser Ser Asn Asp Ile Pro Thr Ser Ile Ile Tyr Arg Ile Val Asp

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Lys Asp Lys Ala Pro Glu Ala Ile Asn Tyr Glu Gln Ile Lys Lys Asp

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Thr Thr Met Gln Ser Phe Tyr Glu Gln Ile Ala Ala Phe Lys Thr Val

515 520 525

Glu Glu Lys Ser Ile Lys Glu Thr Leu Ser Leu Leu Phe Asp Asp Leu

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Lys Ala Gln Lys Leu Asp Leu Ser Lys Ile Tyr Phe Lys Asn Asp Lys

545 550 555 560

Ser Leu Thr Asp Leu Ser Gln Gln Val Phe Asp Asp Tyr Ser Val Ile

565 570 575

Gly Thr Ala Val Leu Glu Tyr Ile Thr Gln Gln Ile Ala Pro Lys Asn

580 585 590

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595 600 605

Glu Lys Ala Lys Tyr Leu Ser Leu Glu Thr Ile Lys Leu Ala Leu Glu

610 615 620

Glu Phe Asn Lys His Arg Asp Ile Asp Lys Gln Cys Arg Phe Glu Glu

625 630 635 640

Ile Leu Ala Asn Phe Ala Ala Ile Pro Met Ile Phe Asp Glu Ile Ala

645 650 655

Gln Asn Lys Asp Asn Leu Ala Gln Ile Ser Ile Lys Tyr Gln Asn Gln

660 665 670

Gly Lys Lys Asp Leu Leu Gln Ala Ser Ala Glu Asp Asp Val Lys Ala

675 680 685

Ile Lys Asp Leu Leu Asp Gln Thr Asn Asn Leu Leu His Lys Leu Lys

690 695 700

Ile Phe His Ile Ser Gln Ser Glu Asp Lys Ala Asn Ile Leu Asp Lys

705 710 715 720

Asp Glu His Phe Tyr Leu Val Phe Glu Glu Cys Tyr Phe Glu Leu Ala

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740 745 750

Pro Tyr Ser Asp Glu Lys Phe Lys Leu Asn Phe Glu Asn Ser Thr Leu

755 760 765

Ala Asn Gly Trp Asp Lys Asn Lys Glu Pro Asp Asn Thr Ala Ile Leu

770 775 780

Phe Ile Lys Asp Asp Lys Tyr Tyr Leu Gly Val Met Asn Lys Lys Asn

785 790 795 800

Asn Lys Ile Phe Asp Asp Lys Ala Ile Lys Glu Asn Lys Gly Glu Gly

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Tyr Lys Lys Ile Val Tyr Lys Leu Leu Pro Gly Ala Asn Lys Met Leu

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850 855 860

Ser Pro Gln Lys Gly Tyr Glu Lys Phe Glu Phe Asn Ile Glu Asp Cys

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Arg Lys Phe Ile Asp Phe Tyr Lys Gln Ser Ile Ser Lys His Pro Glu
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Phe Glu Asn Ile Ser Glu Ser Tyr Ile Asp Ser Val Val Asn Gln Gly
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Leu Phe Tyr Arg Lys Gln Ser Ile Pro Lys Lys Ile Thr His Pro Ala
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Lys Glu Ala Ile Ala Asn Lys Asn Lys Asp Asn Pro Lys Lys Glu Ser
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Val Phe Glu Tyr Asp Leu Ile Lys Asp Lys Arg Phe Thr Glu Asp Lys
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Ile Asn Asn Ile Lys Glu Met Lys Glu Gly Tyr Leu Ser Gln Val Val
1140 1145 1150
His Glu Ile Ala Lys Leu Val Ile Glu Tyr Asn Ala Ile Val Val Phe
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Gln Val Tyr Gln Lys Leu Glu Lys Met Leu Ile Glu Lys Leu Asn Tyr
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Gly Asp Lys Ala Ala Lys Gly Lys Trp Thr Ile Ala Ser Phe Gly Ser
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1315 1320 1325
Arg Glu Val Tyr Pro Thr Lys Glu Leu Glu Lys Leu Leu Lys Asp Tyr
1330 1335 1340
Ser Ile Glu Tyr Gly His Gly Glu Cys Ile Lys Ala Ala Ile Cys Gly
1345 1350 1355 1360
Glu Ser Asp Lys Lys Phe Phe Ala Lys Leu Thr Ser Val Leu Asn Thr
1365 1370 1375

Ile Leu Gln Met Arg Asn Ser Lys Thr Gly Thr Glu Leu Asp Tyr Leu

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Ile Ser Pro Val Ala Asp Val Asn Gly Asn Phe Phe Asp Ser Arg Gln

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Gly Val Arg Ser Ala Gln Glu Leu Glu Arg Ser Val Lys Gly Met Leu

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Pro Trp Gln Gln Leu Ser Gly Val Glu Lys Ala Val Glu Ile Leu Tyr

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1585 1590 1595 1600

Ala Gln Leu Ile Val Thr Val Asp Asn Gly Ile Ser Ser His Ala Gly

1605 1610 1615

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1795 1800 1805

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1925 1930 1935
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1940 1945 1950
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1955 1960 1965
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Leu Arg Arg Gln Ala Cys Gly Arg Met Ser Ile Tyr Gln Glu Phe Val

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Tyr His Gln Phe Phe Ile Glu Glu Ile Leu Ser Ser Val Cys Ile Ser

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370 375 380

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