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Amplification of DNA

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iGEM Gifu

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Our project aims to quantify human fatigue through the quantification of the biomarker human herpesvirus6 using Cas12a, a protein that is fundamental and essential to our project. We ordered the synthesis of composite parts with affinity tags attached to Cas12a, but the sequence was too long to be synthesized as a single of dsDNA. To overcome this problem, we split the parts into three fragments and synthesized them. Here, an adaptor sequence was added to the 5' end by PCR to allow for later assembly.

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Reagent

KAPA HiFi HotStart ReadyMix (x2) (KAPA Biosystems)

Vector primers (IDT) :

- Forward

5'- CCATACTACGTTTGAGGAGGATATCGTTGCCTGAGCGAAGGTCCGGCAAAAAAGGGCAAG -3'

- Reverse

5'- GACAGAGCTCTACGGACTGTAACGCGTTTGGTACTGCAATCCAGAAATCATCCTTAGCG -3'

Fragment primers:

- Fragment 1 Forward

5'- ATTGCAGTACCAAACGCGTTACAGTCCGTAGAGCTCTGTC -3'

- Fragment 1 Reverse

5'- CTCAGGCAGGATGGTTTCAATAATGTCTTTTTTAAACAGAG -3'

- Fragment 2 Forward

5'- TCTGTTTAAAAAAGACATTATTGAAACCATCCTGCCTGAG -3'

- Fragment 2 Reverse

5'- AGTACATGGTATGTAAGTTCGGCGTGCCATGGCTCTTGTC -3'

- Fragment 3 Forward

5'- GACAAGAGCCATGGCACGCCGAACTTACATACCATGTAC -3'

- Fragment 3 Reverse

5'- CTTCGCTCAGGCAACGATATCCTCCTCAAACGTAGTATGG -3'

Template DNA:

- pSB1C3
- pSB1A3
- Cas12a fragment 1 (Twist Bioscience)
- Cas12a fragment 2 (Twist Bioscience)
- Cas12a fragment 3 (Twist Bioscience)

DNase free water

Equipment

Thermal cycler:

- Gene Amp® PCR system 2700 (Applied Biosystems)
- LifeECO ver3.0 (Bioer Technology)

1 Thaw the DNA solution at room temperature, and KAPA HiFi HotStart ReadyMix (x2) on ice.

2 Vortex the reagents, then centrifuge them briefly in a microcentrifuge.

3 Mix the reagents according to the composition in the table below.

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A	B	C
COMPONENT	VOLUME (µl)	CONCENTRATION
KAPA HiFi HotStart Ready Mix(x2)	25	×2
DNA Template	1.0	1.0 µg/µl
Forward Primer	2.5	0.5 µM
Reverse Primer	2.5	0.5 µM
Nuclease Free Water	19.0	
Total Volume	50.0	

4 For each template DNA, select the temperature setting of the thermal cycler listed in the table below.

4.1 Vectors(pSB1C3 and pSB1A3)

A	B	C	D
STEP	TEMP (°C)	TIME	CYCLES
Initial Denature	95	5 minutes	
Denature	98	30 seconds	30
Anealing	68	15 seconds	
Extension	72	1 minutes 30 seconds	
Final Extension	72	5 minutes	
Hold	4		

4.2 Cas12a fragments(1-3)

A	B	C	D
STEP	TEMP(°C)	TIME	CYCLES
Initial Denature	95	5 minutes	
Denature	98	30 seconds	30
Anealing	65	15 seconds	
Extension	72	1 minute	
Final Extension	72	5 minutes	
Hold	4		

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