NOTEBOOK

May 2023

MAY

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PCR (cl2,28a, ADH fragment)

Tuesday,May 4,2023

Overview

- The cl 2,28a, and ADH fragments were obtained by pcr

Experimental equipment

- primer star enzyme,
- DPN1 enzyme
- rcutsmart buffer

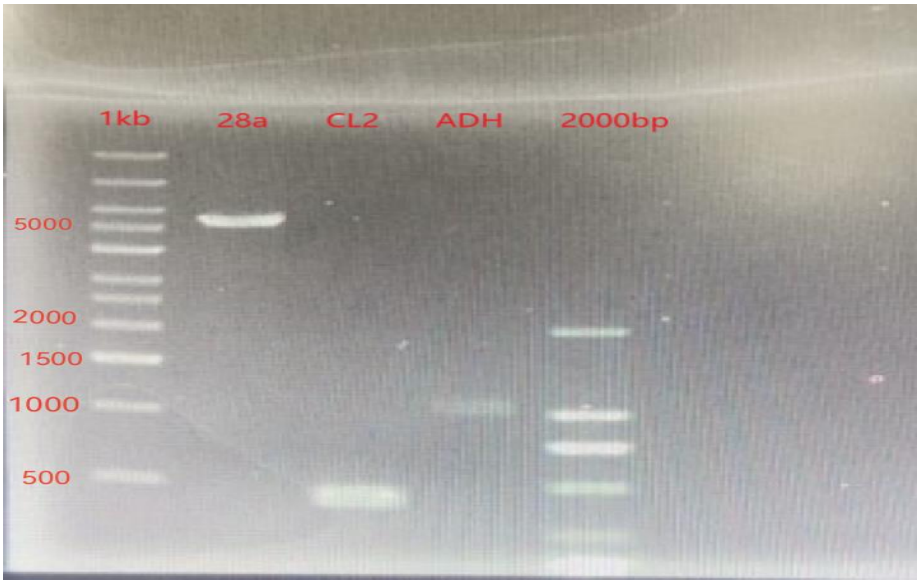
Experimental steps

- 3 ulH_20, 1ul of template, 0.5ul of forward primer (R), 0.5ul reactant (F), 5ul primer star were added in turn to the PCR tubes
- After addition, the corresponding program is set in the PCR instrument for PCR
- After PCR, the nucleic acid glue to verify whether the target product is obtained
- After the verification is completed, the template can be eliminated.

DPN 1 dilution system: 1ul DPN1,1ul rcutsmart buffer, 8 ulH_2O

Temtemplate system: 2ul DPN1 (dilution), 1ul rcutsmart buffer, 2ul H_20 and 5ul PCR products

Experimental results



T5 transformation of 28a-CL2-ADH

Thursday,May 6,2023

Overview

- The pET28a vector, CL2 fragment, and T5 fragments were linked by the T5 enzyme, and the ligated fragments were transferred into DH 5 a competent cells

Experimental equipment

- T5 enzyme
- 4×PBS buffer

Experimental steps

① T5 enzyme dilution system

- 0.5 ul of T 5 enzyme + 4.5ul of sterile water + 5 ul of 4-buffer

The ② T5 enzyme conversion system

- 1ul dilution of the T5 enzyme + xul vector + (4-x) ul fragment

③ T5 conversion operation steps

- 1. Add the carrier and fragment first, and then add the diluted T5 enzyme and wait for about 5min (put it on ice immediately after the addition, and the T5 enzyme is faster at room temperature, 3 nt / min on ice)
- After 2. 5min, 30ul of competent cells were added
- 3. After the addition of competent cells, the ice was immediately bathed for 30min
- 4. 42℃ heat shock for 45s
- 5. Immediately take an ice bath for 2min
- 6. 150ul NZY of medium was added to the ultra-clean workbench
- The cells were incubated in 7. 37℃ thermotemperature shaker for 40min – 60min
- 8. Apply the plate and culture it well in a 37℃ incubator overnight

Breaking bacteria, purification, activity determination (A2, T7,M8)

Fri day,May 14,2023

Overview

- Because of the suspicion of early measurement activity and purification interval time is too long, protein degradation. Therefore, the operation from breaking to measuring activity was completed in a short time.

Experimental equipment

① broken bacteria

- 1×PBS buffer、PMFS

② protein was purified

- Nickel beads, G250 Coomassie brilliant blue, and imidazole at each concentration

③ determination of activity

- The protein, the substrate, 1mM NAD +, and 1 PBS buffer were purified

Experimental steps

① broken bacteria

- 1. Add 1% PMFS to the bacterial solution, and then wash the high pressure cell cruter twice with RO water and 1 PBS buffer (pressure is controlled at 300bar)
- 2. After washing the high pressure cell crushing ter, add target liquid for crushing (pressure control at 800bar~900bar)
- 3. According to the transparency of the broken bacteria liquid, break the broken bacteria for 3 to 6 times.
- 4. After bacteria crushing, pack with 2ml EP tube, and leave one tube without centrifugation (sample preparation). After packaging, at 14000rpm, 10°C

  The conditions were centrifuged for 30min.
- 5. After centrifugation, keep one tube of precipitation and one tube of supernatant (sample preparation). The remainder of the supernatant can be collected.

② protein was purified

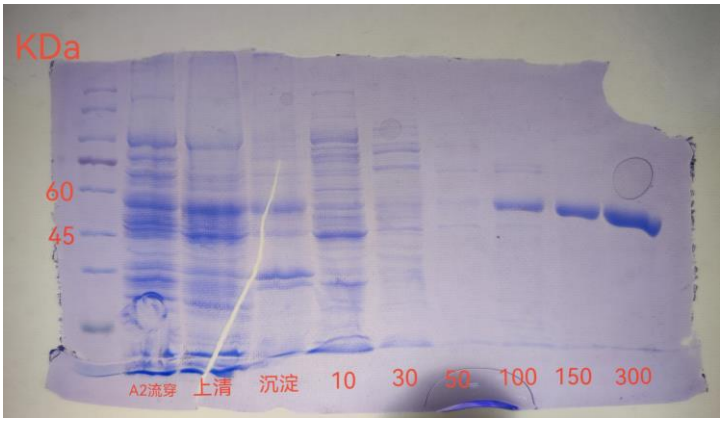
- 6 Appropriate amount of nickel beads were first added to the purified column and then washed twice with RO water and 1 PBS buffer.
- 7. After washing, the nickel beads were activated by adding 5min of 10 mM imidazole to the purified beads supplemented with nickel beads.
- 8. After activating the nickel beads, immediately add the collected supernatant night and place the sealing tube in the silent mixer for 60min (so that the target protein is fully combined with the nickel beads).
- 9. After mixing, take the penetrating fluid and place it on ice.
- 10 with 10 mM imidazole, 30 mM imidazole, 50 mM, 100 mM imidazole, 150 mM imidazole, 300 mM, and 600 mM imidazole. Each concentration of imidazole should be washed until the eluate meets G250 Coomassie blue.
- 11. Make the precipitation, supernatant, flow through, whole, and each concentration of imidazole eluates.40ul loading and 10ul samples were mixed and boiled at 100℃ for 10min.
- 12 After making the samples, the target proteins were tested by SDS-PAGE. During loading, take 10ul of each sample and 2ul of protein marker.
- 13 ultrafiltration based to SDS-PAGE results.

③ determination of activity

- 14. During the ultrafiltration process, use 4000rpm, 10~30min (according to the actual situation)
- 15. After ultrafiltration, the activity of the target protein was determined
- 16. When measuring, add the corresponding reagent according to the activity measurement system (the enzyme should be added last)

Experimental results

A2


KDa
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A2流穿
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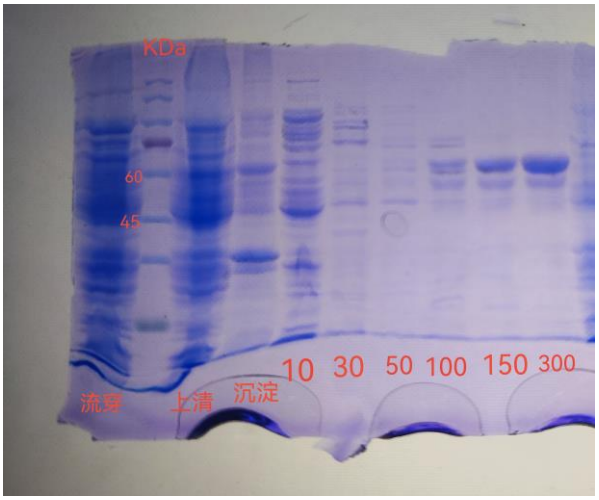
300

T7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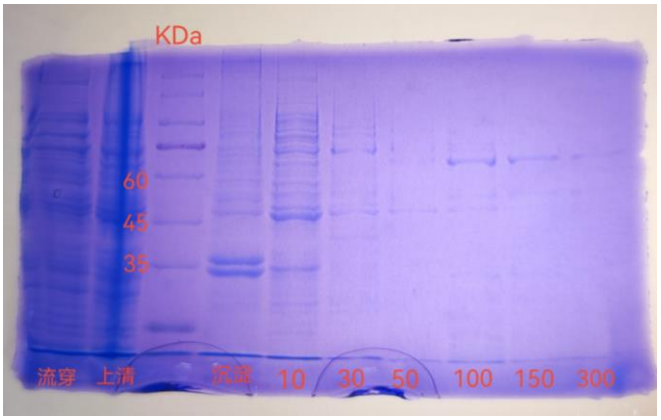
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