Extration of Cas12a protein

Culture:

First, we transformed the obtained MBPhis-AsCas12a plasmid into BL21 (DE3) strains, selected monoclonal colonies, and inoculated them into Amp+LB broth. After cultivating about eight hours later, we transfered 1% inoculum size of bacteria into 350 ml new AMP+LB broth in 1L shake flask, and shaked (220rpm) it for 5-6 h at 37 °C. Then, we added 1 mM iptg, shifted bacteria in the logarithmic growth phase to 16°C shaker and shaked for 16 hours, in order to get the target protein.

Collecting and Crushing:

After culture, the bacteria in the shake flask were collected at 3000rpm, 4 °C contrifugation for 20min, and is resuspend with 40ml PBS. Subsequently, we completely crushed 斜 E. coli 斜 using a high pressure homogenizerthe to separate the protein from the bacteria.

Separation and combination:

To isolate the proteins, the broken solution was centrifuged at 10000g at 4 °C for 50min, precipitating the broken bacteria and other impurities. After discard the supernatant, we added 3ml column volume of his tag binding beads, shake them in vertical shaker at 4°C overnight. Cas12a was combined with a nickel column using his tag.

Elution:

The binding solution was poured through the column, FT was collected, and eluted with 20mM, 40mM imidazole eluent W1 and W2 in 20CV and 10CV volumes, respectively, to wash away non-specific binding proteins and impurities. Then we eluted the target protein with 3CV eluent E1 and E2 with 200mM and 400mM imidazole.

Gel results:

We can see from the results of SDS protein gel that the size of our extracted protein is about 110kD, which is consistent with our target protein. Western blot was further used to verify that our extracted protein was Cas12a.

Chinese Version

Cas12a 蛋白的提取和纯化

培养:

首先将获得的 MBPhis-AsCas12a 质粒转化至 BL21（DE3）菌株中，挑单克隆菌落至 Amp 抗性的 LB 培养基，培养约 8 小时后，以 1%的接种量接至 1L 摇菌瓶中，于 350ml 氨苄抗性 LB 中 37 度 220rpm 培养 5-6 小时，加入 1mMIPTG，将处于对数生长期的大肠杆菌转移至 16 度摇床内培养 16 小时，以便获得目标蛋白。

收集破碎:

培养结束后，将摇菌瓶内菌 3000rpm 与 4 度离心 20min 收集，用 40mlPBS 收集，并用高压匀质仪将大肠杆菌完全破碎，以分离菌体与蛋白。

分离结合:

为分离蛋白质，我们将破碎后溶液于 4 度 10000g 离心 50min，沉淀破碎的菌体以及其他杂质。将上清倾到出，加入 3ml 柱体积的 his tag 结合珠，4 度于垂直摇床结合过夜。将 Cas12a 利用 his tag 与镍柱结合。

洗脱:

将结合液倒入流穿柱，收集 FT，分别用含有 20mM，40mM 咪唑的洗脱液 W1，W2 分别以

20CV 和 10CV 体积流过珠子，洗去非特异性结合蛋白与杂质。再分别用 200mM，400mM 咪唑的洗脱液 E1，E2 用 3CV 洗脱目标蛋白。

跑胶结果：

通过 SDS 蛋白胶结果我们可以看出，我们的目的蛋白大小约为 110kD，与跑胶结果相符。并进一步用 western blot 方法验证，证明所提取出蛋白即为 Cas12a。