MIRZO ULUG‘BEK NOMIDAGI
O‘ZBEKISTON MILLIY UNIVERSITETI
JIZZAX FILIALI



"BIOTEXNOLOGIYANING RIVOJLANISH ISTIQBOLLARI VA MUAMMOLARI"

MAVZUSIDAGI RESPUBLIKA MIQYOSIDAGI ILMIY-TEXNIK ANJUMAN TO‘PLAMI





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МИНИСТЕРСТВО ВЫСШЕГО ОБРАЗОВАНИЯ, НАУКИ И ИННОВАЦИЙ РЕСПУБЛИКИ УЗБЕКИСТАН

ДЖИЗАКСКИЙ ФИЛИАЛ НАЦИОНАЛЬНОГО УНИВЕРСИТЕТА УЗБЕКИСТАНА ИМЕНИ МИРЗО УЛУГБЕКА

СБОРНИК ТЕЗИСОВ РЕСПУБЛИКАНСКОЙ НАУЧНОЙ КОНФЕРЕНЦИИ

«ПЕРСПЕКТИВЫ И ПРОБЛЕМЫ РАЗВИТИЯ БИОТЕХНОЛОГИЙ»

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POLIMERAZA ZANJIR REAKSIYASI UCHUN REKOMBINANT VENT POLIMERAZA FERMENTINING ANALOGINI OLISH

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Molekulyar biologiya fanida polimerazalar asosiy fermentlar sifatida tan olingan bo'lib, ular DNK va RNK sintezini amalga oshirishda muhim o'rin egallaydi. DNK polimerazalari hujayralardagi matritsa DNK replikatsiyasi, ta'miri va rekombinatsiya jarayonlarini bevosita ishtirok etadi [1]. Ushbu fermentlarning biologik jarayonlardagi vazifalari qanchalik muhim bo'lsa, ularning laboratoriya sharoitida DNK polimerazalarining yuqori haroratga chidamli turlari polimeraza zanjiri reaksiyasi (PZR) kabi texnologiyalarni amalga oshirishda katta ahamiyatga ega. Polimeraza zanjirli reaksiyasi DNK molekulalarini ko'paytirish uchun keng qo'llaniladigan texnologiya bo'lib, u 1985-yilda Keri Mullis tomonidan ixtiro qilingan [2]. PZR yordamida atigi bir necha nusxada bo'lgan DNK fragmentlari millionlab nusxaga ko'paytirish mumkin. Ushbu texnologiya inson genomini to'liq o'rganishga qaratilgan yirik ilmiy tashabbus-Inson Genomi Loyihasi (Human Genome Project) doirasida muhim vosita sifatida qo'llanilgan. Ushbu usul genlarni klonlash, sekvenslash, klinik diagnostikada, sud-tibbiy ekspertizasida, va turli boshqa molekulyar biologiya sohalarida katta muvaffaqiyat bilan qo'llanilmoqda.

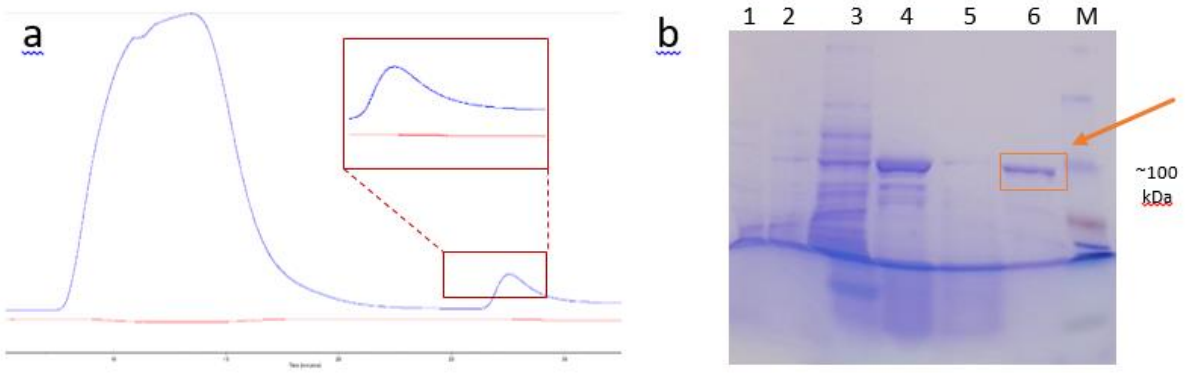
DNK polimeraza bu - tirik organizmlarda DNKning komplementar zanjirini sintez qiluvchi ferment. DNK polimerazalar *in vitro* sharoitda DNK klonlash, sekvensing va shu kabi bir qancha jarayonlarda keng qo`llaniladi [3]. Turli DNK polimerazalarning birlamchi vazifasi yangi DNK zanjirini sintez qilish bo`lsada, ular bir-biridan aniqlik, spetsifiklik, proofreading qobiliyati, termostabillik va tezlik jihatdan farq qiladi.

Eng keng tarqalgan polimeraza – Taq, termofil eubakteriya *Thermus aquanticus*dan olingan bo`lib, PZRda ko`p ishlatiladi. Minutiga 1000 ta nukleotidni sintez qiluvchi bu polimeraza Pfu (*Pyrococcus furiosus*dan topilgan) dan ancha tez bo`lib, proofreading qobiliyati yo`qligi bilan farq qiladi. Pfu esa Taqdan 52 marta aniq ishlaydi va narxi ham 10 barobar qimmat. 3`→5` proofreading qobiliyati va narxining arzonligi jihatidan Vent polimeraza Taq va Pfu polimerazadan ustun turadi. Vent polimeraza *Thermococcus litoralis* gipertermofil arxeyadan ajratilgan bo`lib yuqori termostabillikka ega [4]. Enzimdagi ekzonukleaza faolligi DNK zanjiri sintezidagi xatoliklarni tuzatish, PZR reaksiyasining aniqroq hamda mahsulotning sifatliroq bo`lishini ta`minlaydi.

Material va metodlar: Oqsil ekpressiyasi uchun *E.coli* LEMO shtammi va BBF10K ekpression plazmidasidan foydalanildi. Dastlab, *E.coli* LEMO shtammiga ekpressiya qilingan va oldingi tadqiqotlarda nisbatan samarali ekanligi aniqlangan Vent polimeraza stoklari xona temperaturasida eriguncha saqlandi va 3 mL LB ozuqa muhitida (+ ampitsilin, 50 μg/mL) 37°C haroratda 250 rpm tebranishda bir sutka davomida o'stirildi. So'ngra, ushbu kulturadan 1 mL olinib, 100 mL LB (+ ampitsilin) ozuqa muhitiga ko'chirildi va OD600 ko'rsatkichi taxminan 0,6 ga yetguncha 37°C haroratda 250 rpm tebranishda o'stirildi. Ozuqa muhitiga IPTG qo'shildi va 37°C haroratda, 4 soat davomida termoshakerda (250 rpm) induksiya qilindi. Induksiya tugagach 7068 x g tezlikda, 20 daqiqa davomida 4°C sentrifuga qilindi. Supernatant qismi olib tashlanib, Vent polimeraza cho`kmasi 5 mL A bufer (50 mM Tris HCl, 0.5 mM EDTA) bilan eritildi va 30 daqiqa xona haroratida inkubatsiya qilindi. Keyin eritmaga B bufer (50 mM Tris HCl, 0.5 mM EDTA, 500 mM KCl) dan 5 mL solindi va Vent polimerazani 75°C da suv hammomida 1 soat davomida inkubatsiya qilindi. So'ng oqsil 15 daqiqa davomida 7068 x g da sentrifuga qilindi. Supernantant qismi keyingi bosqich uchun olib qo`yildi. Dastlab affin xromatografiya uskunasining tegishli kanallari ishchi bufer (A bufer) yordamida yuvilib, uskuna ishchi holatga keltirildi. Vent polimeraza oqsilini tozalab olish uchun cOmplete™ His-Tag Purification Column 1 mL affin almashinuv sorbentli kolonkadan foydalanildi. NaCl konsentratsiyasi taxminan 300 mM ga yetkazildi. Kolonka xromotografiya uskunasiga ulangach, A buffer bilan 10 CV/yoki bazal liniyaga tushguncha yuvildi. Keyin 5 hajm B bufer bilan oqsil elutsiya qilindi. Xromatografiyaning barcha bosqichida oqim 1 mL/min tezlikda olib borildi. Xromotografiya bosqichidan chiqqan namunalar 1:3 nisbatda 4xLaemmli buferi bilan aralashtirilib, 95°C haroratda 5 minut davomida denaturatsiyalandi. Denaturatsiyalangan namunalar, 7,5% SDS-PAGE gelida Tris-Glycin-SDS buferi yordamida 120V doimiy kuchlanish rejimida yurgizildi. Elektroforez tugagach, gel 0,05% Coomassie Brilliant Ko'k R-250 bo'yog'i bilan bo`yaldi. Ikkinchi gel Western blot analiz uchun ishlatildi. Vent polimeraza C buferiga o'tkazildi. C buferining tarkibida 50% glitserin va Tris-HCl mavjud bo'lib, bu fermentning barqarorligini oshirish va uzoq muddat saqlash sharoitlarini optimallashtirish uchun tanlangan. Bufer almashtirish jarayoni ultrafiltratsiya usuli bilan Amicon Ultracel 30kDa filtrli sentrifugasida amalga oshirildi. So`ng PZR jarayoni amalga oshirildi va natijani aniqlash uchun 2% agaroza gelidan foydalanildi.

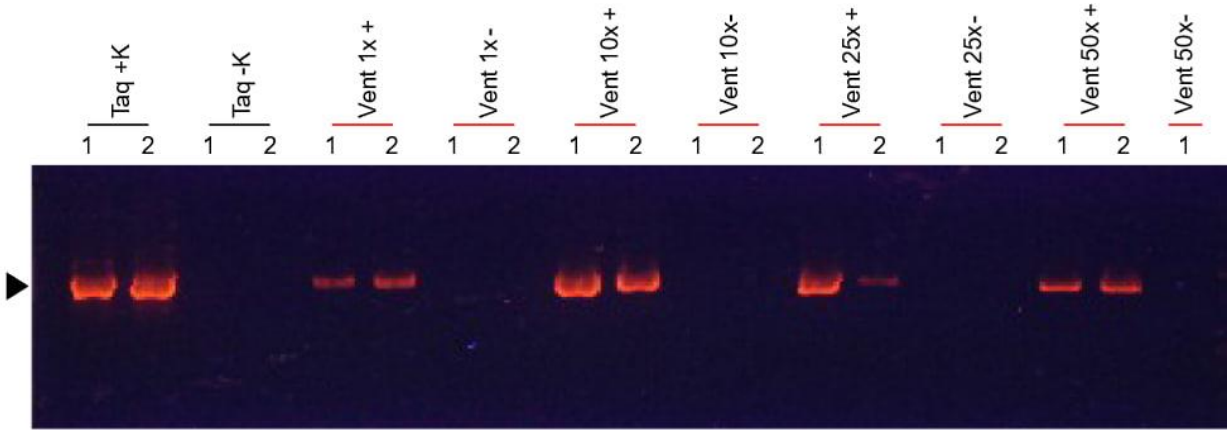
Olingan natijalar: Xromatografiyadan so'ng oqsil konsentratsiyasi 1.163 mg/mL, tozalangach esa 0.318 mg/mL ko'rsatkichi qayd etildi. Oqsil forezi natijalariga va markerlarni hisobga olgan holda, Vent polimeraza toza holda

ajratildi (rasm 1a, 1b) va bu polimerazani PZR jarayoni uchun bemalol ishlatilsa bo`ladi.



1-rasm. a) Xromatogafiya natijasi. Chapdagi pik bog'lanmagan oqsillar, o'ngdagi pik maqsadli vent polimerazaga tegishli; b) Namunalar: 1. Induksiyadan oldingi namuna; 2. Eskpressiya tugagach namuna; 3. Lizisdan so'nggi namuna; 4. Xromatografiya uchun tayyorlangan namuna; 5. Flowtrough; 6. Elutsiya namunasi.

PZR reaksiyasida Brazzein DNKsidan foydalib 400 - 500 bp mahsulot amplifikatsiya qilindi. Vent polimeraza turli konsentratsiyalaridan olingan mahsulot sifati kontrol Taq polimerazalaridan olingan mahsulotga yaqin (rasm 2).



2-rasm. Gel elektroforez natijasi. Mahsulot kattaligi: 400-500 bp

Xulosa: Olingan naijalarga asoslanib, rekombinant Vent polimerazasi muvaqqiyatli ekpressiya qilindi va tozalandi, PZR reaksiyasida muvaffaqiyatli qo'llanildi. 50x suyultirilgan namunasi tavsiya qilindi.

Foydalanilgan adabiyotlar ro`yxati:

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POSSIBILITIES OF INCREASING SALT TOLERANCE IN BARLEY USING CRISPR-CAS9 TECHNOLOGY

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Abstract: This analytical thesis examines the potential of using CRISPR/Cas9 technology to enhance salt tolerance in barley (Hordeum vulgare L.). Ensuring global food security and increasing crop productivity are among the most pressing challenges worldwide. Soil salinization is a major limiting factor in agriculture, significantly reducing crop yields, especially for essential cereal crops such as barley. This thesis analyzes the genes responsible for salt stress response, their molecular mechanisms, and the possibilities of improving their function through gene editing using CRISPR/Cas9 technology. Research indicates that the CRISPR/Cas9 system can enhance stress tolerance in barley by modifying genes related to salt tolerance, such as ABi5, NHX1, HKT1;5, and SOS1 [8, 9]. Additionally, the advantages, limitations, and prospects of this technology in agriculture are discussed. This study contributes to understanding how CRISPR/Cas9 technology can be effectively applied in future research and breeding programs.

Keywords: Barley (Hordeum vulgare L.), salt stress, CRISPR/Cas9, gene editing, ABi5, NHX1, HKT1;5, SOS1, molecular breeding, genetic engineering, agricultural biotechnology.

Introduction: The agricultural sector faces significant challenges in ensuring global food security. According to the Food and Agriculture Organization (FAO) of the United Nations, global food production must increase by at least 70% by 2050 to meet the growing needs of humanity [6]. Factors such as population growth, climate change, and decreasing natural resources have made increasing