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SELECTION OF THE OPTIMAL METHOD FOR ISOLATION OF GEOMIC DNA OF YEAST AND MOLD FUNGI

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In this study, we aimed to isolate genomic DNA (gDNA) from a yeast cell using various methods and choose the cheapest, most convenient, and fastest method among them. For this purpose, based on a thorough review of the literature, three relatively optimal methods were selected based on the use of guanidine thiocyanate, lyticase enzyme, and lithium acetate/SDS reagents. In the research work, the disadvantages and advantages of these methods were studied and compared. The obtained results showed that the isolation method using the lyticase enzyme is not optimal, given the relatively long time and high cost of the enzyme used, the isolation method using lithium acetate/SDS reagents is much simpler, faster and cheaper, and the isolation method using the guanidine thiocyanate is recognized impractical due to poor isolation of genomic DNA. In addition, we also investigated the process of isolating genomic DNA from Candida albicans and molds other than Pichia pastoris. For this, only the lithium acetate/SDS method was used, and this method was shown to be able to isolate high quality genomic DNA from all types of yeasts and molds.

Keywords: guanidine thiosulfate, polymerase chain reaction, *Pichia pastoris*, lyticase enzyme, genomic DNA, lithium acetate, Sodium Dodecyl Sulfate, *Candida albicans*

ПОДБОР ОПТИМАЛЬНОГО СПОСОБА ВЫДЕЛЕНИЯ ГЕНОМНОЙ ДНК ДРОЖЖЕВЫХ И ПЛЕСНЕВЫХ ГРИБОВ

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Целью данного исследования является выделение геномной ДНК (гДНК) из дрожжевой клетки с помощью различных методов и подбор самого удобного в исполнении, быстрого и недорогого метода. Для этой цели провели тщательный обзор литературных данных и выбрали три относительно оптимальных метода, основанных на использовании реагентов гуанидин тиоцианата, фермента lyticase и ацетата лития/SDS. В исследовательской работе были изучены и сопоставлены преимущества и недостатки этих методов. Полученные результаты показали, что метод выделения с использованием фермента lyticase не является оптимальным, в связи с длительным временем выполнения и высокой стоимостью используемого фермента, метод выделения с использованием реагентов ацетат лития/ SDS значительно проще, быстрее и дешевле, а метод разделения с помощью тиоцианатной соли гуанидина признан неэффективным из-за низкого выхода геномной ДНК. Кроме того, мы также исследовали процесс выделения геномной ДНК из Candida albicans и других типов плесневых грибов, кроме Pichia pastoris. Для этого использовался только метод ацетата лития/SDS, и было показано, что этот метод позволяет выделить высококачественную геномную ДНК из всех типов дрожжей и плесени. .

Ключевые слова: гуанидинтиосульфат, полимеразная цепная реакция, *Pichia pastoris*, фермент lyticase, геномная ДНК, ацетат лития, Sodium Dodecyl Sulfate, *Candida albicans*

ACHITQI VA MOG'OR ZAMBRUG'LARIDAN GENOM DNK SINI AJRATISHNING OPTIMAL USULINI TANLASH

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Biz mazkur tadqiqot ishimizda achitqi hujayrasidan uning genom DNK (gDNK) sini turli usullar yordamida ajratib, ulardan eng arzon, qulay va tez usulini tanlab olishni maqsad qildik. Shu maqsadda, adabiyot ma'lumotlarini to'liq o'rganish asosida biz guanidin tiosiyanat, lyticase fermenti va litiy asetat/SDS reagentlari ishlatishga asoslangan nisbatan optimal bo'lgan 3 xil usulni tanlab oldik. Tadqiqot ishida ushbu usullarning kamchilik va ustun tomonlari o'rganilib solishtirildi. Olingan natijalar shuni ko'rsatdiki, lyticase fermenti tasirida ajratish usuli nisbatan uzoq vaqt olishi va ishlatilgan fermentning narxi qimmatligi inobatga olinib maqbul usul emasligi, litiy asetat/SDS reagentlari yordamida ajratish usuli ancha sodda, tez va arzonligi, guanidin tiosionat tuzi yordmida ajratish usuli esa, gDNK yomon ajraganligi sababli maqsadga muvofiq emasligi aniqlandi. Bundan tashqari biz, Pichia pastorisdan boshqa Candida albicans va begona tipdagi mog'or zambrurlarinidan genom DNK ni ajratish jarayonlarini ham tadqiq qildik. Bunda jarayon faqat litiy atsetat/SDS usulida amalga oshirildi va mazkur usul bilan barcha achitqi va mog'or zambrug'laridan sifatli genom DNK sini ajratish mumkinligi isbotlandi.

Kalit so'zlar: Guanidin tiosulfat, Polimeraza zanjir reaksiyasi (PZR), *Pichia pastoris*, Litikaza fermenti, gDNK (genomDNK), litiy asetat, SDS(Sodium Dodecyl Sulfate), *Candida albicans*

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Kirish

Metilotrofik *Pichia pastoris* achitqisi geterologik oqsillarni ishlab chiqarish uchun keng qo'llaniladigan mikroorganizmlardan biri hisoblanadi [1-5]. *Pichia pastoris* ekspresyon tizimi boshqa tizimlardan arzon ozuqaviy muhitda ko'paytirilganda sezilarli biomassaning to'planishi, endotoksinlar va pirogenlarning yo'qligi, yuqori darajadagi rekombinant oqsillar sintezlanishi kabi afzalliklarga ega [6-10]. Achitqi hujayralaridan

genom DNK ni (gDNK) ajratishning an'anaviy usullari fermentativ degradatsiya [11] yoki shisha sharchalar bilan doimiy chayqatish [12], so'ngra odatda detergent bilan hujayralarni lizislash va gDNKni fenol-xloroform bilan ekstraksiya qilishdan iborat. Bundan tashqari xovonchada maydalash, mexanik gomogenlash, fermentativ ishlov berish va fransuz press texnologiyasi va boshqa maxsus jihozlardan foydalanib achitqi hujayra devorini buziladi [13-22]. DNK molekulasini ajratish uchun isitish / sovutish tezligini ta'minlaydigan va natijada DNKning amplifikatsiyasi uchun mos keladigan samarali qurilma ishlab chiqilgan [23]. Ke va boshqalar tomonidan bir bosqichda amalga oshiriladigan hujayra lizisi va DNKni amplifikatsiyasini o'z ichiga olgan yangi qurilma taklif qilingan [25].

Achitqi va mog'ordan umumiy gDNKni tayyorlash uchun oddiy va tezkor protokol Borman va boshqalar tomonidan taklif qilingan [18]. Filtr qog'ozi (Whatman FTA) DNK immobilizatori matritsasi sifatida ishlatilgan. Matritsa to'liq quvvatda mikroto'lqinli pechga qo'yildi, keyin Whatman FTA yuvish reagenti bilan yuvildi, so'ngra TE buferi bilan ikki marta yuvildi. FTA filtr qog'ozlari, aslida, kimyoviy moddalar bilan singdirilgan tolali matritsalar, masalan, xelatatorlar va mikroorganizmlarni lizis va faolsizlantiradigan denaturatsiya qiluvchi birikmalardir [17, 18]. Shunday qilib, uni butunlay kimyoviy moddalarsiz ekstraksiya protokoli deb hisoblash mumkin emas. Zambrug' DNKsini tayyorlash uchun issiqlik bilan ishlov berish orqali shisha sharchalrdan foydalanadi va namuna 20 daqiqa davomida 95 °C gacha isitiladi. Borman va boshqalar tomonidan amalga oshirilgan muzdan tushirish jarayoni (2006) namunalarni suyuq azotda muzdan tushirishning olti sikli amalga oshirilgan. Liguori va boshqalar tomonidan ishlab chiqilgan issiqlik protsedurasida fosfat-buferli tuz eritmasidan foydalanilgan, so'ngra steril distillangan suvda qayta eritilgan va 3 daqiqa davomida 95 °C ga qizdirilgan va DNKni cho'ktirish uchun sentrifugalangan [26]. De Baere va boshqalar tomonidan qo'llaniladigan DNK-ekstraksiya uchun qaynatish va muzlatish protokolida, hujayralar distillangan suvda eritiladi va 95 °C da 15 daqiqa davomida qaynatiladi va -70 °C da muzlatish uchun qo'yiladi. Namunalar xona haroratida eritiladi va hujayra qoldiqlarini olib tashlash uchun sentrifuga qilinadi [27]. Freschi va boshqalar tomonidan tasvirlangan qaynash jarayonida namunalar sentrifugadan o'tkaziladi, keyin steril distillangan suvda qayta eritiladi, 10 daqiqa davomida 95 ºC ga qizdiriladi, muzda sovutiladi va 13000 x g da 3 daqiqa davomida sentrifuga qilindi. Shu tariqa supernatant PZR (PCR) tahlili uchun ishlatilgan [28]. Deak va boshqalar tomonidan tasvirlangan oddiy qaynatish usullari hujayralarni yig'ish, qaynatish jarayonidan keyin hujayra qoldiqlarini yo'q qilish va jami cho'kilgan DNKni granulalash uchun sentrifugadan foydalaniladi [29]. Ushbu so'nggi olti protsedura Borman va boshqalar tomonidan tasvirlanganidan ancha sodda va tezroq edi [18,19]. Ammo bu protokollar muhim qadam sifatida sentrifugalashni talab qildi va ulardan ba'zilari namunalarni qayta ishlash uchun alohida e'tibor talab qildi.

Material va metodlar

Pichia pastoris genom DNK sini Litikaza fermenti yordamida ajratish.

P. pastoris hujayralari yangi tayyorlangan YPD (1% Yeast extract, 2% Peptone, 2% Dextrose) ozuqa muhitida 10 ml ozuqa muhitda 30 °C da optik zichligi OD_{600} 5-10 gacha o'stiriladi. Xona haroratida 5-10 daqiqa davomida 1500 x g da sentrifugalash orqali hujayralar cho'ktiriladi. Sentrifugalash orqali hujayralarni 10 ml steril suv bilan yuviladi va cho'kkan hujayralarni 2 ml SCED (1 M sorbitol, 10 mM sodium citrate, pH = 7,5, 10 mM EDTA, 10 mM DTT) buferida qayta eritiladi. 0,1-0,3 mg miqdorda zimoliyaza (yoki lyticase) fermenti qo'shilib yaxshilab aralashtiriladi. Hujayra sferoplastiga erishish uchun 37 °C da 50 daqiqa davomida inkubatsiya qilinadi. 2 ml 1% SDS qo'shilib, muloyimlik bilan aralashtiriladi va muzga (0 dan 4 °C gacha) 5 daqiqaga qo'yiladi. Ustiga 1,5 ml 5 M kaliy asetat, pH 8,9 qo'shilib muloyimlik bilan aralashtiriladi. Namunalar 4 °C da 5-10 daqiqa davomida 10 000 x g da sentrifuga qilinadi va supernatant saqlanadi. Supernatant boshqa steril probirkaga olinib 2 hajm 96% etanol qo'shiladi va xona haroratida 15 daqiqa davomida inkubatsiya qilinadi. So'ng 4 °C da 20 minut davomida 10 000 x g da sentrifuga qilinadi. Cho'kmani 0,7 ml TE buferida, pH 7,4 ga muloyimlik bilan qayta eritiladi va teng hajmdagi fenol:xloroform (1:1 v/v) bilan bilan ekstraksiya qilinib, keyin teng hajmdagi xloroform:izoamil spirti (24:1) bilan yuviladi. Suvli qatlam boshqa probirkalarga olinadi. Har bir probirkaga 1/2 hajm 7,5 M ammoniy asetat, pH 7,5 va 2 hajm etanol

qo'shiladi. Quruq muzga 10 daqiqa yoki -20 °C da 60 daqiqaga saqlanadi. So'ngra 4 °C da 20 daqiqa davomida 10 000 x g sentrifuga qilinadi va cho'kma bir marta 1 ml 70% etanol bilan yuviladi. Namunalar havoda qisqa vaqt quritiladi va har birini 50 mkl TE buferda, pH 7,5 eritiladi. DNK namunasining konsentratsiyasi aniqlanadi. Namunalarni -20 °C da alohida yoki foydalanishga tayyor bo'lgunga qadar birlashtirilgan holda saqlash mumkin.

***Pichia pastoris* genom DNK sini LiAc va SDS usulida ajratish.**

Pichia pastoris achitqi shtammi tanlab olinib, jarayonlar quyidagicha amalga oshirildi.

P. pastoris hujayralari yangi tayyorlangan YPD (1% Yeast extract, 2% Peptone, 2% Dextrose) ozuqa muhitida ikki kun davomida Petri likopchalarida o'stirilib, ulardan taxminan 8-10 ta yakka holdagi koloniyalar ajratib olindi. Koloniyalar 100 mkl 0,2 M litiy atsetat va 1% SDS eritmalarida eritildi. Namunalar termoblokda 65 °C haroratda 10 min davomida ushlandi. Probirkalar termoblokdan olinib ustiga 300 mkl 96% etanol, 1/10 hajm 5 M atsetat ammoniy solinadi va yaxshilab aralashtiriladi. DNK suspenziyasi 14 000 ay/min tezlikda 2 min davomida sentrifuga qilinadi. Hosil bo'lgan cho'kma qoldirilib, suyuq qismi (supernatant) pipetka yordamida olib tashlandi va xona haroratida 30 min davomida og'zi ochiq holda quritildi. Cho'kkan DNK ning ustiga 100 mkl TE pH = 8,0 bufferidan solinib, yaxshilab aralashtirildi. Namuna 14 000 ay/min da 1 min sentrifuga qilinib, suyuq qismi boshqa steril probirkaga ajratib olindi. Olingan namunadagi DNK miqdori va sifati spektrofotometrik usulda (260/280 nm) tahlil qilinib, DNK miqdori o'rtacha 1-2 mkg/mkl ekanligi aniqlandi.

Hujayradan gDNK ni Guanidin tiosionat tuzi yordmida ajratish.

Lizislovchi buffer eritmaning tarkibi: 4M Guanidin tiosulfat, 50 мM Tris HCl pH = 8, 0,5 mM EDTA, 0,1 M β-merkaptoetanol, 0,5% Sorkozil. gDNK ni ajratish jarayoni quyidagicha amalga oshirildi. Petri likopchasida o'sib turgan achitqi hujayrasidan olinib 100 mkl suvda eritildi. So'ngra uning ustiga 300 mkl lizislovchi bufer eritma solindi. Namuna 65 °C da 20 daqiqa davomida ushlandi. Shundan so‘ng namunalar olinib ularning ustiga 400 mkl dan izoproponol solindi. Probirkalar sentrifugaga olinib 14 000 ay/min da 10 daqiqa davomida sentrifuga qilindi. So‘ngra namunalar sentrifugadan olinib supernatant qismi olib tashlandi. Cho'kmani yuvish maqsadida namunalar ustiga 500 mkl dan 70% etanol solindi. Namunalar 14 000 ay/min 5 daq davomida sentrifuga qilindi va yana supernatant qisim olib tashlandi. Cho'kmaga 45 min davomida laminar xona ichida quritildi va namunalar 100 mkl disterlangan H_2O da eritildi. gDNK namunalari gel elektroforez usuli yordamida baholandi.

Polimeraza zanjir reaktsiyasi (PCR).

Klonlangan transformantlarni tekshirish yoki kerakli genni amplifikatsiya qilish uchun PZR (PCR) usuli qo’llaniladi. PZR aralashmasi quyidagi tarkibdan tashkil topgan: 1X PZR bufer, 3 mM $MgCl_2$, 0.8 mM dNTPs, xar bir praymerdan 0,8 mM, 2,5 yunit Taq Polimeraza, 50ng DNK solinib xajm steril suv bilan 25 mkl gacha yetkaziladi. PZR jarayoni quyidagi siklda olib boriladi: denaturatsiya 5 min 95 °C, initsiatsiya 60 °C 30 sek, elangatsiya 72 °C 1min va reaksiya to’liq yakunlanishi uchun 72 °C 7 min. Jarayonning takrorlanishlar soni 35 martadan iborat.

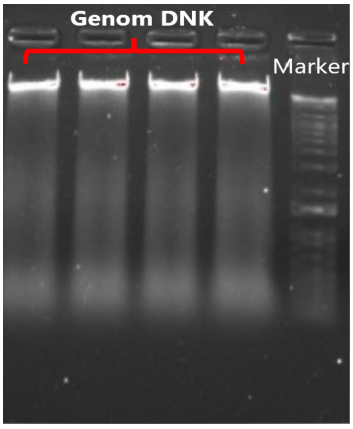
Natijalar va muhokamasi

***Pichia pastoris* genom DNK sini ferment yordamida ajratish.**

Biz mazkur tadqiqot ishimizda achitqi hujayrasidan uning genom DNK sini turli usullar yordamida ajratib, ulardan eng arzon, qulay va tez usulini tanlab olishni maqsad qilganmiz. Buning uchun biz adabiyotlarni chuqur o'rganish natijasida nisbatan optimal bo'lgan 3 xil – guanidintiosionat detergenti, *lyticase* fermenti va litiy atsetat/SDS reagentlari yordamida ajratish usullarini tanlab oldik. Tadqiqot ishida ushbu usullarning kamchilik va ustun tomonlari o'rganilib solishtirishga harakat qilindi. Achitqi hujayralari mustahkam hujayra devorlari bilan o'ralgan. Undan genom DNK sini ajratib olish uchun mazkur devorni yorish zarur bo'ladi. Achitqi hujayra devori asosan glyukanlardan iborat bo'lib, quruq massaga nisbatan taxminan 50-60% ni tashkil qiladi. Ulardan eng ko'p qisimni β-1,3-D-glyukan egallab glyukanlarning 60-95% ni tashkil qiladi[30] Mazkur β-1,3-D-glyukan bilan o‘ralgan achitqi hujayra devorini yorish uchun bir qator fermentlardan foydalaniladi, jumladan bularga Zymolyase, Lyticase va Glusulaselarni misol qilishimiz mumkin [31-33]. Bizda laboratoriyamizda *Arthrobacter luteus* bakteriyasidan ajratib olin-

gan *Lyticase* (Sigma Aldrich) fermenti bo'lganligi uchun tahlil jarayonida undan foydalandik.

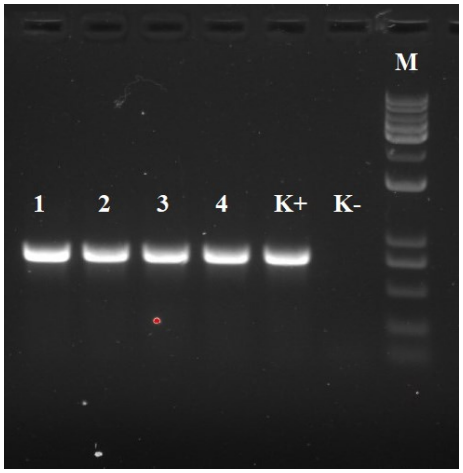
Chashka Petrida o'sib turgan achitqi hujayralarini olib eppindorf probirkasida eritdik va metodlar bo‘limida keltirilgan protokol asosida jarayonni amalga oshirdik. Ajratilgan DNK molekulasining mavjudligini ko'rish maqsadida agaroza gelidagi elektroforezni amalga oshirdik (1-rasm).



1-Rasm. Ajratib olingan genom DNK ning 1 % li agaroza gelidagi elektrofofez tahlili.

Mazkur achitqi shtami o'zida gepatit B virusining S genini saqlaydi. Shu sababli toza holda ajratib olingan DNK namunalari undagi genning mavjudligini va qanchalik toza holda ajratib olinganligini tekshirish maqsadida uning standart PZR tahlini amalga oshirdik (2-rasm). Uning identifikatsiyasi uchun quyidagi praymerlardan foydalanildi. PZR jarayonining borish tartibi va protokoli metodlar bo‘limida keltirilgan.

5’ α-factor – 5’-TACTATTGCCAGCATTGCTGC -3’
3’ AOX1 – 5’-GCAAATGGCAT TCTGACATCC -3’



2-Rasm. PZR amplifikatlarining elektroforez tahlili. Bunda 1-5 tahlil jarayonidagi namunalar, K+ musbat namuna, K- manfiy namuna.

K+ sifatida *Pichia pastoris* genomiga kiritilgan PIC3.5-S plazmidasi ishlatildi. Manfiy namuna uchun esa oddiy steril disterlangan suv ishlatildi. Yuqoridagi elektroforez tahlil natijadan ko‘rinib turibdiki, biz *Pichia partoris* hujayrasidan ajratib olgan genom DNK tarkibida unga kiritilgan gen mavjud va ajratilgan genom DNK toza va sifatli ajratilgan bo'lib, bu usul tahlil jarayonlari uchun mos kelishi mumkin. Lekin, bu usulning borish davomiyligi ancha uzun bo‘lganligi va nisbatan qimmatroq reaktivdan foydalanilganligi sababli to'la maqbul usul hisoblanmaydi.

Achitqi genom DNK sini liytiy asetat va SDS yordamida ajratish.

Litiy asetat laboratoriyada DNK va RNKning gel elektroforezi uchun bufer sifatida ishlatiladi. Shuningdek, u DNK transformatsiyasida DNK ni achitqi devoridan o'tkazish uchun ham ishlatiladi. LiOAc ning foydali ta'siri DNK, RNK va oqsillarni denaturatsiya qilishida uning xaotrop ta'siridan kelib chiqadi, deb xisoblanadi [34].

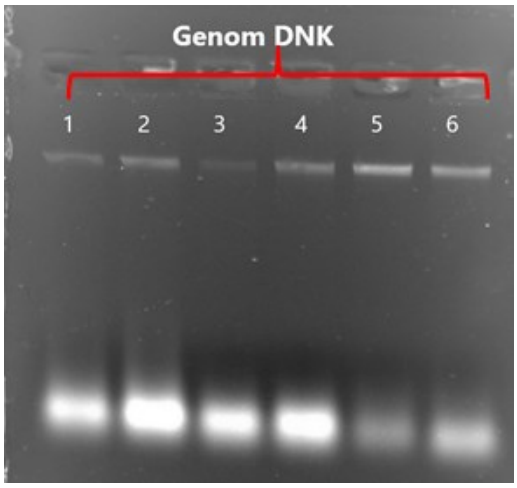
SDS (natriy dodesil sulfat) tozalash jarayonlarida qo'llaniladi va odatda RNK va/yoki DNK ekstraksiyasi paytida hujayralarni parchalash va SDS-PAGE texnikasida elektroforezga tayyorlashda oqsillarni denaturatsiya qilish uchun komponent sifatida ishlatiladi [35].

Yuqorida ta'kidlaganimizdek achitqidan genom DNK sini ferment yordamida ajratish nisbatan ko'p vaqt olishi va reaktivlarning qimmatligi sababli biz, nisbatan arzon va tez amalga oshiriladigan usulni tanlashni maqsad qildik. Buning uchun biz, bor yo'g'i ikki xil SDS va litiy asetatdan foydalangan holda va kam vaqt oralig'ida genom DNK ni ajratishni maqsad qildik va jarayonni quydagicha amalga oshirdik.

Mazkur jarayonda ham qayta ekilgn achitqi shtammlari orasidan ixtiyorit 4 ta koloniya tanlab olinadi va ular 500 mkl steril suv saqlovchi probirkalarga solinadi. So'ngra namunalar sentrifugada 2 min davomida 10 000 ay/min d cho'ktirilib ustidagi suyuqlik olib tashlanadi.

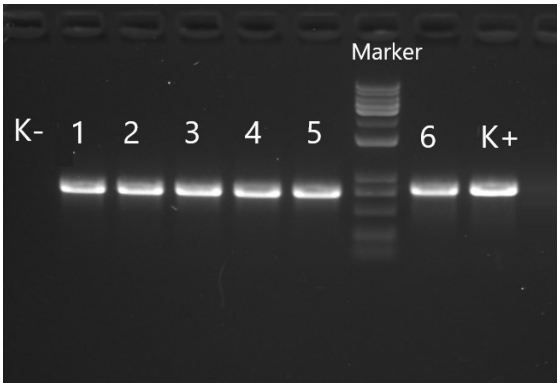
Xosil bo'lgan cho‘kmalarning ustiga tarkibida 1 % SDS va 0,2N litiy asetat saqlovchi eritmadan solinadi. Namunalarni termoblokda 65 °C haroratda 10 min davomida ushladik. So'ngra probirkalar termoblokdan olinib ustiga 300 mkl 96% etanol, 1/10 hajm 5M atsetat ammoniy solindi va yaxshilab aralashtirildi. DNK suspenziyasi qilinadi va hosil bo'lgan cho‘kma qoldirilib, suyuq qismi (supernatant) pipetka yordamida olib tash-

landi va 60 °C haroratida 5 min davomida og'zi ochiq holda quritildi. Namunalar bufferili erituvchida yaxshilab eritilib olindi, agaroza gelidagi elektroforez jarayoni amalga oshirildi (3-rasm).



3-rasm. Ajratilgan DNK molekulasining 1% agaroza gelidagi elektroforez tahlili.

Ajratilgan DNK molekulasining qanchalik toza va sifatli ajratilganligini aniqlash uchun yuqoridagi kab PZR jarayonini amalga oshirdik. Uning identifikatsiyasi uchun quyidagi praymerlardan foydalanildi. PZR jarayonining borish tartibi va protokoli material va metodlar bo'limida keltirilgan. PZR jarayoni tugagandan so'ng namunalar olinib elektroforez tahlili olib borildi. Elektroforez jarayoni 1 % agaroza gelida olib borildi (4-rasm).

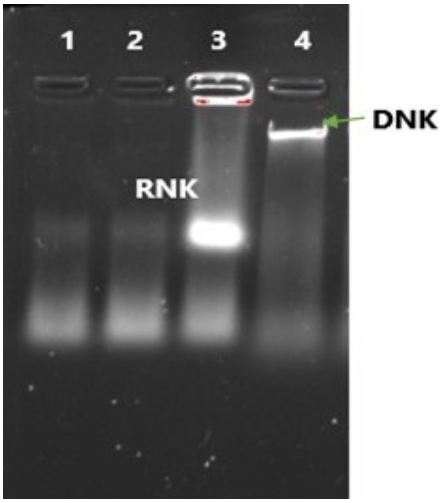


4-rasm. PZR amplifikatlarining elektroforez tahlili. Bunda 1-6 tahlil jarayonidagi namunalar, K+ musbat namuna, K- manfiy namuna.

PZR tahlil natijalari shuni ko‘rsatdiki, *Pichia pastoris*dan uning genom DNK sini SDS va litiy asetat usulida ajratilganda uning tarkibida biz kiritgan gen mavjudligi va namunalarnig toza va sifatli ekanligi aniqlandi . Demak, mazkur usul o‘zining sodda va arzonligi bilan bir qatorda DNK ni toza holda ham ajratib olish imkoniyatini beradi.

Hujayradan gDNK ni Guanidin tiosionat tuzi yordmida ajratish.

Guanidin rangsiz kristall modda bo‘lib, namlikning singishi tufayli havoda suyultiriladi. Guanidin tuzlari oqsillarni denaturatsiya qiluvchi ta'siri bilan mashhur. Guanidin gidroxloridi eng mashhur denaturant hisoblanadi. Uning 6 M eritmasi deyarli barcha oqsillarni denaturatsiyaga uchratish qobilyatiga ega. Guanidin tiosiyanat ham uddi shu maqsadda ishlatiladi. Shu sababli biz achitqi hujayrasidan gDNK sini guanidin tiosiyanat tuzi yordamida ajratishni maqsad qildik. Buning uchun maxsus lizislovchi eritma ishlatiladi. Jarayon metodlar bo’limida keltirilgan protokol asosida amalga oshirildi. Tanlangan usul yordamida ajratilgan DNK molekulasini baholash maqsadida uning agaroza gelidagi gel elektroforezini amalga oshirdik (5-rasm).



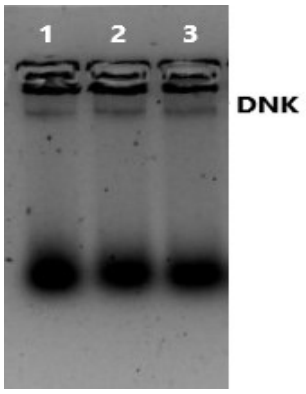
5-rasm. Ajratilgan DNK molekulasining elektroforez tahlili.

Bu yerda: 1-3 guanidin tuzi yordamida ajratilgan namunalar, 4-SDS va LiAc yordamida ajratilgan namuna.

Rasmdan ko'rinib turibdiki, namunalar guanidin usulida ajratilganda gDNK molekulasining ajrab chiqmaganligini ko‘rishimiz mumkin. Biz solishtirish maqsadida SDS va LiAc usulida ajratilgan gDNK molekulasini ham qo'ydik va bunda gDNK molekulasining yaxshi ajralganligini ko'rishimiz mumkin. Yuqorida olib borilgan tajribadan shuni xulosa qilishimiz mumkinki, guanidin tiosionat tuzi yordamida achitqilardan gDNK ajratish maqsadga muvofiq emas ekan.

Bundan tashqari biz, *Pichia pastoris*dan tashqari *Candida albicans*, (O'simlik moddalari

kimyosi instituti molekulyar genetika laboratoriyasi kolleksiyasida saqlanadi) va begona turdagi mog'or zambrug'laridan gDNK ni ajratish jarayonlarini ham sinab ko'rdik. Bunda biz faqat yaxshi natija bergan SDS va LiAc usulidan foydalandik. Jarayon yakunlangandan so'ng ajratilgan DNK molekulalari elektroforez tahlil usulida baholandi (6-rasm).



6-rasm. Ajratilgan DNK molekulalarining elektroforez tahlili.

Elektroforez tahlilidan ko'rinib turibdiki, gDNK molekulalari 3 xil turdagi achitqi va mog'or zambrug'laridan SDS va LiAc usulida yaxshi ajralib chiqqan va deyarli bir xil natijani namoyon qilgan. Demak mazkur usul orqali achitqi va mog'or zambrug'laridan ularning gDNK larini ajratish mumkin.

Xulosa

Mazkur tadqiqot ishida achitqi va mog'or zambrug'laridan ularning genom DNK larini ajratishning eng arzon, qulay va tez usulini tanlab olishni maqsad qilindi. Shu maqsadda, mazkur ish doirasida amalga oshirilgan tadqiqotlar o'rganilib chiqildi va ular orasidan keng qo'llaniladigan usullar tanlab olindi. Bunda biz guanidin tiosiyanat, *lyticase* fermenti va litiy asetat/SDS reagentlari ishlatish orqali amalga oshirilgan ishlarni ko'rib chiqdik. Tadqiqot ishida ushbu usullarning kamchilik va ustun tomonlari o'rganilib solishtirishtirildi. Olingan natijalar shuni ko'rsatdiki, *lyticase* ferment tasirida ajratish usuli nisbatan uzoq vaqt olishi va ishlatigan fermentning narxi qimmatligi inobatga olinib maqbul usul emasligi, litiy atsetat/SDS reagentlari yordamida ajratish usuli ancha sodda, tez va arzonligi, guanidin tiosionat tuzi yordmida ajratish usuli esa, gDNK yomon ajraganligi sababli maqsadga muvofiq emasligi aniqlandi. Litiy atsetat/SDS reagentlari yordamida ajratish usuli barcha achitqi zambrug'lari uchun universal bo'lib, ajratilgan genom DNK molekulasi keying tahlil jarayonlari uchun to'liq yaroqli ekanligi aniqlandi.

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