BIOLOGIE CELULARA SI MOLECULARA

LP06. Izolare acizi nucleici

Biologie Celulara si Moleculara

Modul Biologie celulara:
Notiuni microscopie
Evaluare organite celulare;
Culturi de celule;

Modul Biologie moleculara:
Izolarea ADN şi ARN;
Amplificarea ADN/ARN
Electroforeza
Tehnici de detectare a mutaţiilor:
- ASO.
- RFLP.
- DGGE.
- Real-Time PCR.
- Secvenţiere.

ADN şi ARN non-self.

Metode de izolare a acizilor nucleici. Principii.

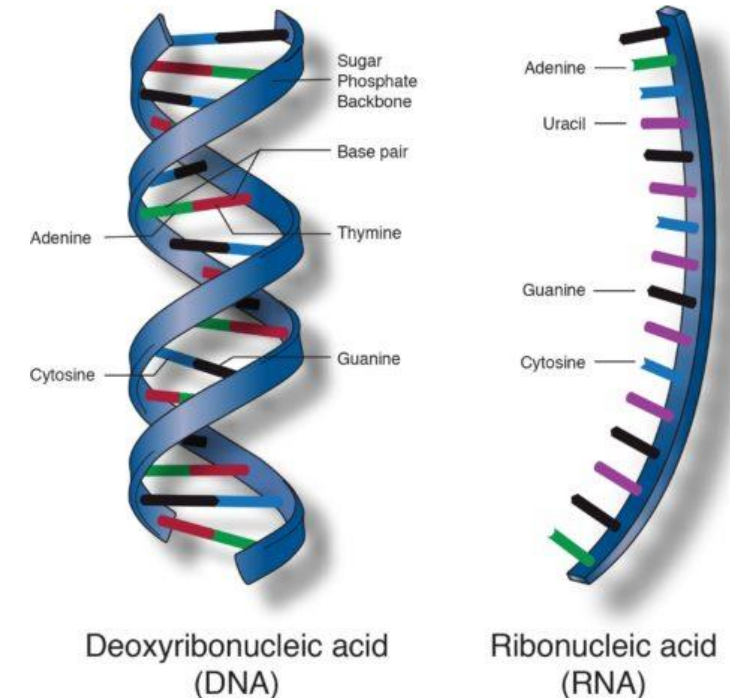
Consideratii

- tipurile diferite de probe necesita metode diferite de izolare.

- tipuri diferite de probe: tesuturi umane (lichide sau solide), bacterii, plante.
- tipuri diferite de probe: proaspat recoltate, pastrate in diferite conditii (congelate, fixate in parafina), degradate (ex. mumii)
- tipuri diferite de protocoale de lucru: manual, automat
- tipuri diferite de protocoale de lucru in functie de principiul metodei

- sunt similitudini intre protocoale izolare ADN si ARN

- conditii obligatorii pentru a utiliza laboratorul.
- urmarirea unui protocol de lucru



Deoxyribonucleic acid (DNA)

Ribonucleic acid (RNA)

Patologie

- Afectiuni digestive maligne (gastrice, hepatice, pancreatice, colorectale)
- Patologie infectioasa (virus hepatitic B, virus hepatitic C, virusul papiloma uman)
- Ginecologie
- Nefrologie
- etc…

Tipuri de tesuturi umane din care putem izola ADN

Sange – recoltat in tuburi cu anticoagulant

Biopsii de tesut– recoltat in solutie stabilizatoare

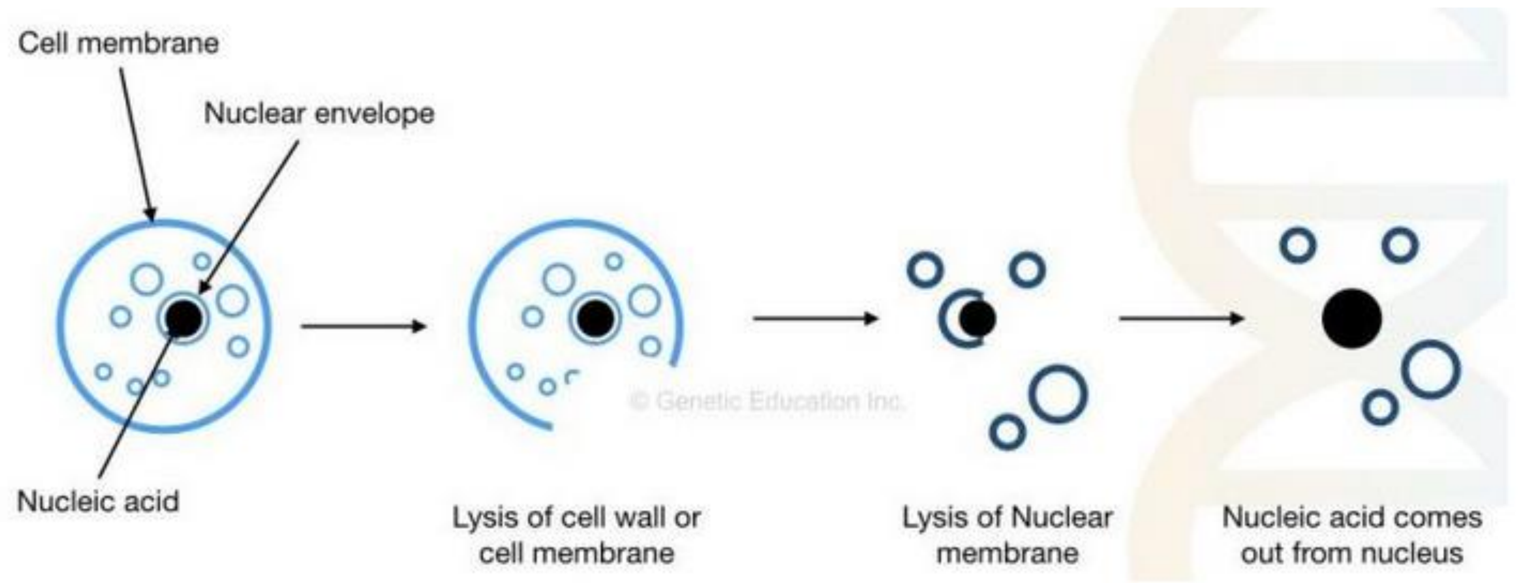
Tesut parafinat

Lichid amniotic

Vilozitati coriale(CVS)

Principiul izolarii ADN-ului

- ***Liza membranelor***
  - se ţine cont de natura celulei.
- ***Indepartarea proteinelor***
- ***Captarea/eliberarea acizilor nucleici***
- ***Spalare acizilor nucleici***
- ***Rehidratare***



The image represents the general outline of how DNA can be extracted from the cell.

Mechanical lysis

Chemical lysis

Enzymatic lysis

Cell wall or cell membrane Lysis

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Nuclear envelope lysis

Proteinase K, SDS, phenol and chloroform

Centrifugation

Protein digestion and Removal of cell debris

Isoamyl alcohol, Ethanol

Salt: NaCl / sodium acetate

Precipitation of DNA

TE buffer

D/W

Dissolving DNA

DNA extraction methods
Chemical DNA extraction methods
Physical DNA extraction methods
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Organic DNA extraction methods
Inorganic DNA extraction methods
Magnetic bead DNA extraction
Paper DNA extraction method
Phenol-chloroform DNA extraction method
Proteinase K DNA extraction method
Silica gel based DNA extraction method
Salting out method

Etapele izolarii ADN

Procedeu manual:

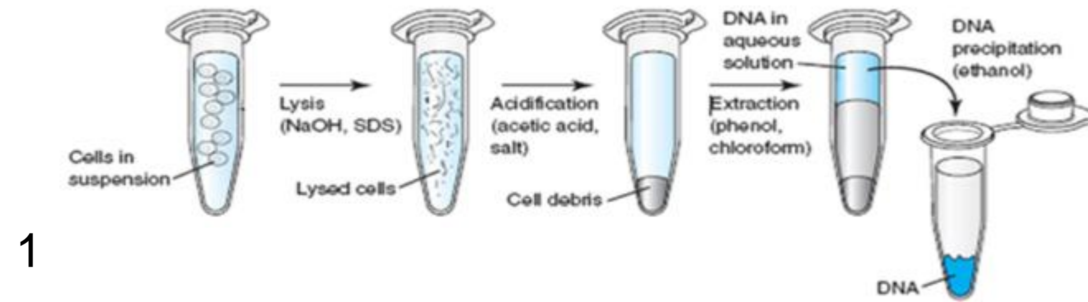
1. izolare organica

2. izolare pe baza de membrane de siliciu

3. izolare pe baza de particule magnetice

https://geneticeducation.co.in/different-types-of-dna-extraction-methods/

1



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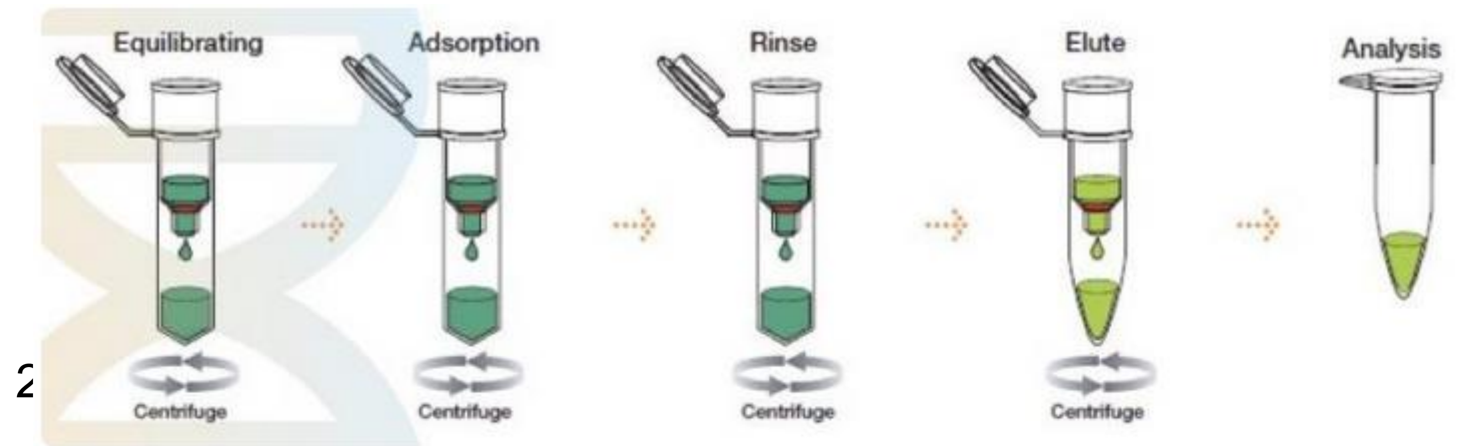

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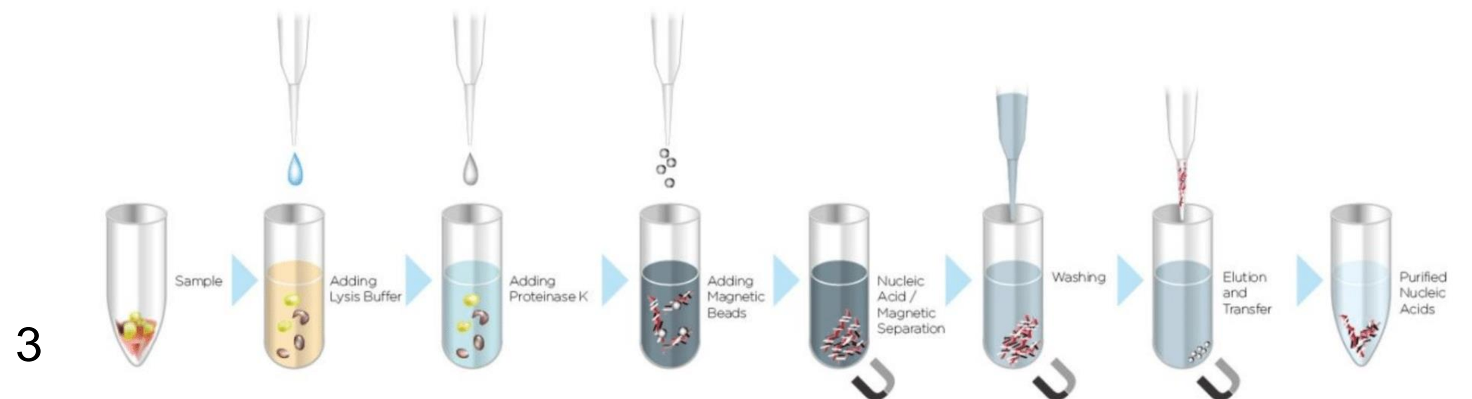

Etapele izolarii ADN

Solutii folosite

liza celulara

liza nucleara

precipitare proteica

precipitare ADN

spalare ADN

rehidratare

DNA extraction step	Chemical
Lysis of cell wall/ cell membrane and Lysis of nuclear membrane	Tris, $MgCl_2$, EDTA, NaCl, SDS, CTAB, Triton X100
Digestion of protein	CTAB, SDS, phenol, chloroform, urea, guanidium isothiocyanate, guanidium thiocyanate, N-Lauroyl sarcosine
Precipitation of DNA	Isopropanol, ethanol, methanol, NaCl, sodium acetate
Washing of DNA	Any alcohol (ethanol commonly used)
Dissolving DNA	TE buffer, distil water

Etapele izolarii ADN

Exemplu de procedeu manual pe baza de solutii

liza celulara

liza nucleara
precipitare proteica

precipitare ADN in izopropanol

spalare ADN cu etanol, uscare

rehidratare cu apa ultrapure sau solutie tampon TE

sânge periferic

liză celulară

Centrifugare
lizat celular

Sol. liză nucleară
Sol. de precipitare
a proteinelor

nuclei celulari

Centrifugare

supernatant
conținând ADN

proteine
denaturate

supernatant(ADN)
tansferat în
izopropanol

Precipitarea ADN

Centrifugare

Spalarea ADN
Etanol 70%

Rehidratarea ADN

Apa/TE

Etapele izolarii ADN

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Wizard® Genomic DNA Purification Kit

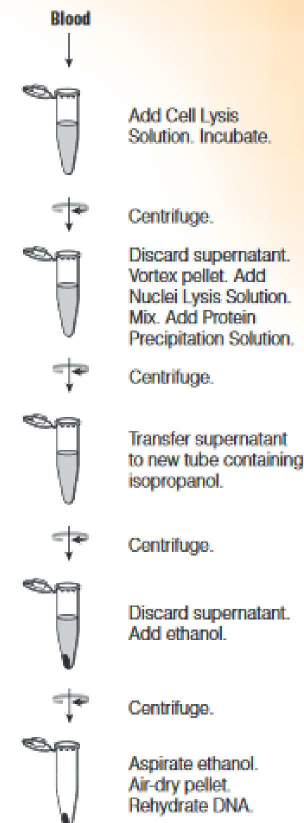
INSTRUCTIONS FOR USE OF PRODUCTS A1120, A1123, A1125 AND A1620.

Quick PROTOCOL

Isolation of Genomic DNA from Whole Blood

Sample Size	Lysis Solution Cell	Lysis Solution Nuclei	Protein Precipitation Solution	Isopropanol	DNA Rehydration Solution
300µl	900µl	300µl	100µl	300µl	100µl
1ml	3ml	1ml	330µl	1ml	150µl
3ml	9ml	3ml	1ml	3ml	250µl
10ml	30ml	10ml	3.3ml	10ml	800µl

As little as 20µl can be processed with this system. Please see Technical Manual #TM050, Section 3.C.



Red Blood Cell Lysis

1. Using volumes from the table above, combine the appropriate volumes of Cell Lysis Solution and blood. Mix by inversion.
2. Incubate for 10 minutes at room temperature.
3. Centrifuge:
   ≤300µl sample 13,000–16,000 × g*; 20 seconds
   1–10ml sample 2,000 × g; 10 minutes
4. Discard supernatant. Vortex pellet.

Nuclei Lysis and Protein Precipitation

5. Using volumes from the table above, add Nuclei Lysis Solution and mix by inversion.
6. Add Protein Precipitation Solution; vortex for 20 seconds.
7. Centrifuge:
   ≤300µl sample 13,000–16,000 × g*; 3 minutes
   1–10ml sample 2,000 × g; 10 minutes

DNA Precipitation and Rehydration

8. Transfer supernatant to a new tube contaning isopropanol (using volumes from table above). Mix.
9. Centrifuge:
   ≤300µl sample 13,000–16,000 × g*; 1 minute
   1–10ml sample 2,000 × g; 1 minute
10. Discard supernatant. Add 70% ethanol (same volume as isopropanol).
11. Centrifuge as in Step 9.
12. Aspirate the ethanol and air-dry the pellet (10–15 minutes).
13. Rehydrate the DNA in the appropriate volume of DNA Rehydration Solution for 1 hour at 65°C or overnight at 4°C.

*Maximum speed on a microcentrifuge.

Additional protocol information is available in Technical Manual #TM050, available online at: **www.promega.com**

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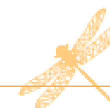

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Etapele izolarii ADN

Exemplu de procedeu manual pe baza de solutii

liza celulara

liza nucleara

precipitare proteica

precipitare ADN in izopropanol

spalare ADN cu etanol, uscare

rehidratare cu apa ultrapure sau solutie tampon TE

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Quick PROTOCOL

Isolation of Genomic DNA from Animal Tissue and Tissue Culture Cells

Prepare Tissues

Tissue Culture Cells: Centrifuge at 13,000–16,000 × *g** for 10 seconds. Wash the cell pellet with PBS, vortex and then add 600µl of Nuclei Lysis Solution and mix by pipetting.

Animal Tissue: Add 10–20mg of fresh or thawed tissue to 600µl of chilled Nuclei Lysis Solution and homogenize for 10 seconds. Alternatively, use 10–20mg of ground tissue. Incubate at 65°C for 15–30 minutes.

Mouse Tail: Add 600µl of chilled EDTA/Nuclei Lysis Solution to 0.5–1cm of fresh or thawed mouse tail. Add 17.5µl of 20mg/ml Proteinase K and incubate overnight at 55°C with gentle shaking.

Lysis and Protein Precipitation

1. Add 3µl of RNase Solution to the cell or animal tissue nuclei lysate and mix. Incubate for 15–30 minutes at 37°C. Cool to room temperature.
2. Add 200µl of Protein Precipitation Solution. Vortex and chill on ice for 5 minutes.
3. Centrifuge at 13,000–16,000 × *g** for 4 minutes.

DNA Precipitation and Rehydration

4. Transfer supernatant to a fresh tube containing 600µl of room temperature isopropanol.
5. Mix gently by inversion.
6. Centrifuge at 13,000–16,000 × *g** for 1 minute.
7. Remove supernatant and add 600µl of room temperature 70% ethanol. Mix.
8. Centrifuge as in Step 6.
9. Aspirate the ethanol and air-dry the pellet for 15 minutes.
10. Rehydrate the DNA in 100µl of DNA Rehydration Solution for 1 hour at 65°C or overnight at 4°C.

*Maximum speed on a microcentrifuge.

Additional protocol information is available in Technical Manual #TM050, available online at: **www.promega.com**

Cells or tissue with Nuclei Lysis Solution.

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Quick PROTOCOL

Isolation of Genomic DNA from Yeast Cultures or Plant Tissue

Prepare Yeast Lysate

1. Pellet cells from 1ml of culture by centrifugation at 13,000–16,000 × g* for 2 minutes.
2. Suspend the cell pellet in 293µl of 50mM EDTA.
3. Add 7.5µl of 75 units/µl lyticase and mix gently.
4. Incubate for 30–60 minutes at 37°C. Cool to room temperature.
5. Centrifuge as in Step 1. Discard the supernatant.
6. Add 300µl of Nuclei Lysis Solution. Proceed to **Protein Precipitation and DNA Rehydration,** Step 1 (below).

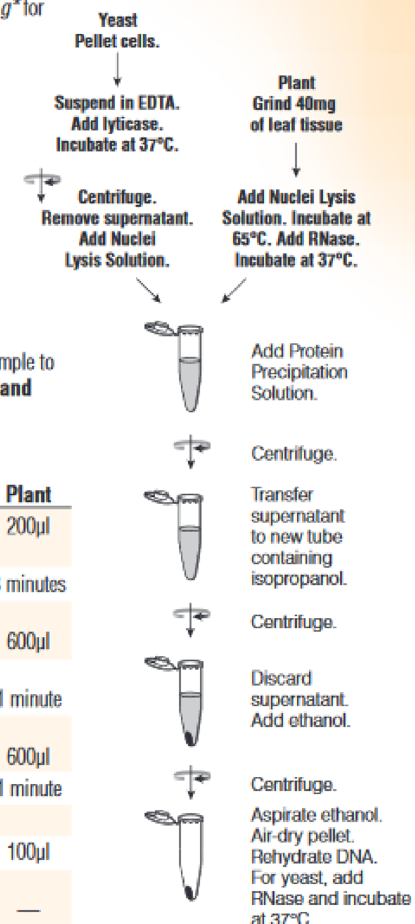
Prepare Plant Lysate

1. Grind approximately 40mg of leaf tissue in liquid nitrogen.
2. Add 600µl of Nuclei Lysis Solution. Incubate at 65°C for 15 minutes.
3. Add 3µl of RNase Solution. Incubate at 37°C for 15 minutes. Cool sample to room temperature for 5 minutes. Proceed to **Protein Precipitation and DNA Rehydration,** Step 1 (below).

Protein Precipitation and DNA Rehydration

	Yeast	Plant
1. Add Protein Precipitation Solution. Vortex. **For yeast only:** Incubate 5 minutes on ice.	100µl	200µl
2. Centrifuge at 13,000–16,000 × g*.	3 minutes	3 minutes
3. Transfer supernatant to clean tube containing room temperature isopropanol.	300µl	600µl
4. Mix by inversion and centrifuge at 13,000–16,000 × g*.	2 minutes	1 minute
5. Decant supernatant and add room temperature 70% ethanol.	300µl	600µl
6. Centrifuge at 13,000–16,000 × g*.	2 minutes	1 minute
7. Aspirate the ethanol and air-dry the pellet.		
8. Add DNA Rehydration Solution.	50µl	100µl
9. **For yeast only:** Add RNase. Incubate at 37°C for 15 minutes.	1.5µl	—
10. Rehydrate at 65°C for 1 hour or overnight at 4°C.		

*Maximum speed on a microcentrifuge.



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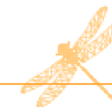

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Sample → DNA → PCR

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DNA extraction
- Proteinase K DNA extraction method
- Silica gel based DNA extraction method
- Paper DNA extraction method
- Salting out method
- Phenol-chloroform DNA extraction method
- Magnetic bead DNA extraction

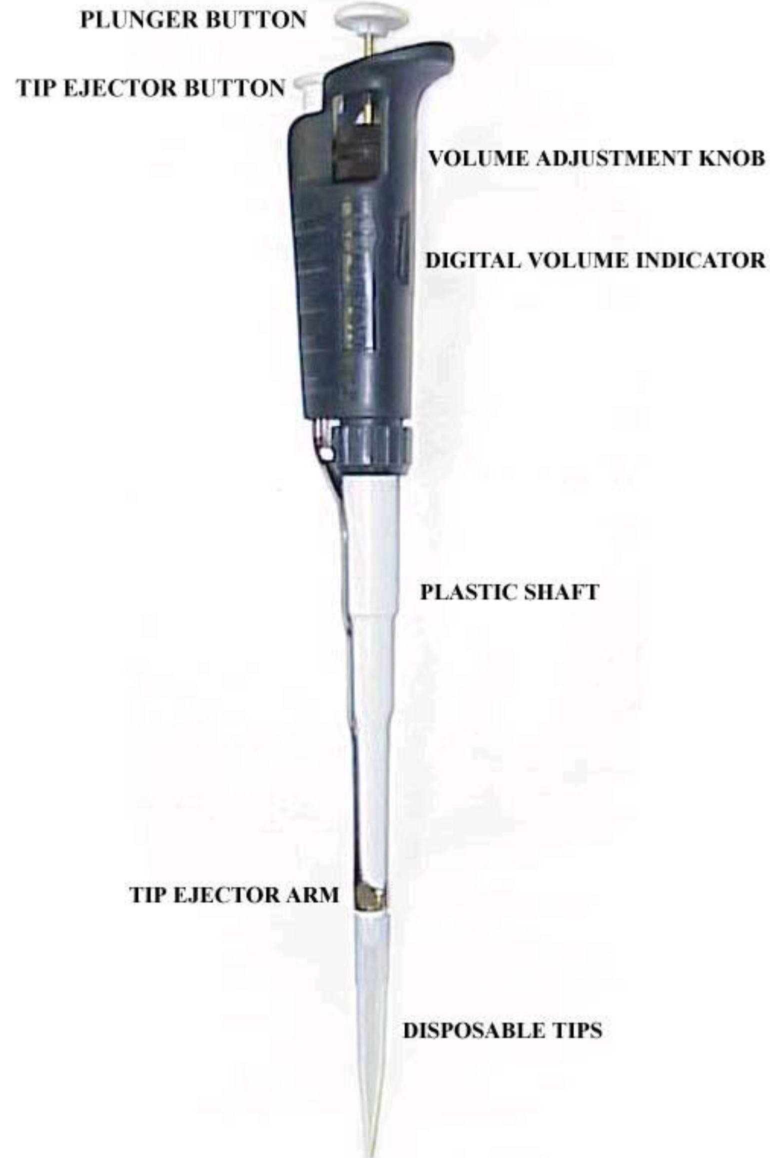
DNA quantification
- Fluorometric analysis
- Spectrophotometric analysis

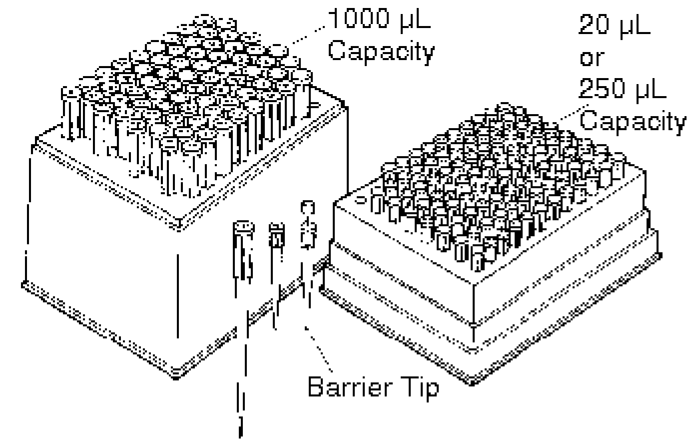
Downstream applications
- DNA sequencing
- DNA micro array
- AFLP
- VNTR
- Marker assistant selection

The general outline of DNA extraction to PCR.

Conditii obligatorii pentru a utiliza laboratorul

- Acces
- Protectia personalului - echipamentul necesar pentru biosiguranta
- Proceduri de lucru
- Circuitul de lucru in laborator
- Protocoale de lucru


Parts of the Automatic Pipettor
PLUNGER BUTTON
TIP EJECTOR BUTTON
VOLUME ADJUSTMENT KNOB
DIGITAL VOLUME INDICATOR
PLASTIC SHAFT
TIP EJECTOR ARM
DISPOSABLE TIPS

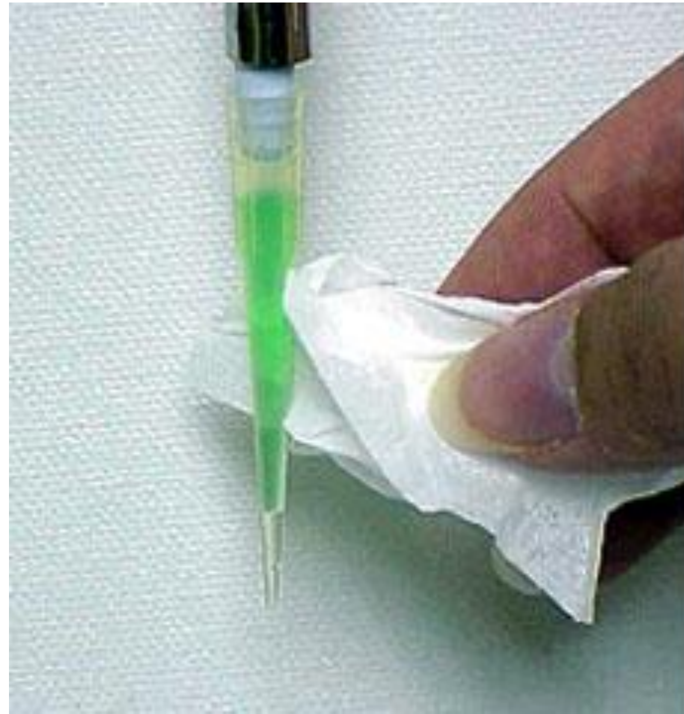
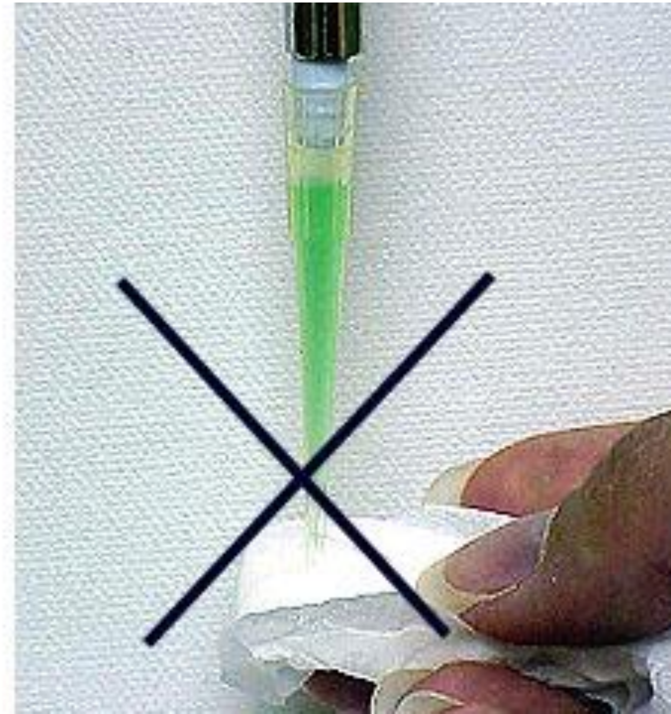

1000 µL
Capacity
20 µL
or
250 µL
Capacity
Barrier Tip

Reglarea volumului necesar de aspirat se realizeaza inainte de a atasa varful.

Reglarea volumului necesar de aspirat se realizeaza inainte de a atasa varful.

- Dupa atasarea varfului, pipeta se tine in pozitie verticala (in special cand exista aspirat pe varf si nu numai).

Daca se contamineaza varful accidental (atingere de peretii tubului, atingere de manusa), se inlocuieste cu alt varf steril. Varful se schimba dupa fiecare proba. Pipeta se introduce in lichid cu pistonul apasat pana la prima treapta, urmeaza aspirarea lenta si dispersia lenta, apasant pistonul pana la treapta a doua (pentru a elimina si volumul rezidual.

DISCUȚII