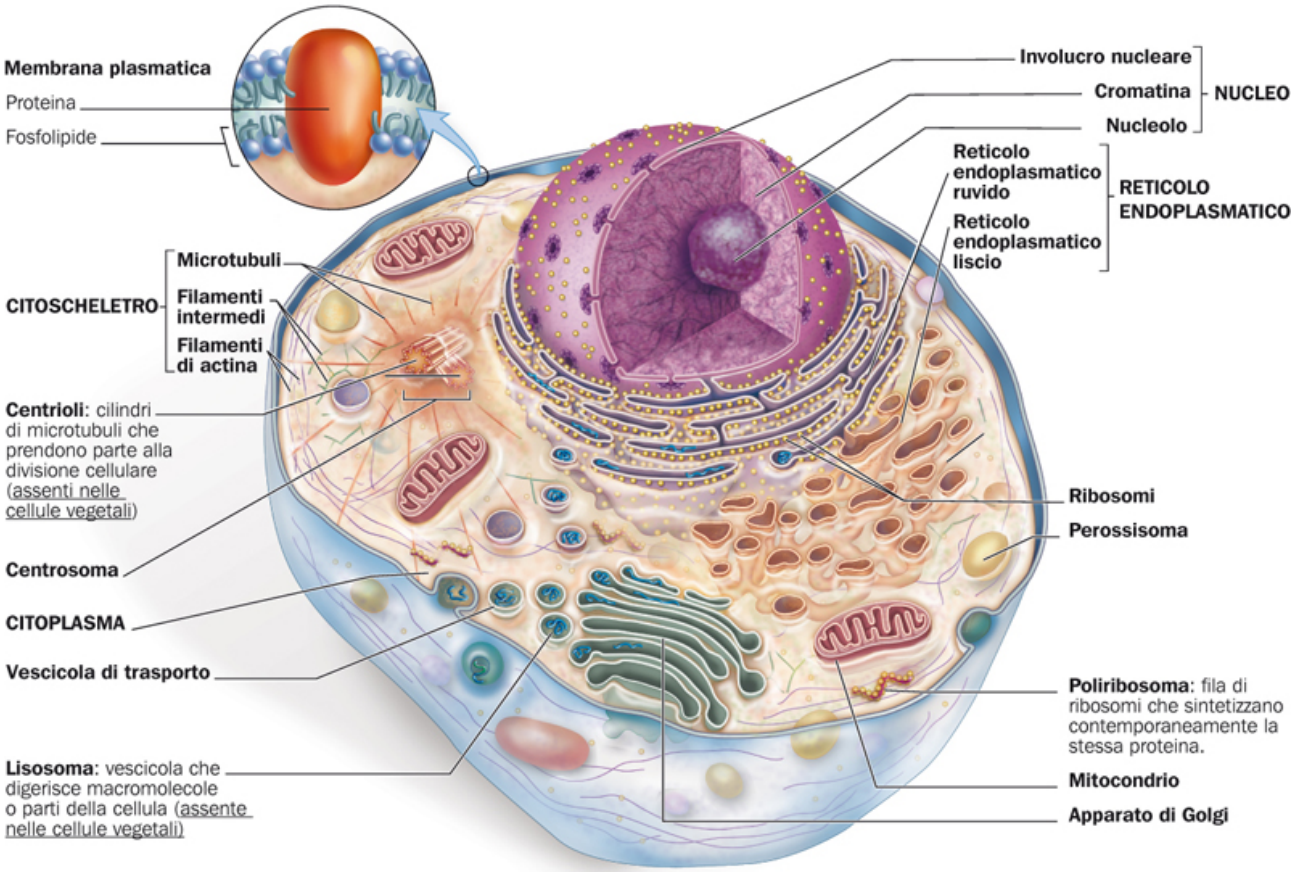
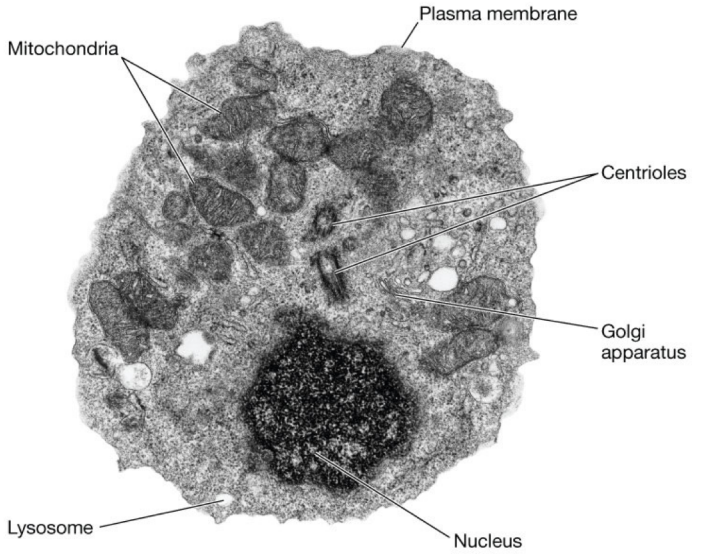
# *1. RECOMBINANT DNA TECHNIQUES*

# LA CELLULA EUCARIOTE




**10-100µm**

**Genoma humano:**
**3,289,000,000 nucelotidi**

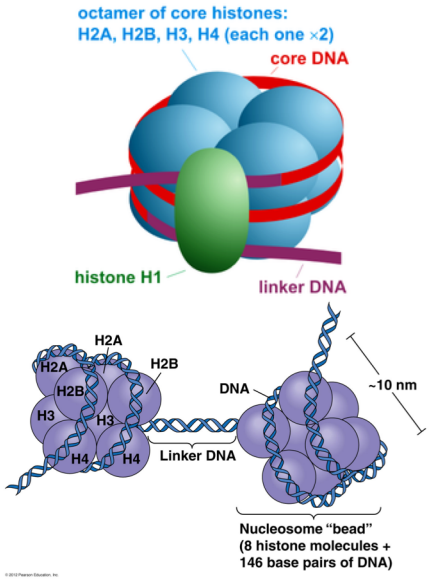
- Dimensioni: circa dieci volte piu' grandi delle cellule procariotiche (10-100 µm)
- La **membrana plasmatica** racchiude il materiale cellulare, lo separa dall'ambiente e regola il passaggio di sostanze cellula/esterno
- **Compartimentazione interna**: all'interno della membrana si trova il **citoplasma**, l'insieme del contenuto cellulare, comprendente il **citosol** (soluzione acquosa di piccole e grandi molecole) ed una serie di **organuli**, compartimenti funzionalmente specializzati delimitati da membrana o comunque strutturalmente separati (Apparato di Goghi; Mitocondrio; Reticilo endoplasmatico)

# LA CROMATINA

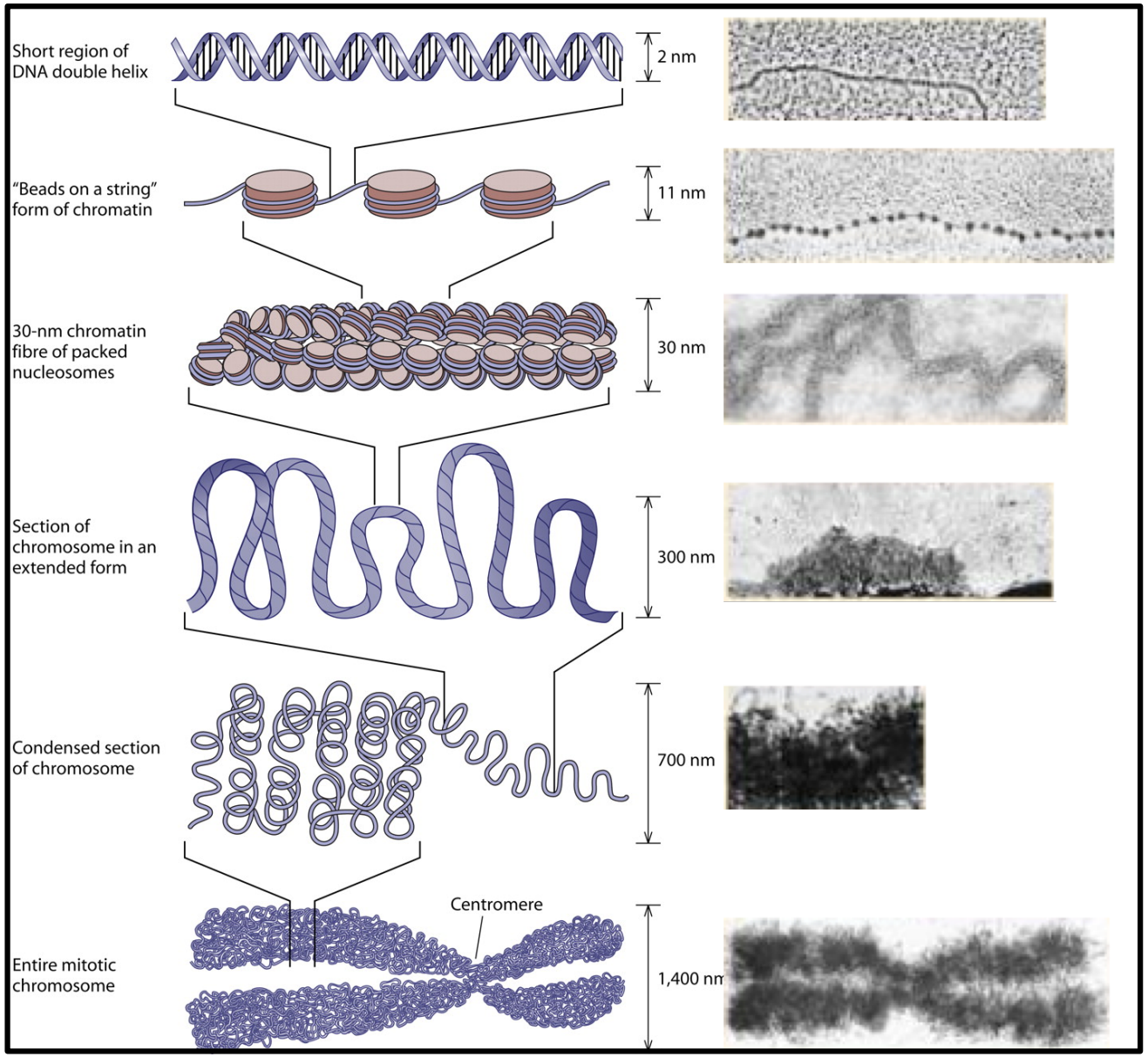
La **cromatina** è la forma in cui gli acidi nucleici si trovano nella cellula.

**Funzione:**

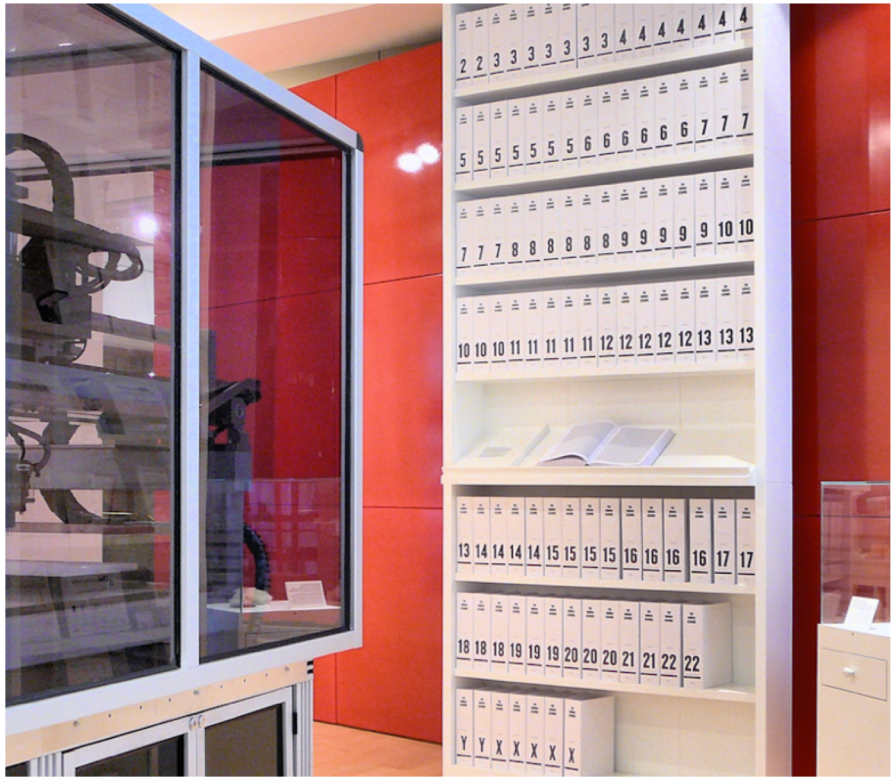
- impacchettamento del DNA
-rafforzare il DNA per permettere la mitosi
- prevenire danni al DNA
-controllare la replicazione del DNA e l'espressione (attivita) del gene

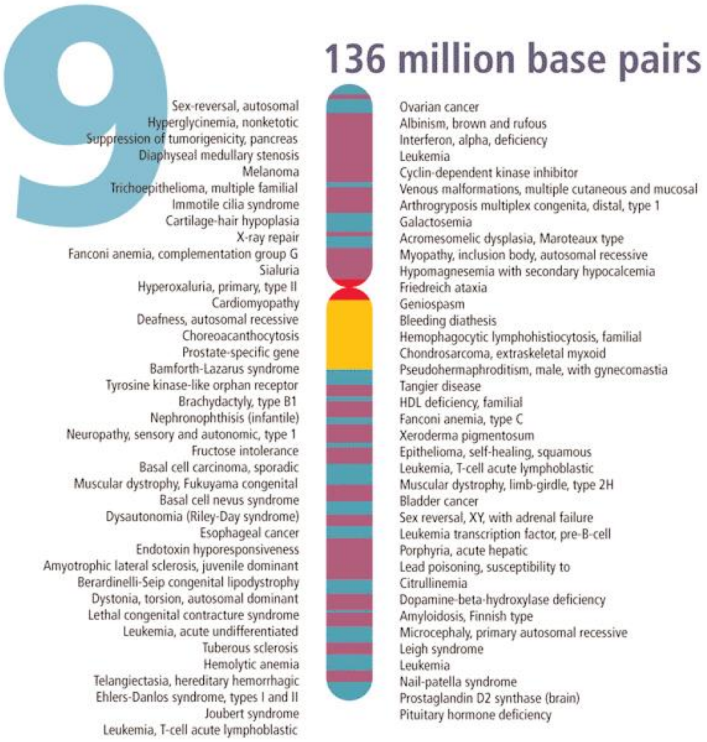



**Nucleosome:**
**8 histone proteins**
**2 giri di DNA (146 nt)**



# Genomes






**Genoma umano aploide: 3.2 x $10^9$ bp (3200000000 bp)**

- → 22 autosomi
- → eterocromosomi (X ed Y)
- → 23000 geni

**Dimensione dei cromosomi: 45-275 Mb;**

- → 2.9 x 109 bp: eucromatina = attivo
- → Genoma noto: >90% dell'eucromatina.

**L'utillizo della infromazione genetica:**

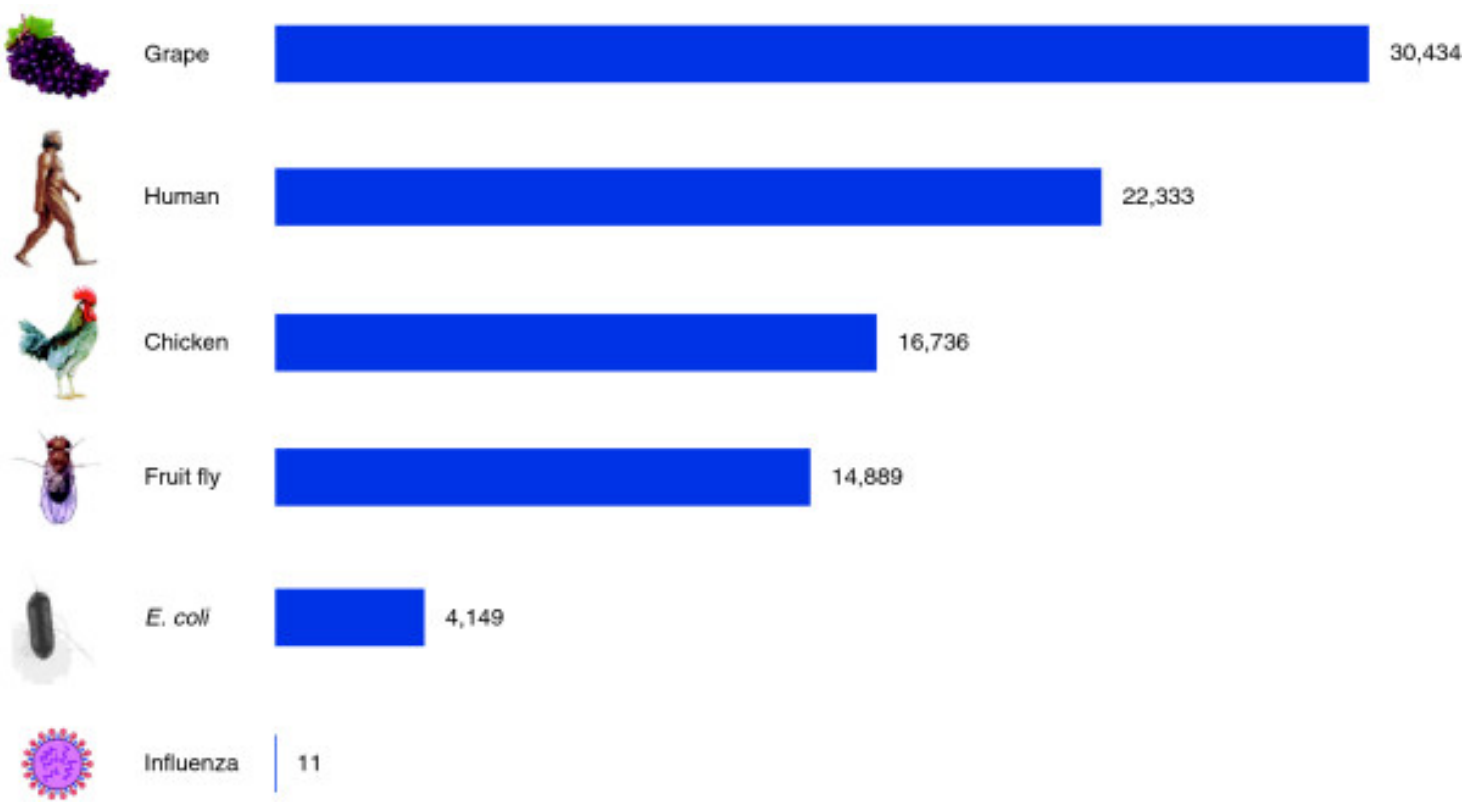
**5.000-10.000 geni espressi da ogni cellula**

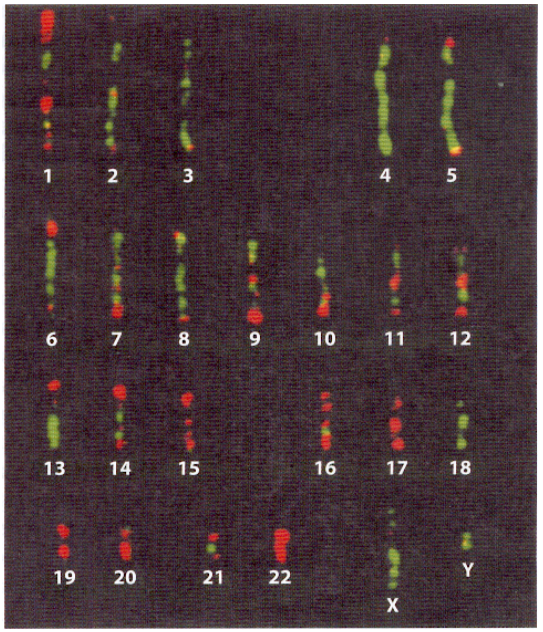
**□ 100.000 specie proteiche diverse per modificazioni post-traduzionali**

**□ $10^8$ specie proteiche diverse nel genere umano (plasma: *proteoma di proteomi)***

***ENORME COMPLESSITA***

# Gene numbers in different organisms



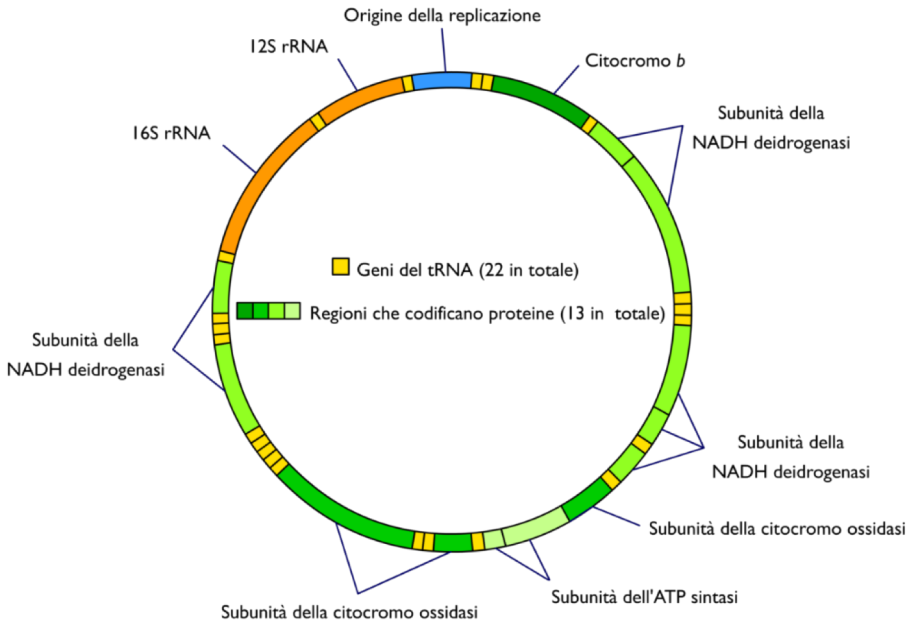

**Mappa genica umana**

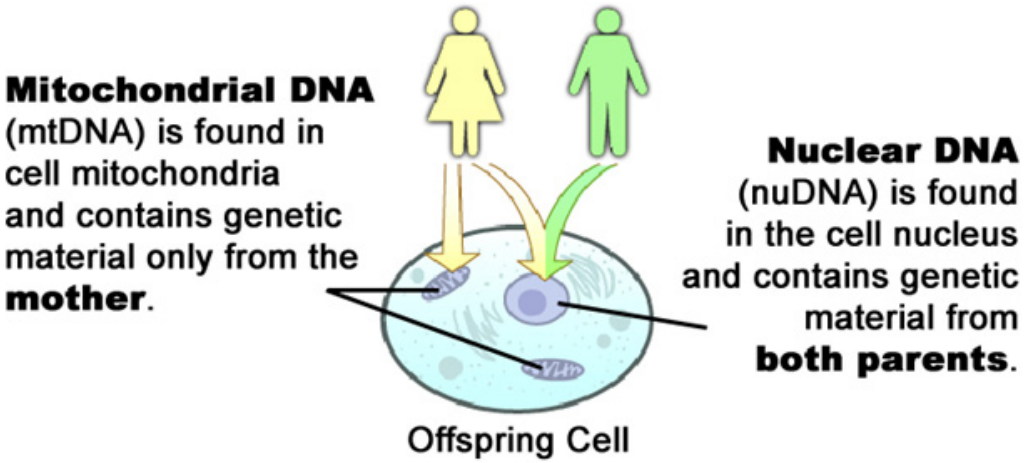
Le regioni in rosso indicano porzioni dei cromosomi ad alta densità genica (ad esempio i cromosomi 15, 16, 17, 19, 20 e 22).

Altri cromosomi come 4, 18, X e Y mostrano una colorazione rossa molto debole e sono poveri di geni.

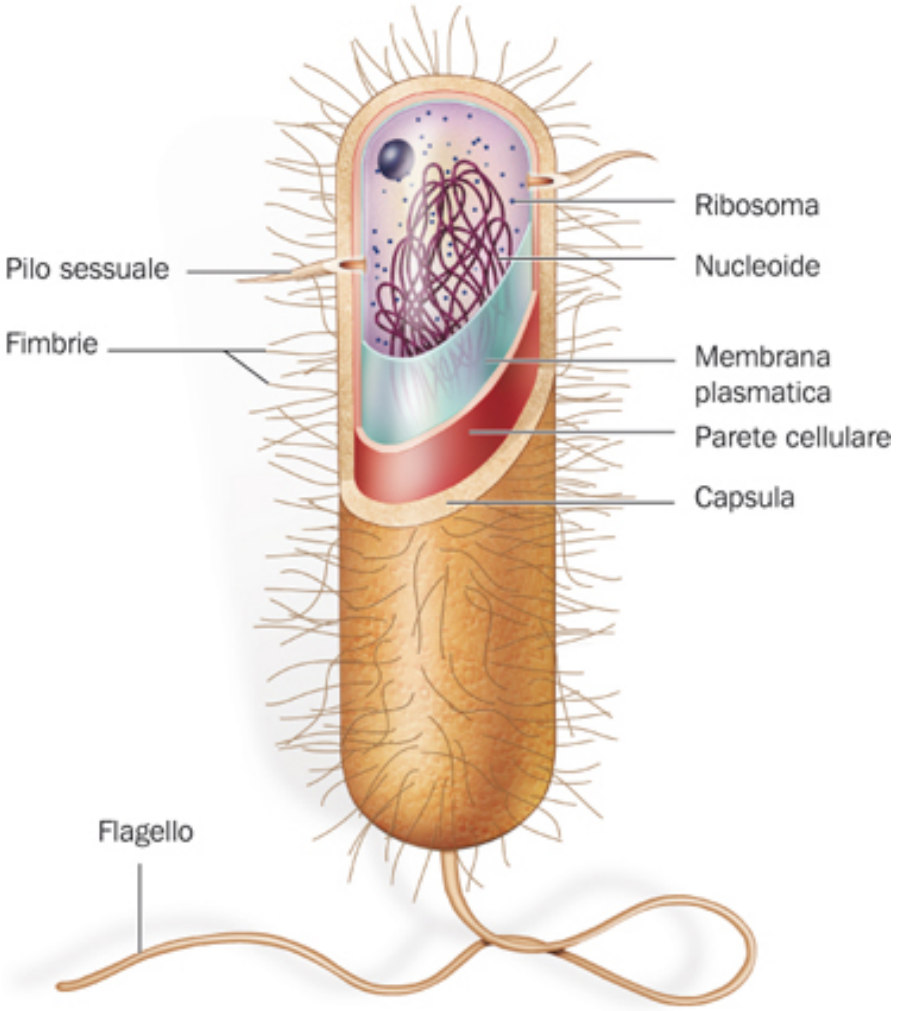
# MITOCHONDRIAL DNA

DNA mitocondriale  dell'uomo:
16569 paia di basi e 37 geni (codificano per 13 polipeptidi sintetizzati dal ribosoma mitocondriale
22 tRNA e 2 rRNA), coinvolti nella produzione di proteine necessarie alla respirazione cellulare.



# PROCARIOTI




Le cellule procariotiche (da *pro*, prima e *karyon*, nucleo) sono **prive di un nucleo** racchiuso da una membrana.

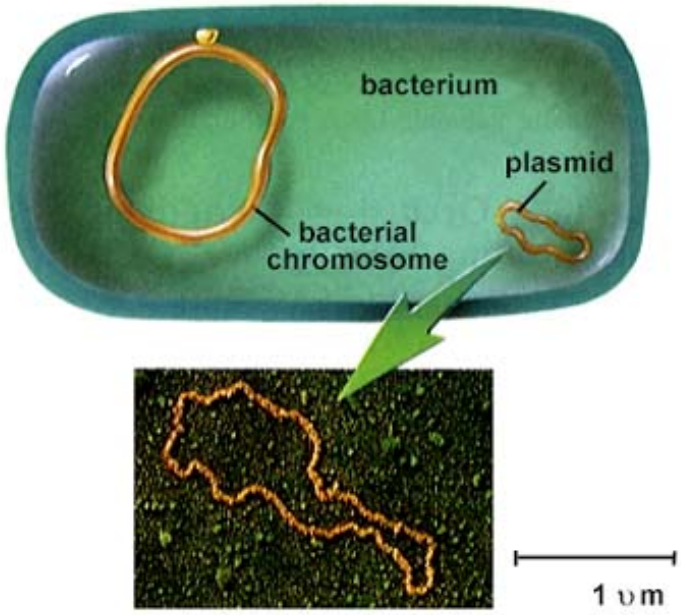
Gli organismi unicellulari costituiti da cellule procariotiche, i **procarioti**, sono classificati in due domini:

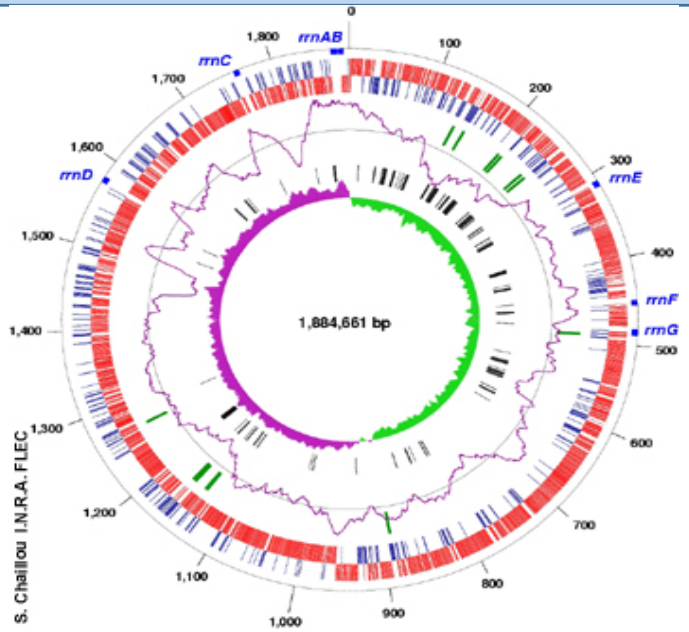
- ***Archaea* (archei);**
- ***Bacteria* (batteri).**

# PROCARIOTI

Il materiale genetico, il DNA, e' organizzato in un **singolo cromosoma circolare,** localizzato nell'area nucleare o **nucleoide**, una regione della cellula non delimitata da membrana.

**1-2 μm** (1.000.000 μm = 1m)




- In aggiunta al DNA principale i batteri possono contenere piccole molecole di DNA circolare, dette **plasmidi**, che codificano per enzimi catabolici, per la resistenza ad antibiotici o legati a meccanismi per lo scambio di materiale genetico tra organismi.
- **Genoma: 130.000 – 14.000.000 nucleotidi**

**I plasmidi hanno origine di replicazione e possono essere presenti in molte copie in un batterio.**

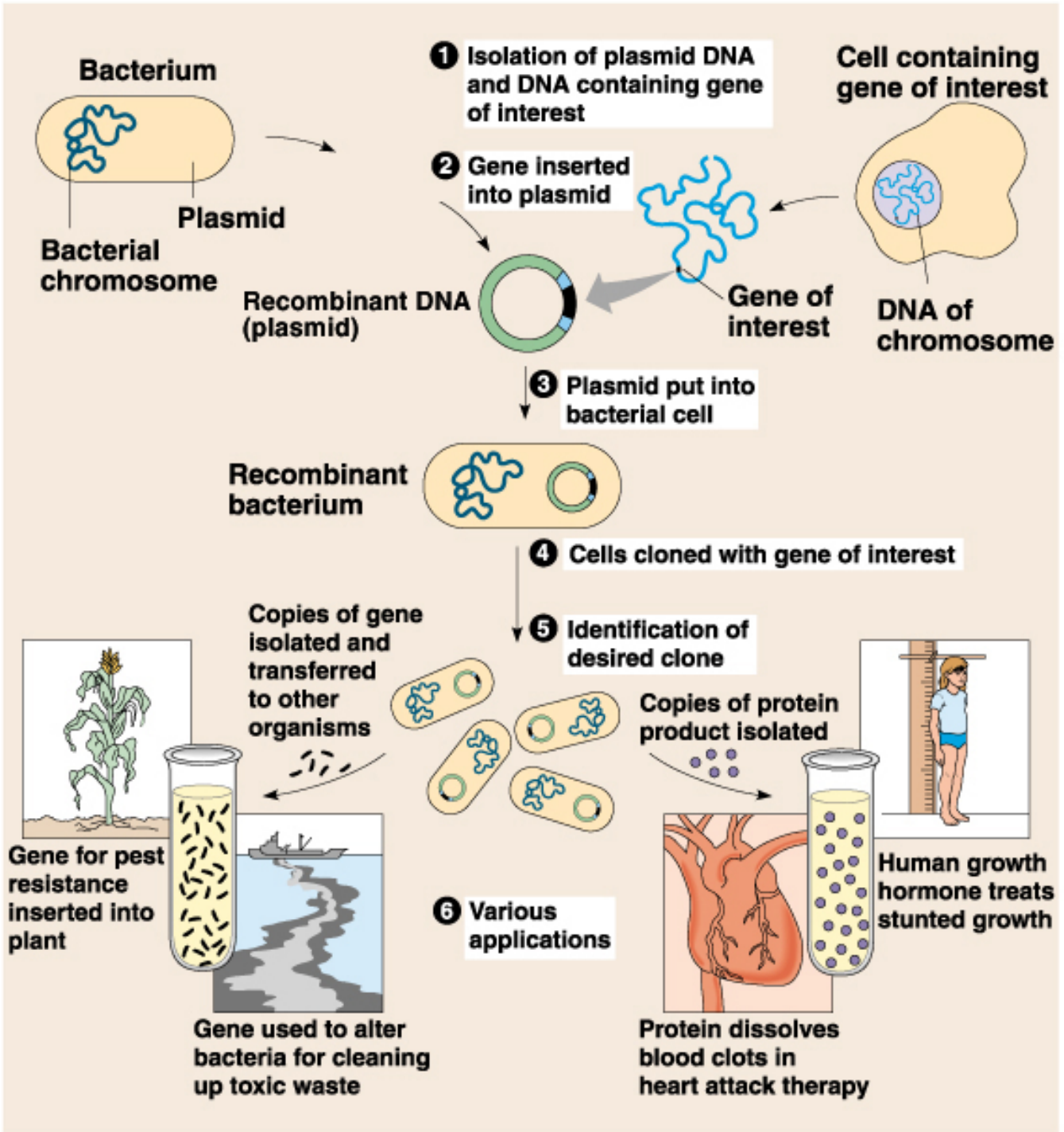
**Plasmidi servono in laboratorio come veicoli, chiamati anche vettori, per amplificare le molecole di DNA per scopi di ricerca e biotecnologici**

# DNA RICOMBINANTE

**tecnica che permette di**

- **ottenere brevi segmenti di DNA clonati e di studiarne la sequenza nucleotidica**
- **di trasferirli nel genoma di altre cellule**
- **di controllare l'incorporazione e l'espressione del DNA clonato**
- **di introdurre mutazioni nel DNA e di studiarne gli effetti**

# A General Strategy to study or use recomobinant DNA

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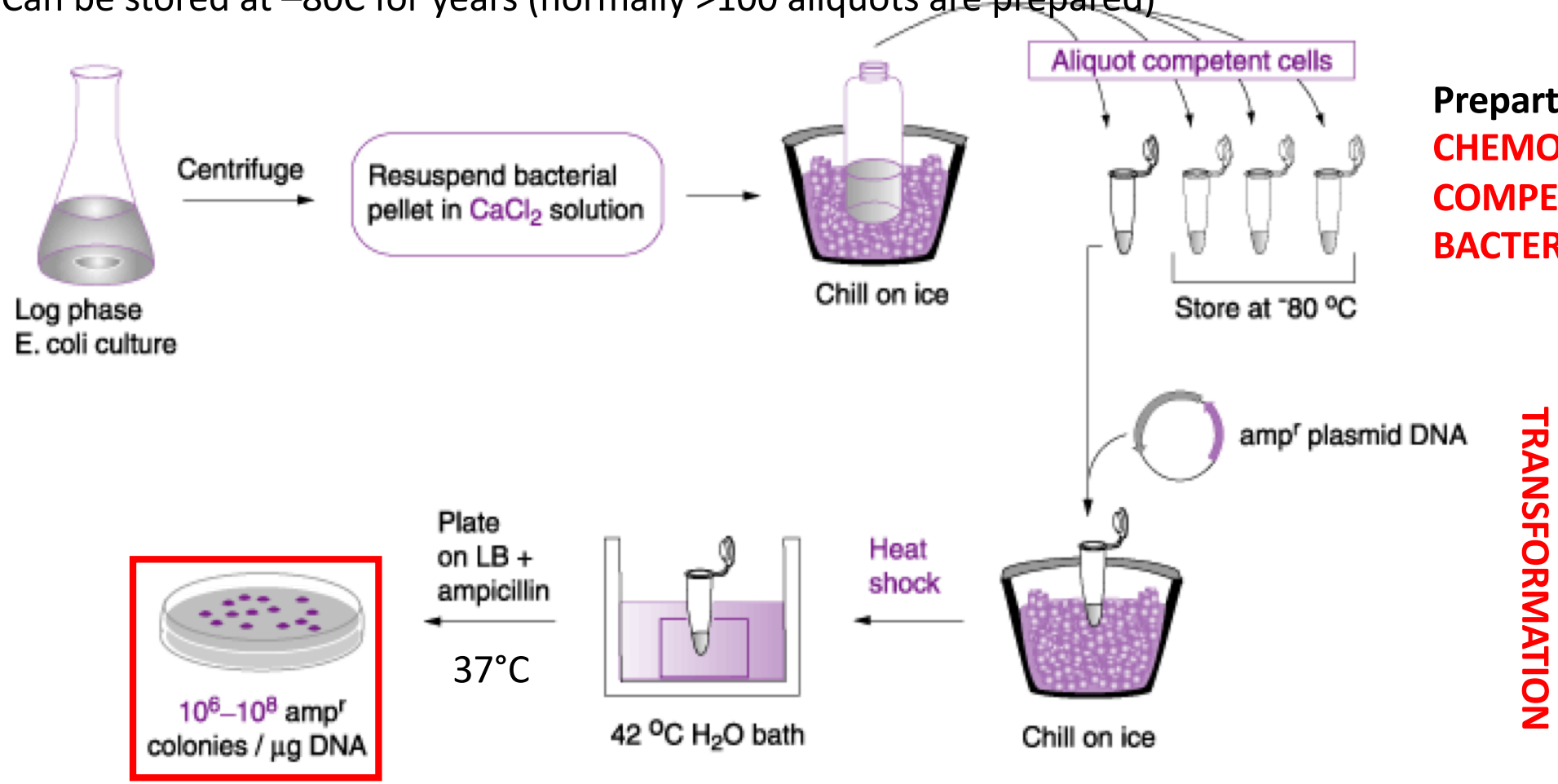
# TRANSFORMATION: TRANSFER OF PLASMID INTO BACTERIA

$CaCl_2$ and cold environment makes membrane permeable without killing the cells

= **CHEMOCOMPETENT BACTERIA - metodo del $CaCl_2$ – (calcio cloruro )**

(Can be stored at –80C for years (normally >100 aliquots are prepared)

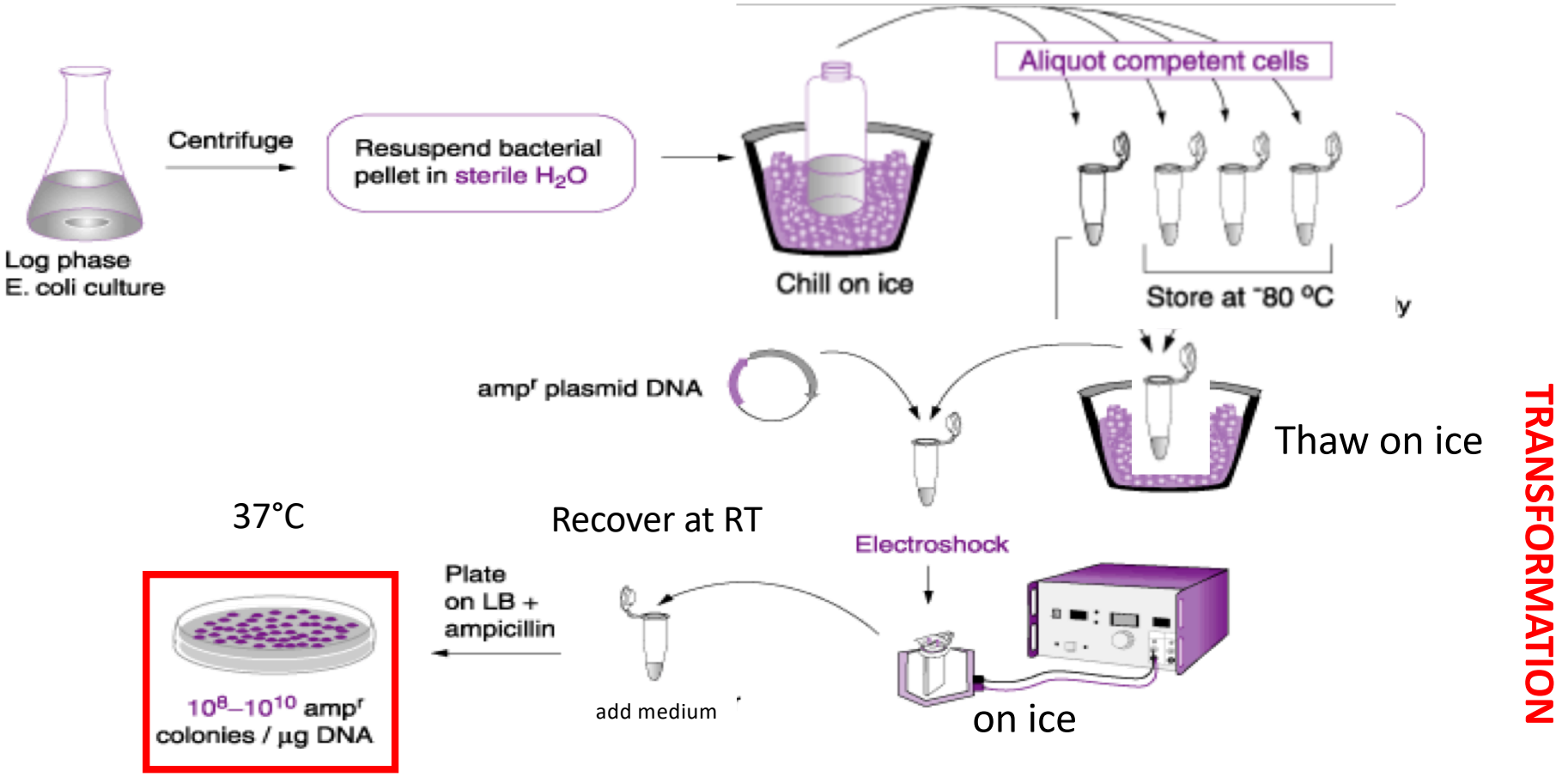



**Compent bacteria are put on ice until bacteria are thawn; add ligation product; induce heat shock (42°C); DNA can enter the bacteria;**

**add liquid media to allow bacteria to recover; plate on media plate containing amplicilin (37°C)**

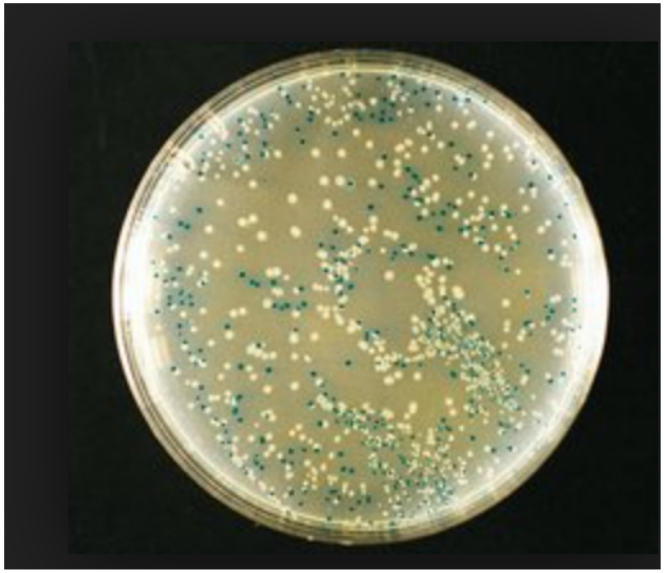
# TRANSFORMATION: TRANSFER OF PLASMID INTO BACTERIA

$H_2O$ and cold environment makes membrane permeable without killing the cells
= **ELECTROCOMPETENT BACTERIA**
(Can be stored at –80°C for years (normally >100 aliquots are prepared))




**Compent bacteria are put on ice until bacteria are thawn; add ligation product; induce electroshock; DNA can enter the bacteria;**
**add liquid media to allow bacteria to recover; plate immediately on media plate containing amplicilin**

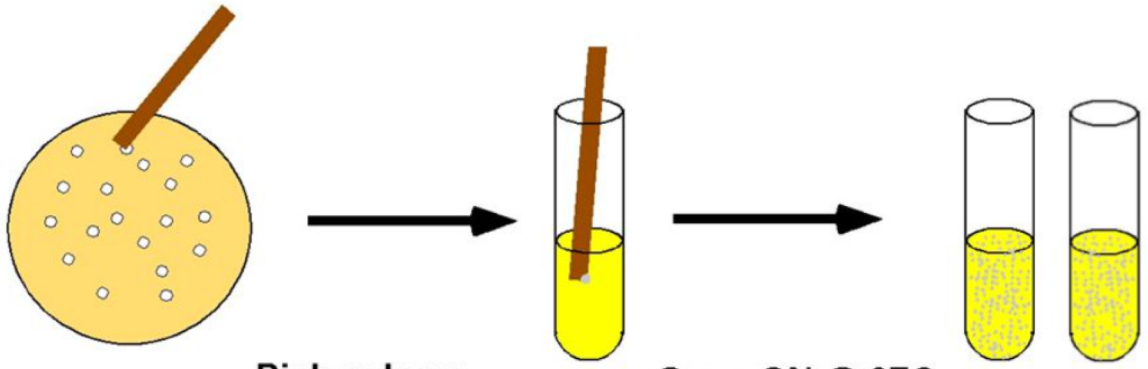
# DNA PREPARATION AND CONTROL DIGEST



In general: pick 6-10 white colonies with sterile pipette tip

## Preparation. Grow the bacteria

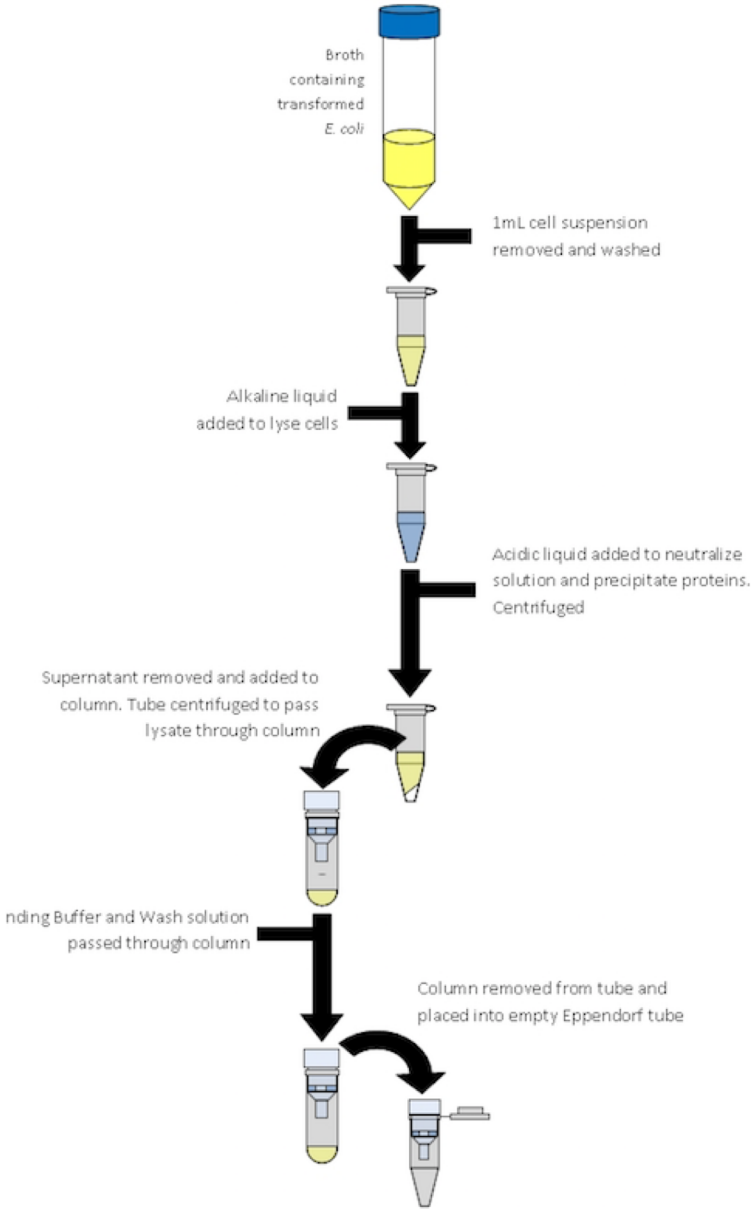
Grow an overnight (ON) culture of the desired bacteria in 2-5 ml of LB medium containing the appropriate antibiotic for plasmid selection. Incubate the cultures at 37°C with vigorous shaking.




Next day: harvest bacteria by centrifugation and prepare plasmid DNA

# 2. DNA PREPARATION AND CONTROL DIGEST

## Alkaline lysis with columns




1ml of overnight culture Removed, spinned and supernatant removed. Bacteria pellet resuspendet in buffer that does not kill cells

Note: Alkaline liquid: mix of NaOH and SDS if DNA is too long in solution with high pH: Hydolysis → destroyed

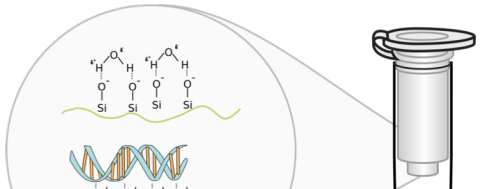
**The lysate is neutralized by the addition of acidic potassium acetate;** The high salt concentration causes Sodium dodecyl sulfate to precipitate, and the denatured proteins, chromosomal DNA, and cellular debris become trapped in salt–detergent complexes.

Plasmid DNA, being smaller and covalently closed, renatures correctly and remains in solution
**Centrifugation an high speed** (ca. 13.000 rpm); cell debries and genomic DNA precipitate; small DNA molecules (plasmid remain in supernatant)

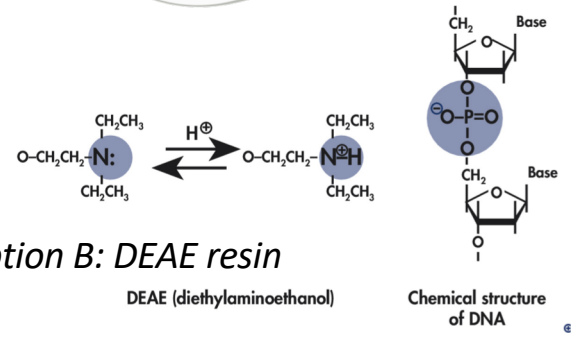
At high salt conditons at low pH (6,5), phosphosugar backbone binds silica resin

### *Option A: Silica resin*

irregular tridimensional framework of alternating silicon and oxygen atoms ($SiO_2$) with nanometer-scale voids and pores

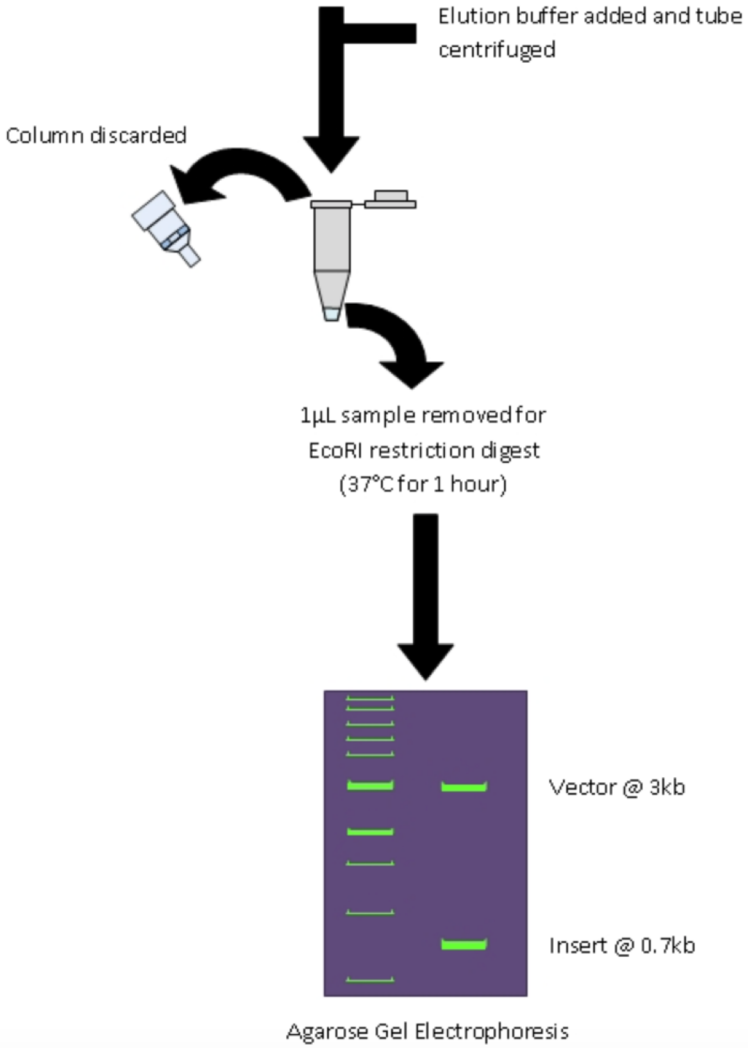


### *Option B: DEAE resin*




Resin in column is positivley charged: Binds negative charge of plasmid DNA backbone

# 2. DNA PREPARATION AND CONTROL DIGEST

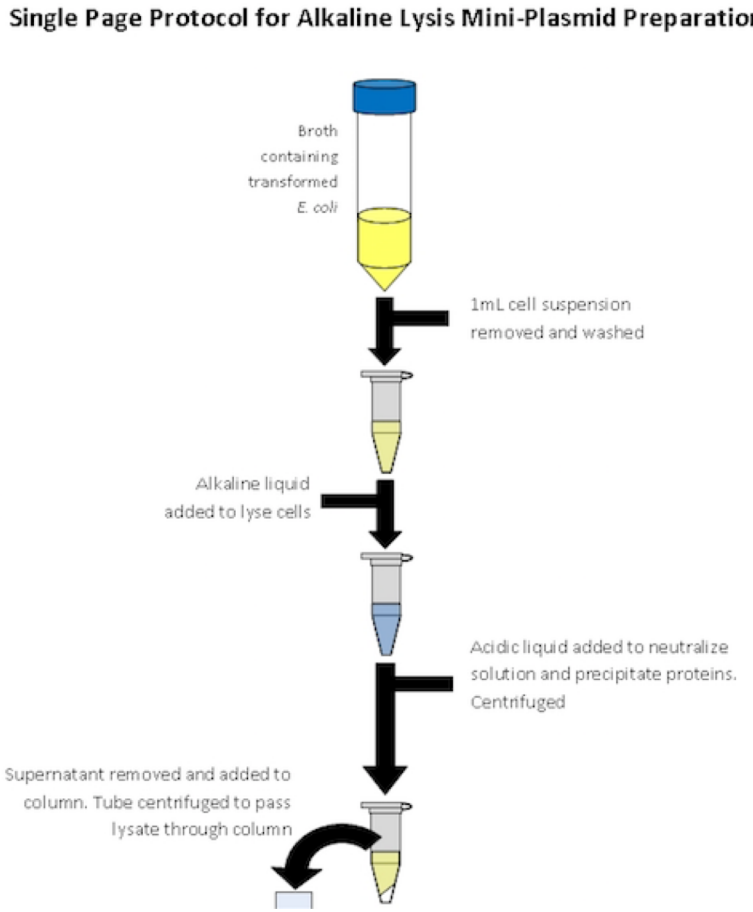



**Elution of DNA at low salt conidtions and at increase pH (7,0)**

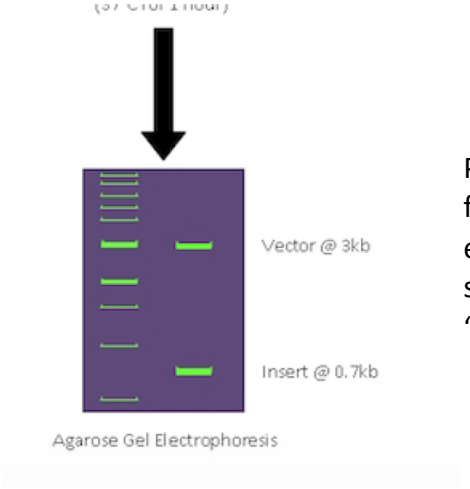
The use of columns results in very pure plasmid DNA.
"**sequence grade**"

# 2. DNA PREPARATION AND CONTROL DIGEST

## Alternative method without columns




- put supernatant in new tube
- add salt (final 0,5M NaCl)
- add Isopropanol
- put at -20C for 1 hour
- centrifuge
- plasmid DNA will preciptate




Plasimid is not very clean; sufficent for digestion with restriction enzyemes; not usable for DNA sequencing
"**not sequencing grade**"

→ **Much cheaper; you can test many colonies for correctness of plasmid**
→ **Takes some more time**