

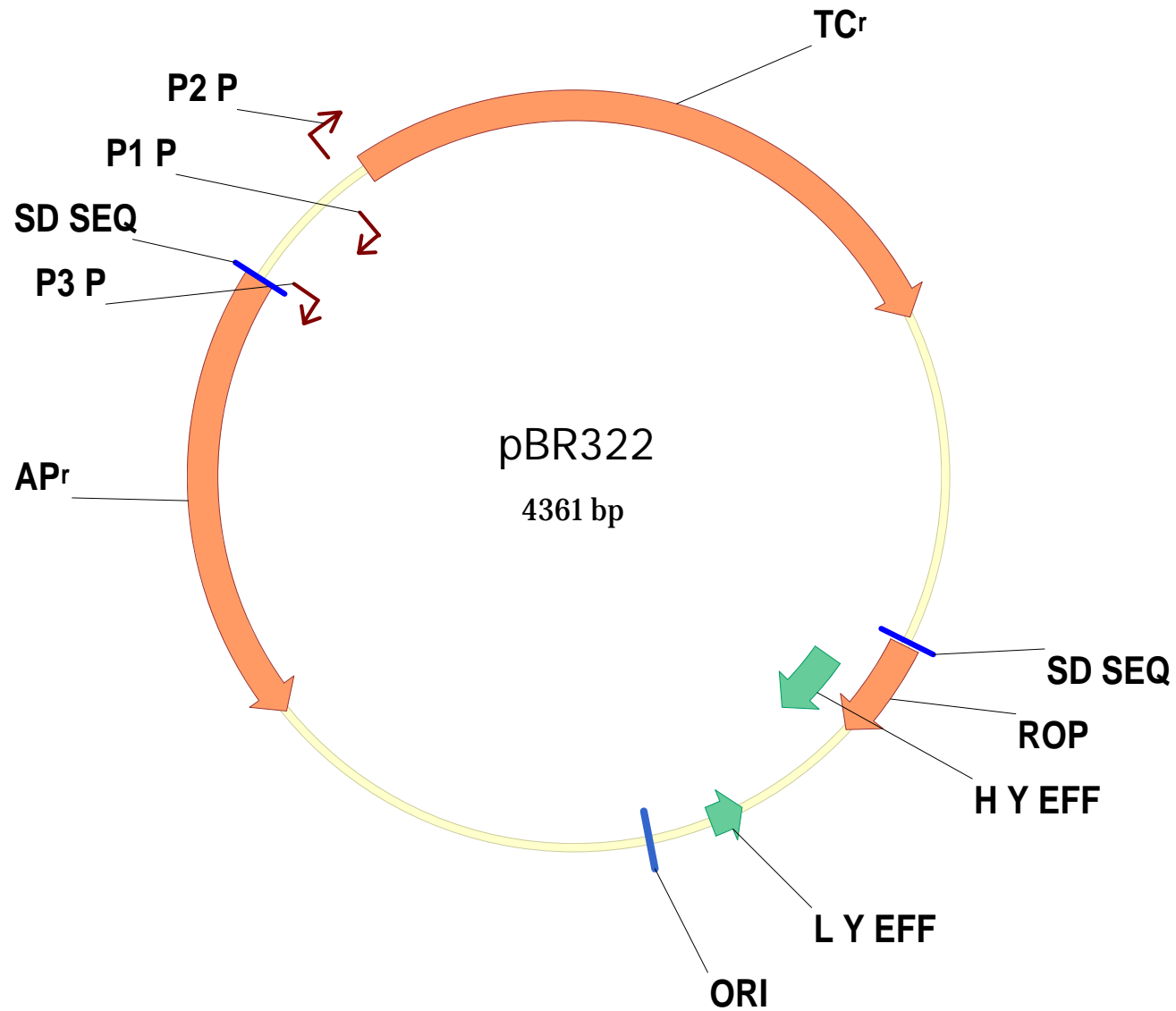


# *Principles behind plasmid purifications*

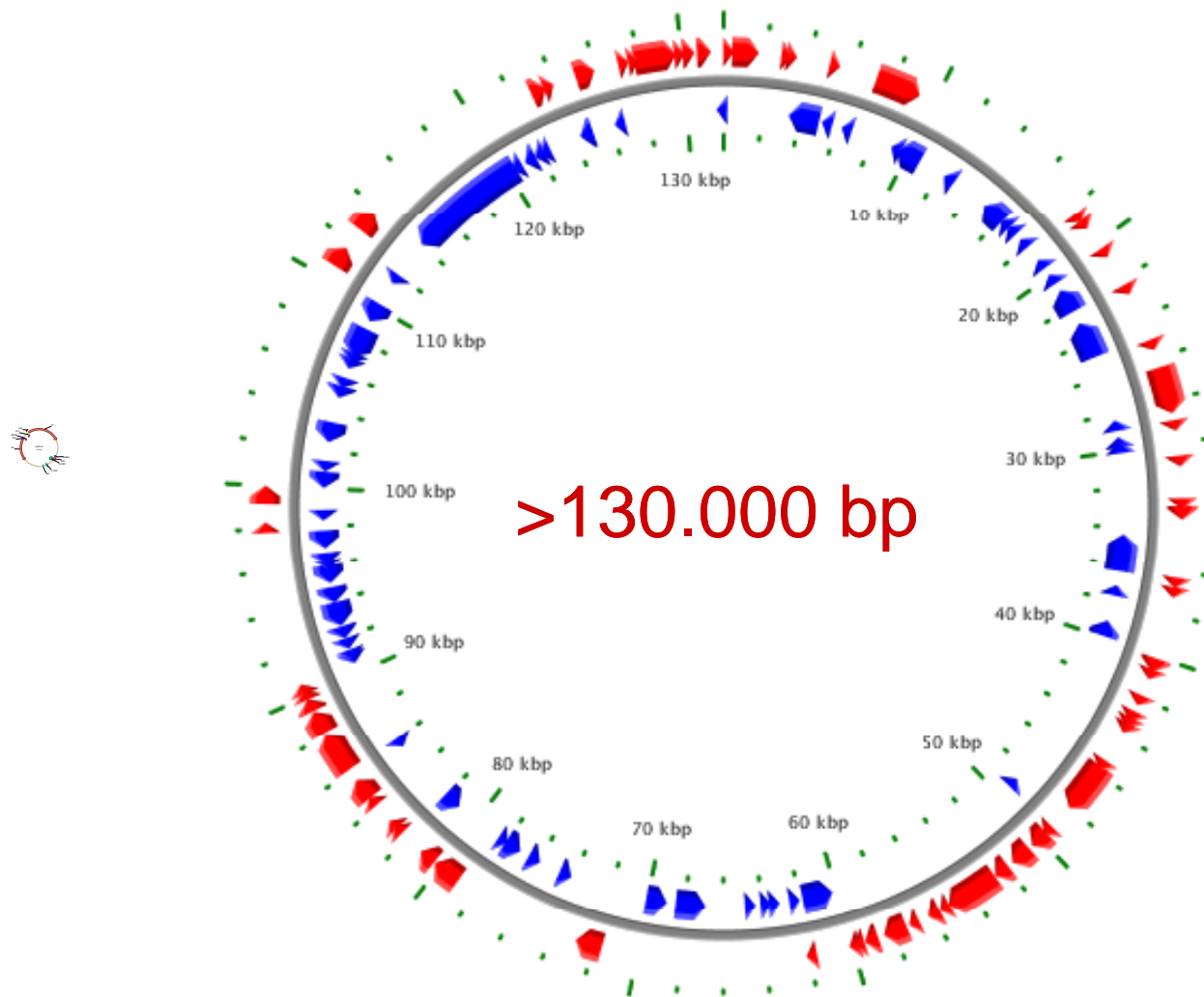
Senior scientist Henrik Hasman, DTU – National Food Institute

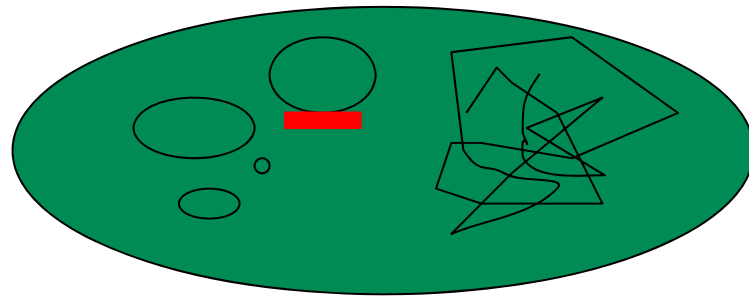
## Genome sizes

- How big is bacterial chromosome?  
1,500,000 - 5,000,000 ( $10^6$ ) bases
- How big is the human chromosome?  
3,300,000,000 ( $10^9$ ) bases
- How big is a plasmid?  
2,000 – 400,000 ( $10^3$  -  $10^5$ ) bases

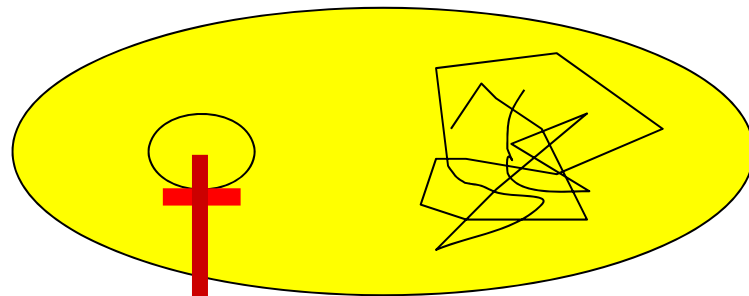


*Pseudomonas syringae* pv. *phaseolicola* 1448A large plasmid, c...





Conjugation or  
Electroporation



Selection on relevant antibiotic

Purify for further work

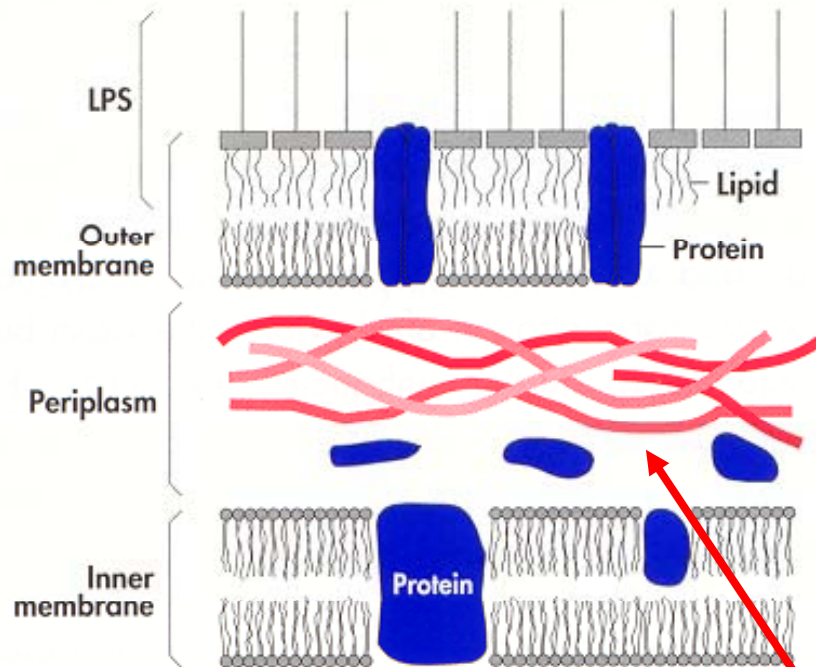
## The principle behind plasmid purifications $G^-$

- Alkaline lysis of cells  $\longrightarrow$  NaOH + SDS (+ RNase)
- Neutralization and precipitation of debris  $\longrightarrow$  KAc + spin
- Binding to resin
- Wash and elution of DNA  $\longrightarrow$  buffer
- Concentration of DNA  $\longrightarrow$  Isopropanol + spin

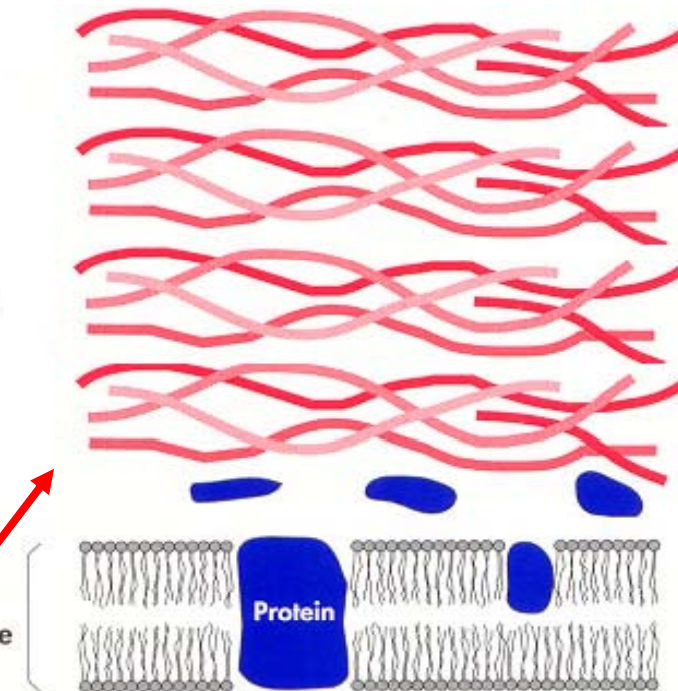


# Bacterial cell wall

## Gram negative



## Gram positive

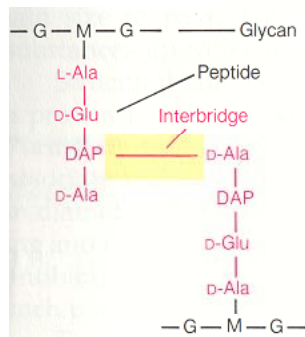
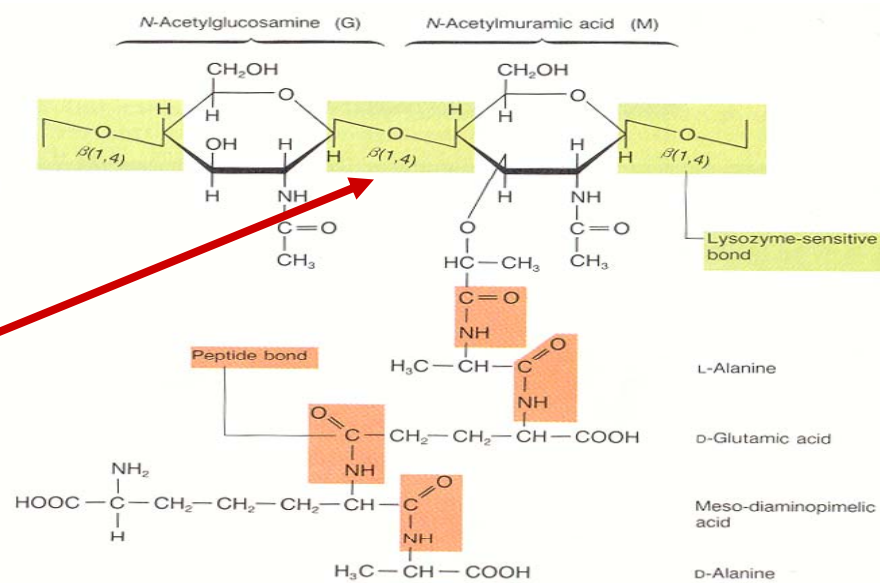


Peptidoglycan

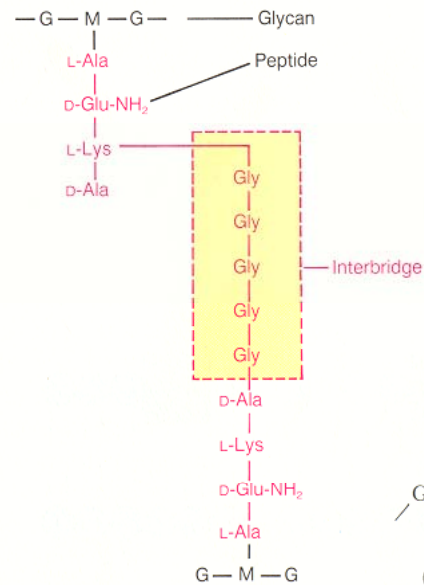
## The principle behind plasmid purifications $G^+$

- Degrading of cell wall  $\longrightarrow$  Lysosyme
- Alkaline lysis of cells  $\longrightarrow$  NaOH + SDS (+ RNase)
- Neutralization and precipitation of debris  $\longrightarrow$  KAc + spin
- Binding to resin
- Wash and elution of DNA  $\longrightarrow$  buffer
- Concentration of DNA  $\longrightarrow$  Isopropanol + spin

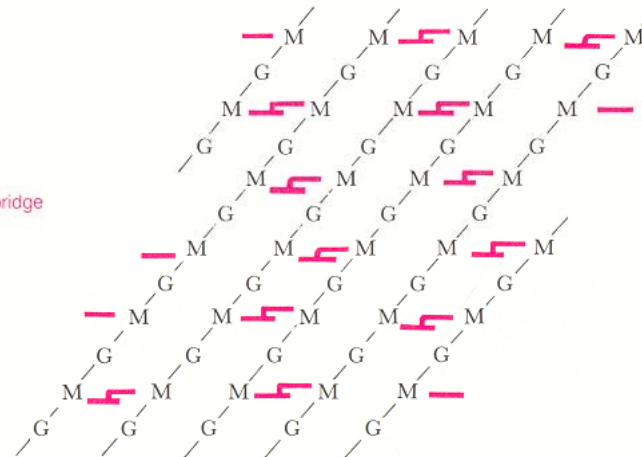
# Lysosyme



(a) *Escherichia coli*  
(Gram negative)



(b) *Staphylococcus aureus*  
(Gram positive)



(c)



# Alkaline lysis of the cells

Grow bacteria in antibiotics (to preserve the plasmid)

Centrifuge the cells and then:

- Resuspend pellet in P1 buffer (TE-buffer + RNase)

RNase removes the RNA (at a later stage)

- If Gram<sup>+</sup>: add lysosyme or lysostaphin (*Staphylococcus*)

Lysosyme/lysostaphin destroys the cell wall

- Add P2 buffer (NaOH + SDS)

SDS destroys the cell membrane

NaOH denatures the DNA

- Finally: add P3 buffer (KAc)

KAc renatures plasmid DNA (neutral pH)

Precipitates KDS containing "cell debris" and chromosomal DNA

## Plasmid size influences the choice of plasmid kit!

### Plasmid < 15 kb (relatively small plasmid)

- Qiagen spin miniprep kit (27104/27106) only Gram<sup>-</sup>
- Qiagen plasmid Mini kit (Tip-20) + lysosyme/staphin to Gram<sup>+</sup>
- Qiagen plasmid Midi kit (Tip-100) + lysosyme/staphin to Gram<sup>+</sup>
- Nucleobond AX + lysosyme/staphin to Gram<sup>+</sup>

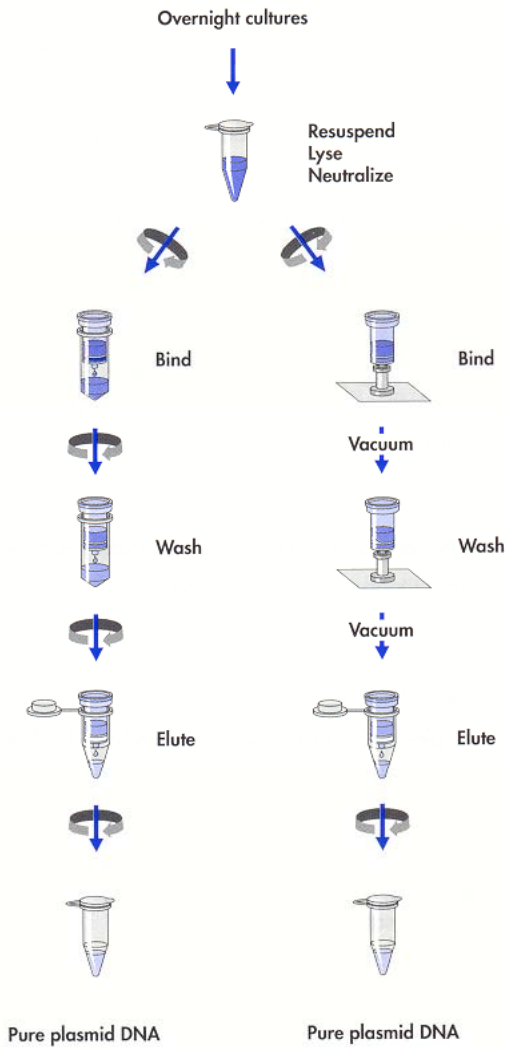
### Plasmid > 15 kb (relatively large plasmid)

- Qiagen plasmid Midi kit (TIO-100) + lysosyme/staphin to Gram<sup>+</sup>
- Nucleobond AX + lysosym/staphin to Gram<sup>+</sup>

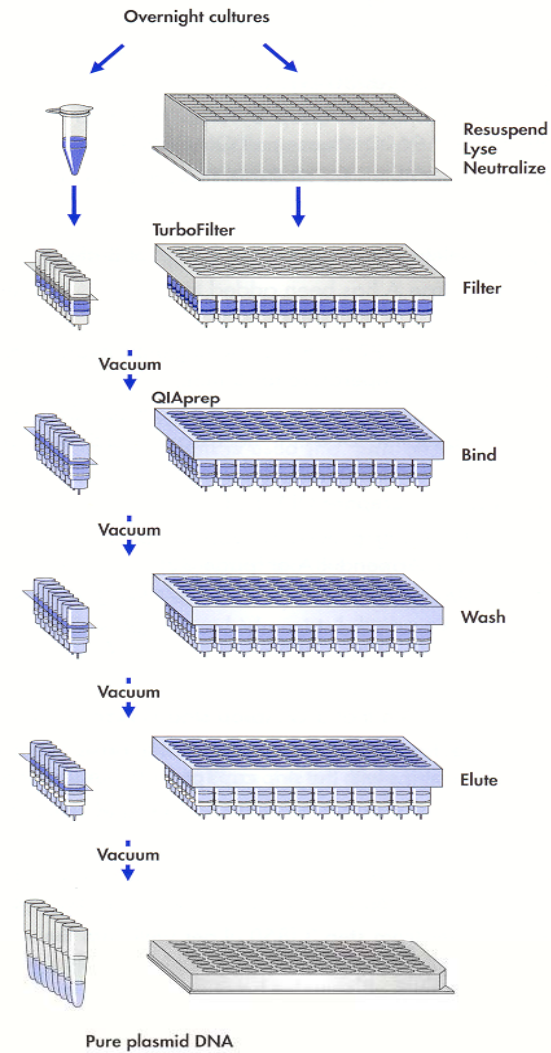


# Qiagen spin miniprep kit

## QIAprep Spin Procedure in microfuges      on vacuum manifolds

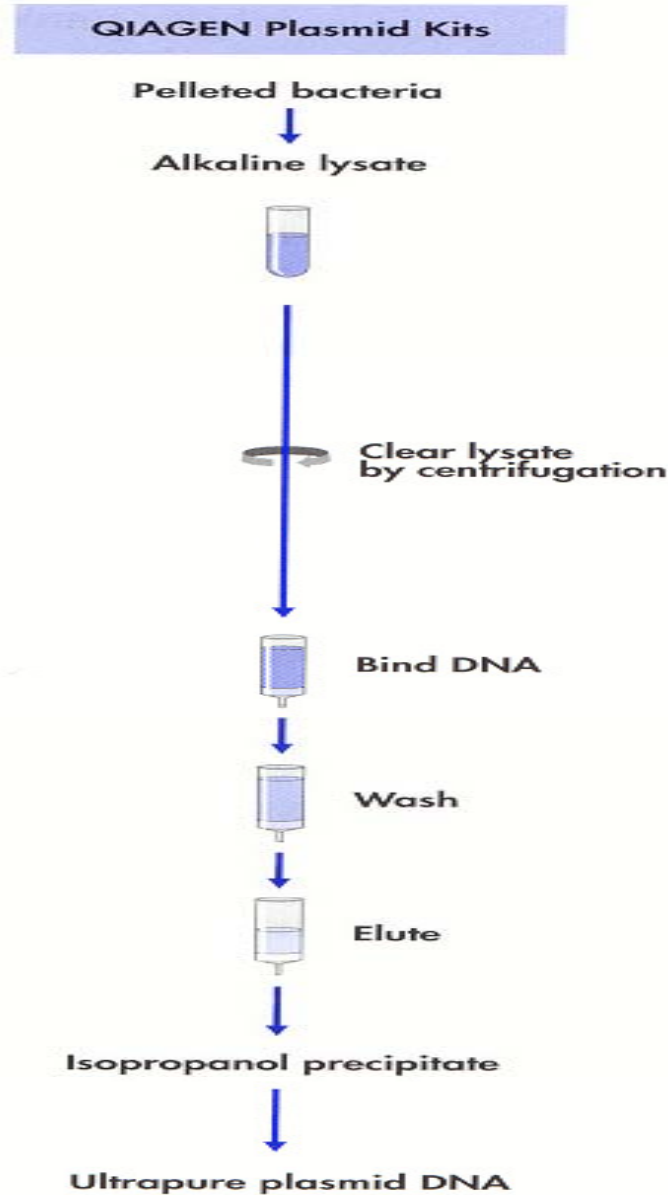


## QIAprep 8 and 96 Turbo Procedure

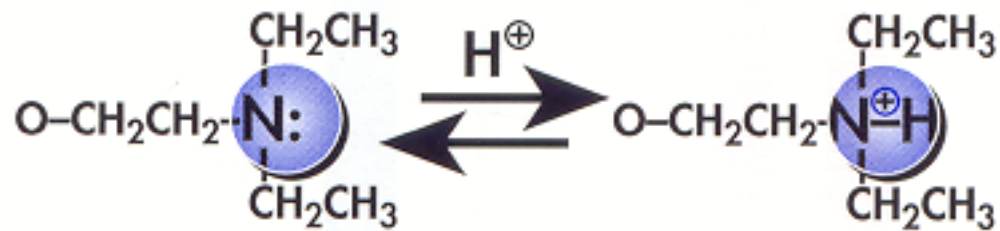




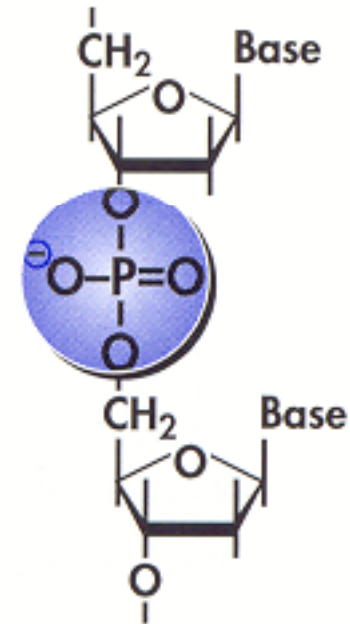
# Qiagen plasmid Midi kit



## Qiagen Resin

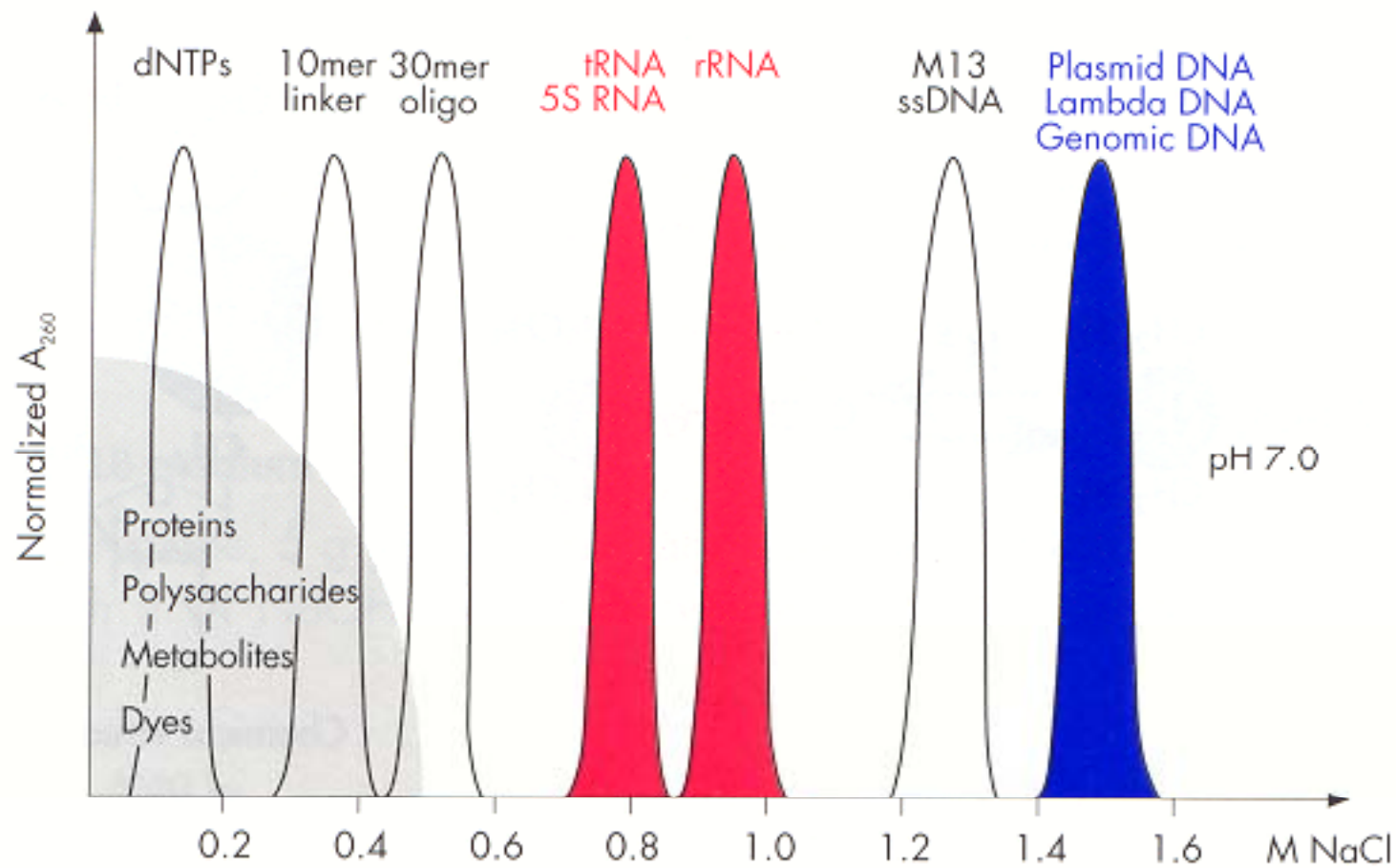


DEAE (diethylaminoethanol)



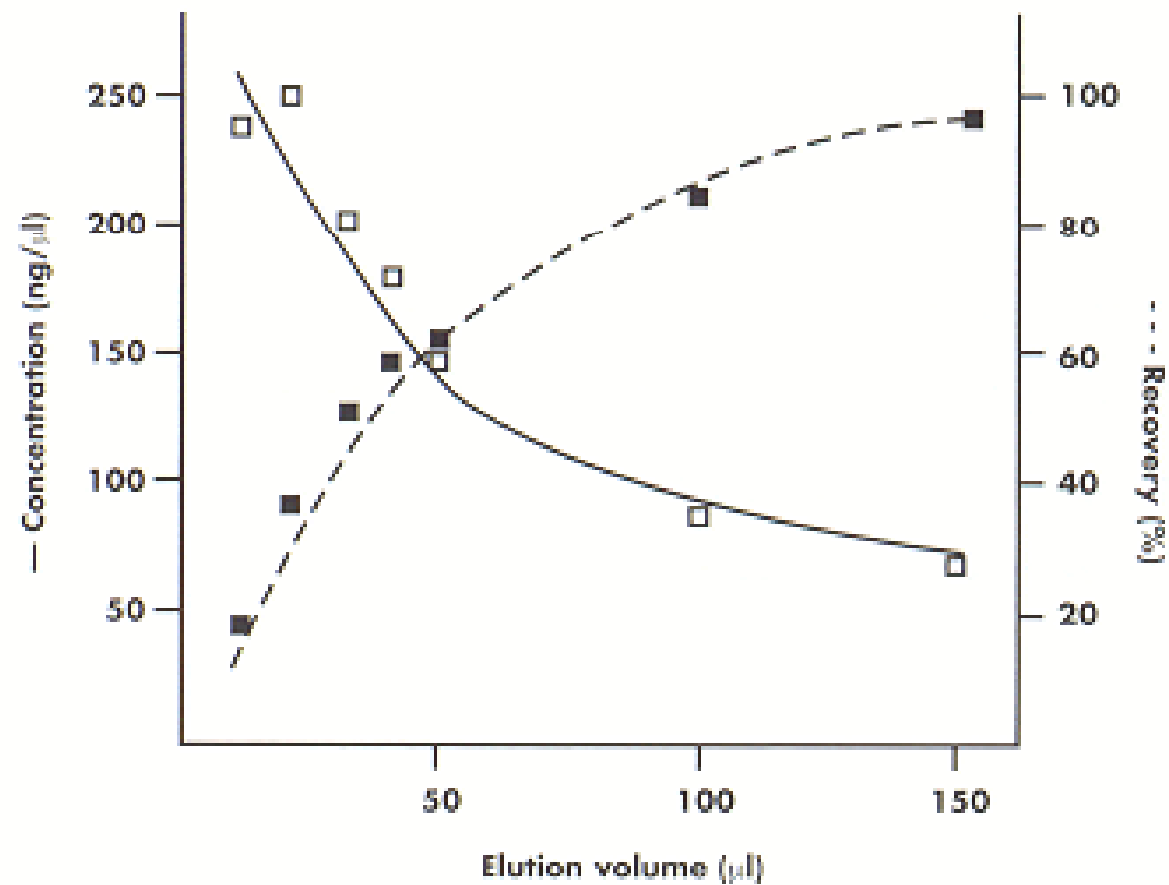
Chemical structure  
of DNA

## Wash and elution



# Elution

## QIAprep Spin



## Precipitation of DNA

Requires alcohol and salt.

The elution buffer already contains salt!

### Isopropanol precipitating:

0,7 volume + 0,1 volume 3 M Natrium Acetat (pH 4,8)

5 ml sample =>  $0,7 * 5 = 3,5$  ml Isopropanol and (0,5 ml NaAc)

### Ethanol precipitation:

2 volume + 0,1 volume 3 M Natrium Acetat (pH 4,8)

5 ml sample =>  $2 * 5 = 10$  ml Ethanol (and 0,5 ml NaAc)

Then spin at 9.600 g for 30 minutes

Carefully wash with 70 % EtOH and spin again for 2 minutes

# Comparison

## Qiagen spin miniprep kit

Gram<sup>-</sup>

< 15 kb

10 - 20 µg DNA

30 - 60 minutes

20 (96) samples pr run

App. 20 kroner/pr collum

## Qiagen plasmid Midi kit

Gram<sup>+</sup> and Gram<sup>-</sup>

Up to 200 kb

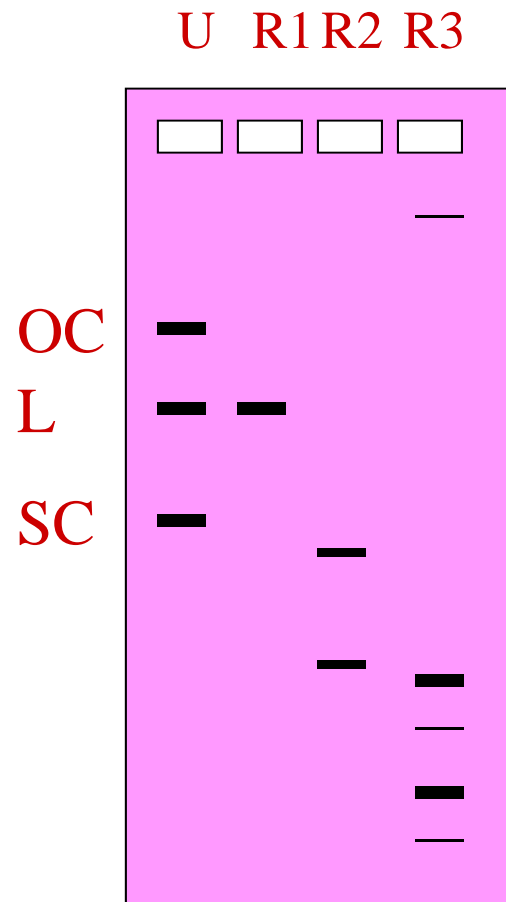
100 µg DNA

3 - 6 hours

6 - 12 samples pr run

App. 50 kroner /pr collum

# Downstream processing of the DNA



## Pitfalls:

- Large plasmids has a low copy number
- The strain can contain both large and small plasmids
- The restriction enzyme does not cut
- The restriction enzyme cut too much
- The method does not apply to this strain
- The strain does not contain a plasmid



1 2 3 4 5 6 7 8 9 10 11 12 13

