

3 其它純化或分離方法 Other purification methods

3.1 製備式電泳 Preparative electrophoresis

蛋白質色帶由原態電泳中直接切除出來

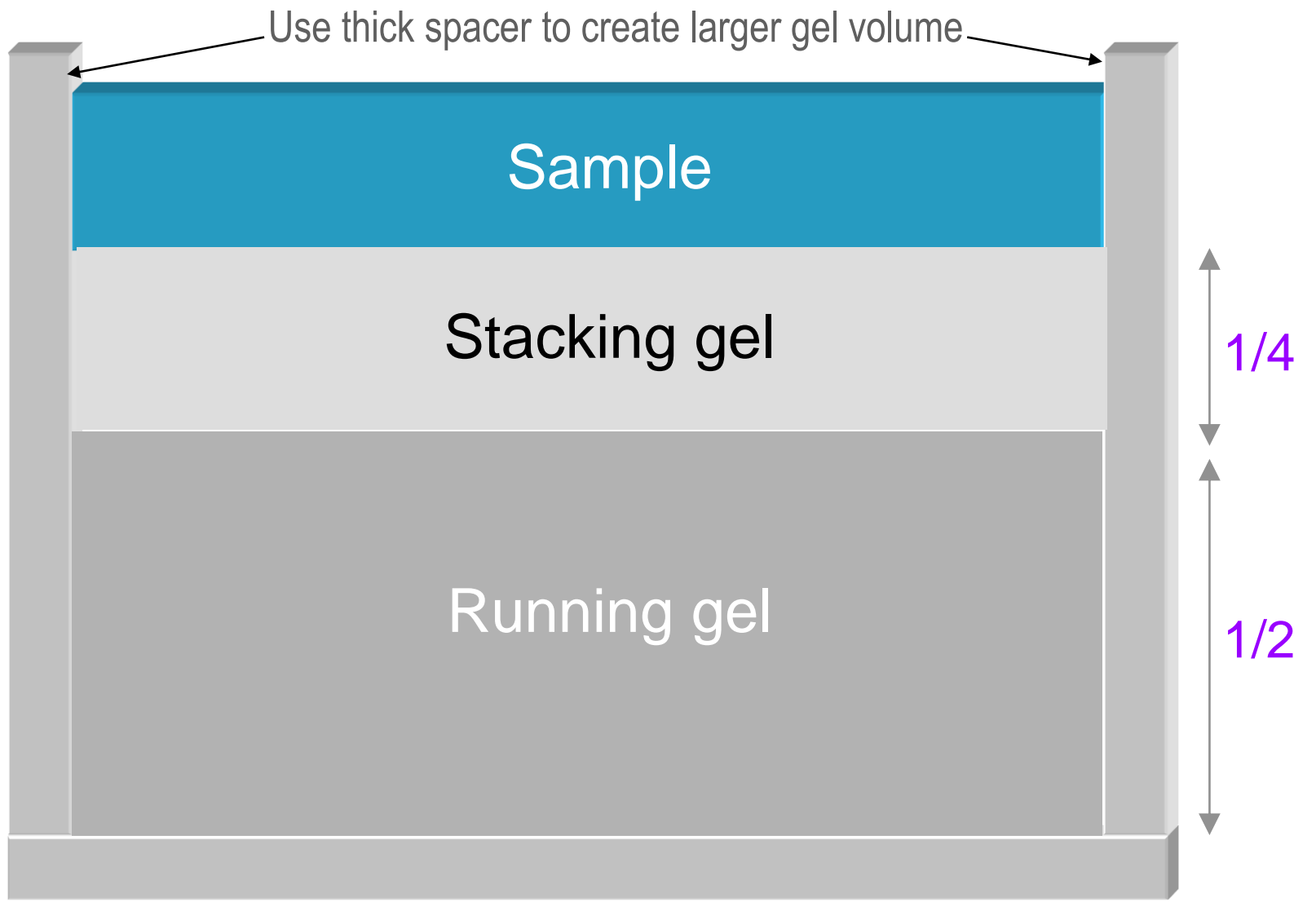
3.2 超高速離心法 Ultracentrifugation

以各種分子的沉降係數不同來進行分離

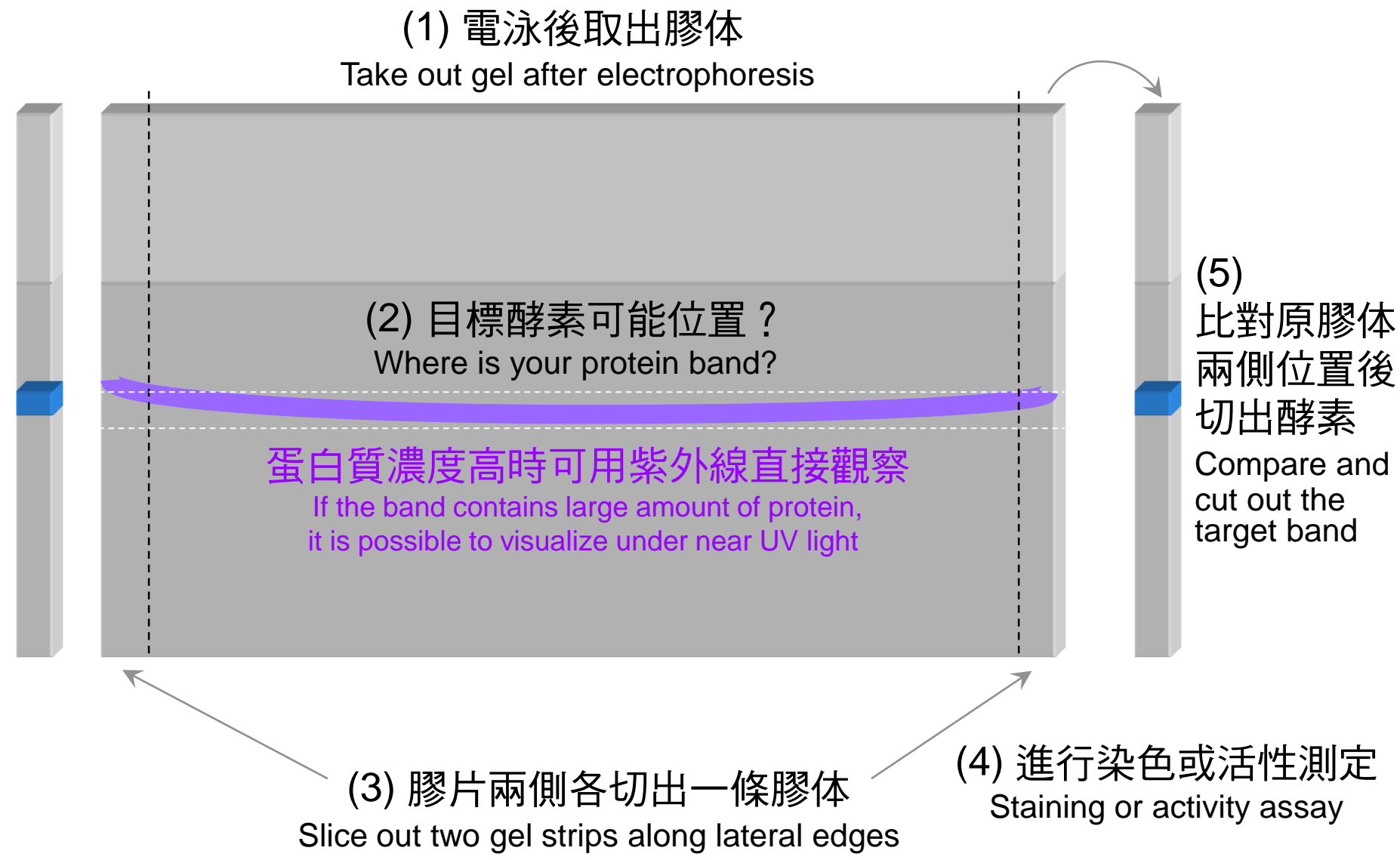
3.3 超微薄膜過濾法 Ultrafiltration

超微薄膜可以用來脫鹽及濃縮蛋白質

3.1 製備式電泳膠片 Preparative gel format



■ 製備式電泳操作 Detect protein band on the gel



3.2 超高速離心 Sedimentation coefficient



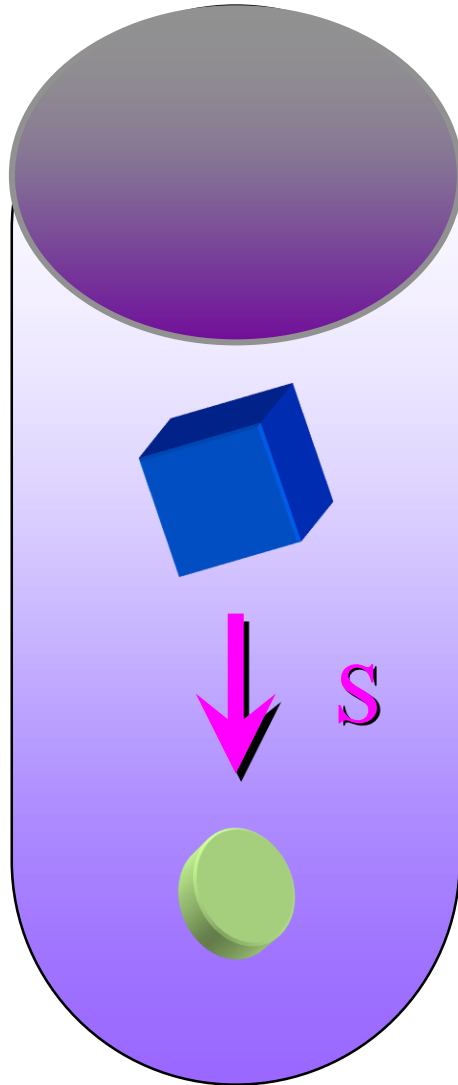
4

Svedberg unit

粒子在密度梯度離心時的沈降速率

The sedimentation velocity of a particle when it is centrifuged in a density gradient

密度梯度



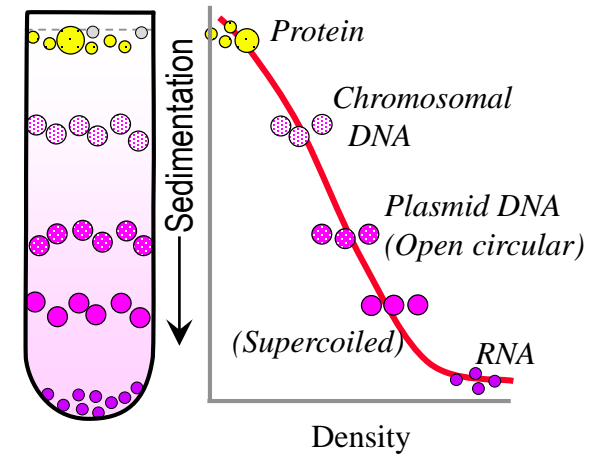
分子量 molecular weight

分子密度 molecular density

分子組成 molecular composition

分子形狀 molecular shape

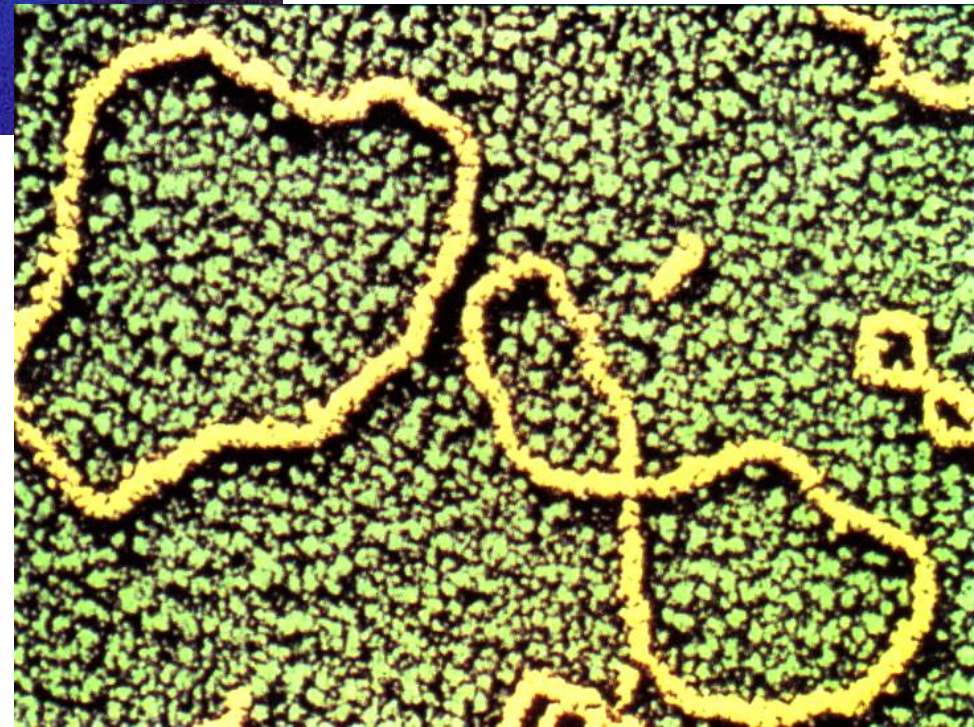
CsCl gradient ultracentrifugation



以超高速離心大量製備質體

Ultracentrifugation is used to prepare plasmid in large scale

Plasmid DNA
(Open circular)



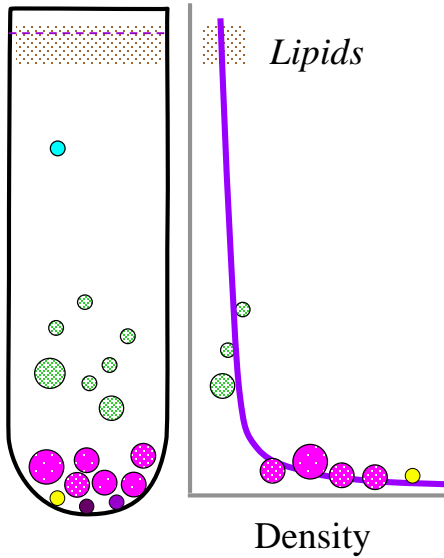
■ 兩種超高速離心比較 Two ultracentrifuge types

Centrifuge	Sedimentation Velocity	Sedimentation Equilibrium
also called →	Zone Centrifugation	Isopycnic Equilibration
Gradient formation	Precast (sucrose, glycerol)	During centrifugation (CsCl)
	Shallow gradient, lower density	Steep gradient, higher density
Suitable samples	Similar density, different MW	Similar MW, different density
	Protein	Nucleic acid / cell organelle
Centrifugation conditions	Lower speed, not complete sedimented, stop at proper time	Completely sediment to where the density is equilibrated, high speed, long running time
中文名稱	區帶離心法	等密度平衡離心法

各種高速離心法比較 Comparison of centrifuges

High speed

Gravity Centrifugation
(No density)



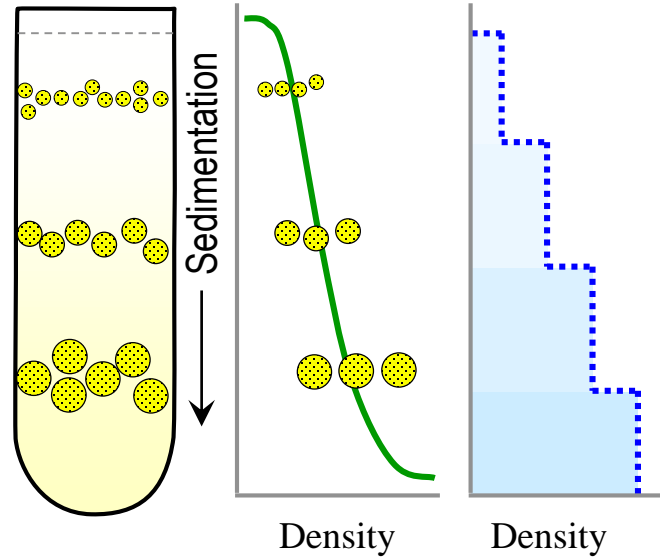
一般的重力離心僅把顆粒與溶液分離開來

Utilize gravity force to separate particles from the solution

Ultracentrifugation

Zone Centrifugation

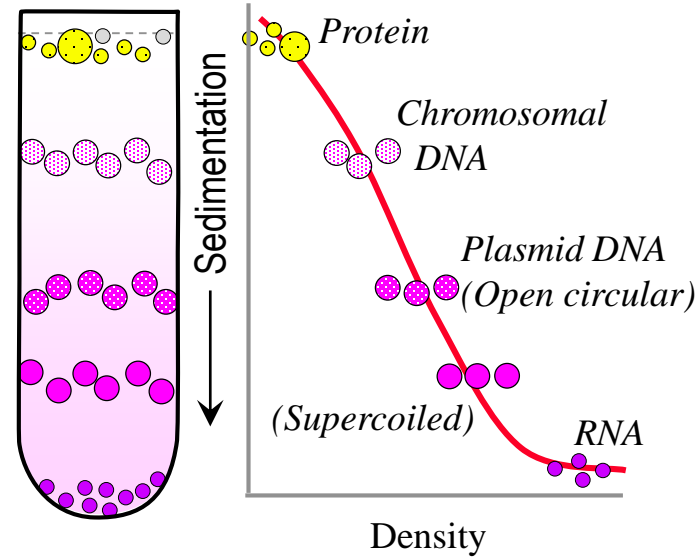
(Precast) → (step-wise)



樣本：多為蛋白質
密度相似、分子量不同者

Sample: protein (similar density, but different in MW)

Isopycnic Equilibration
(CsCl gradient forming)

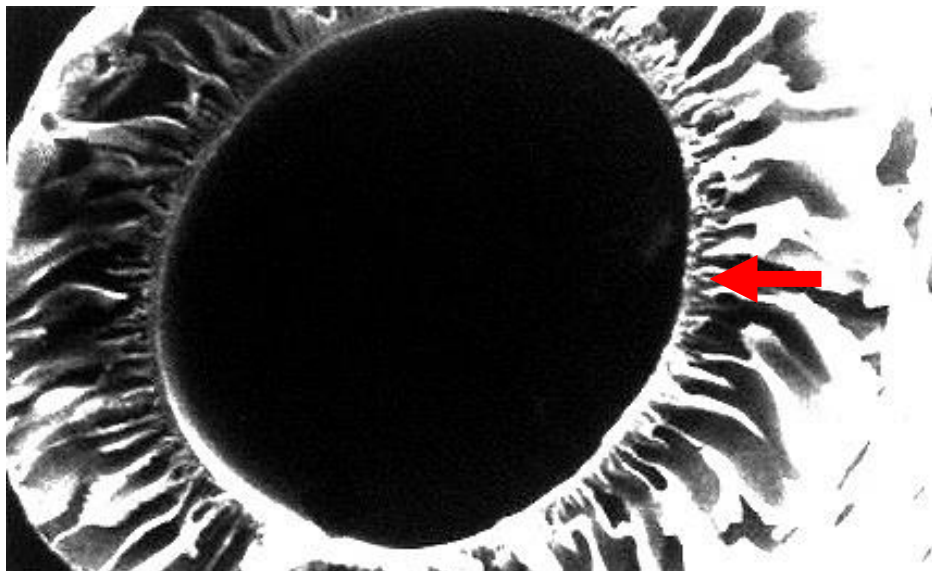
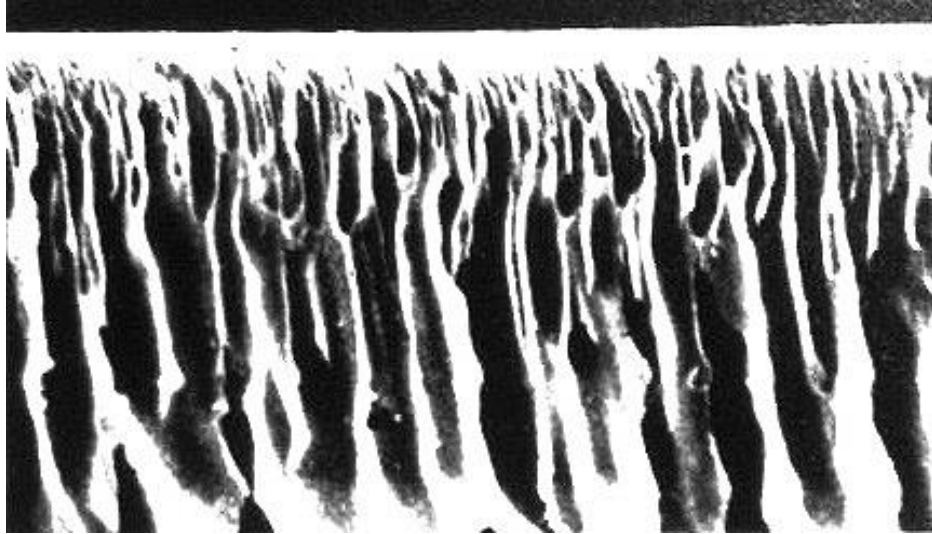
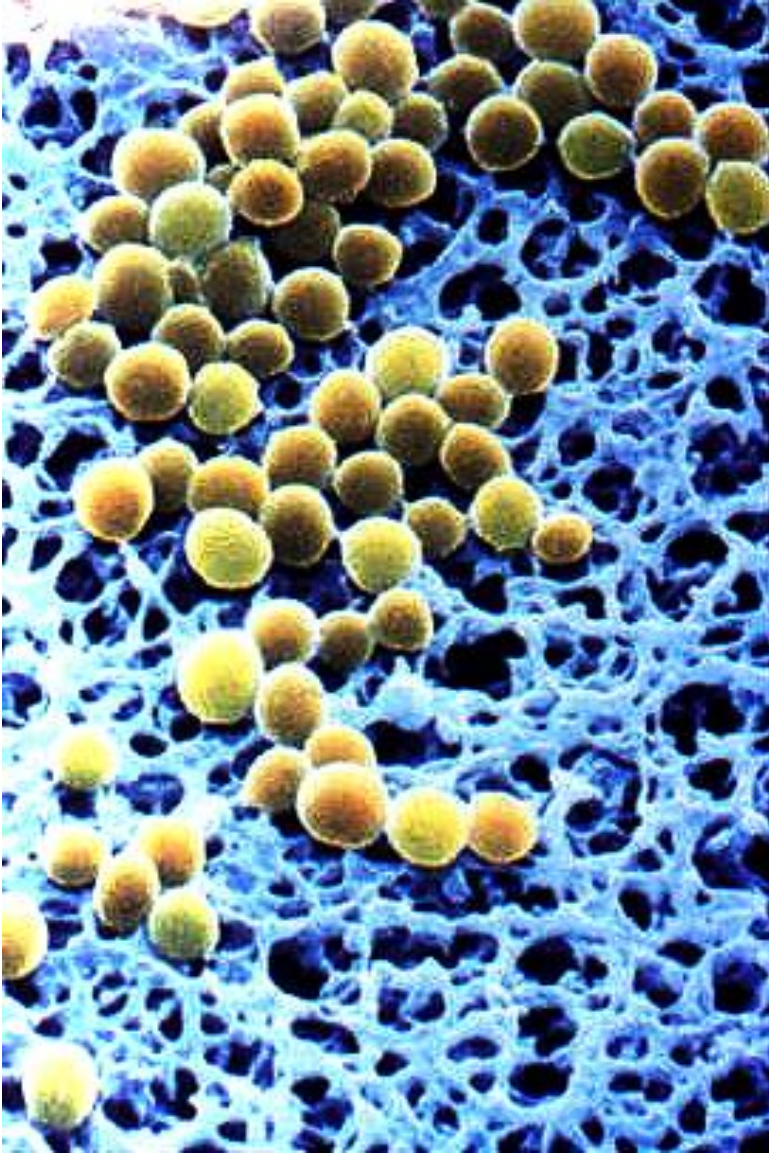


樣本：多為核酸
密度不同、分子量相似者

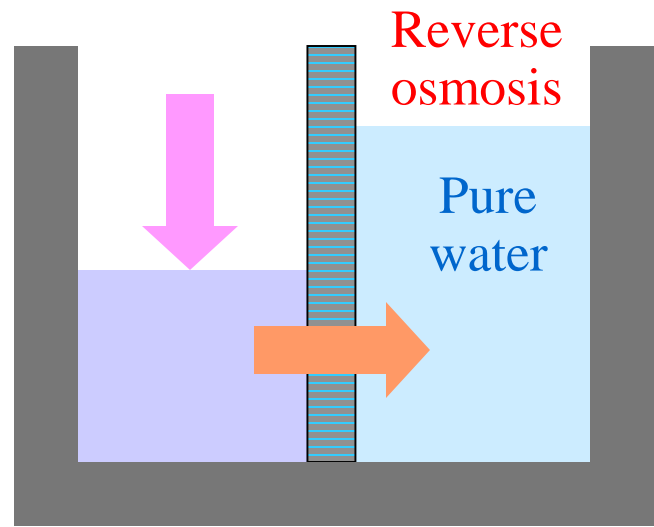
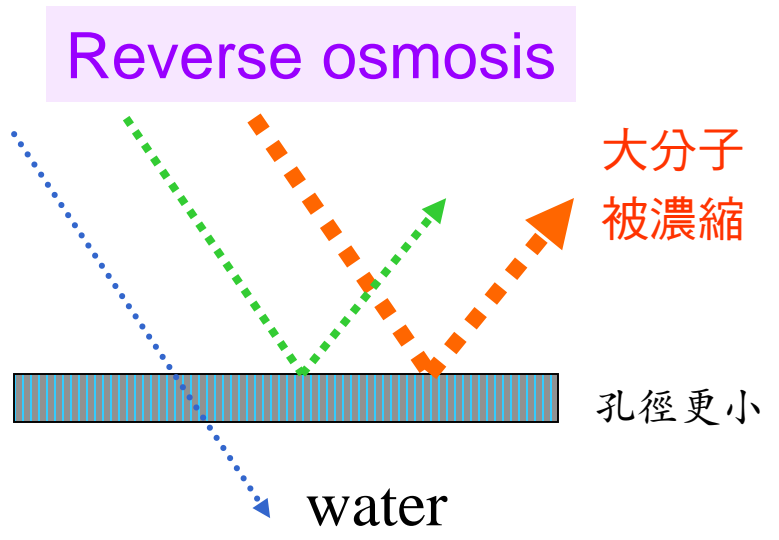
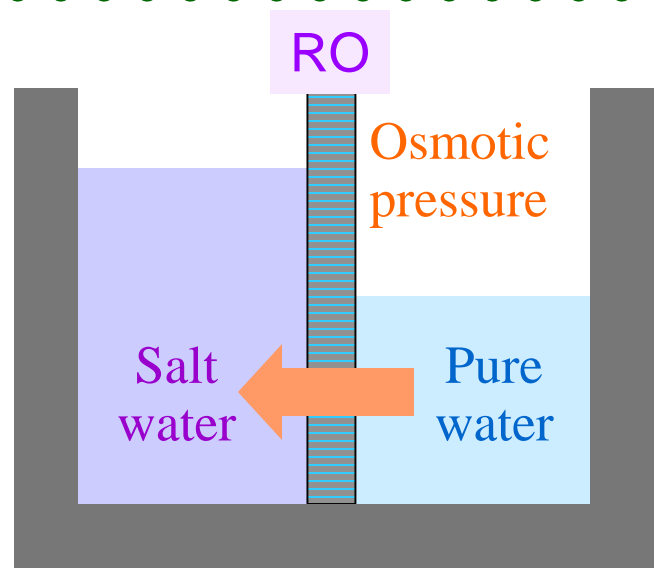
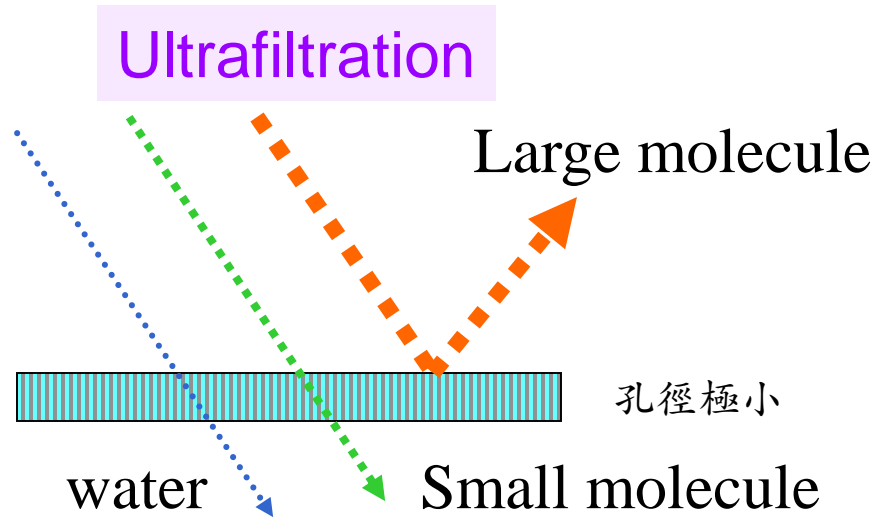
Sample: nucleic acid (similar MW, but different in density)

Lower density ↔ Higher density

3.3 超微薄膜技術 Ultrafiltration technology



超微薄膜及逆滲透 Ultrafiltration and RO

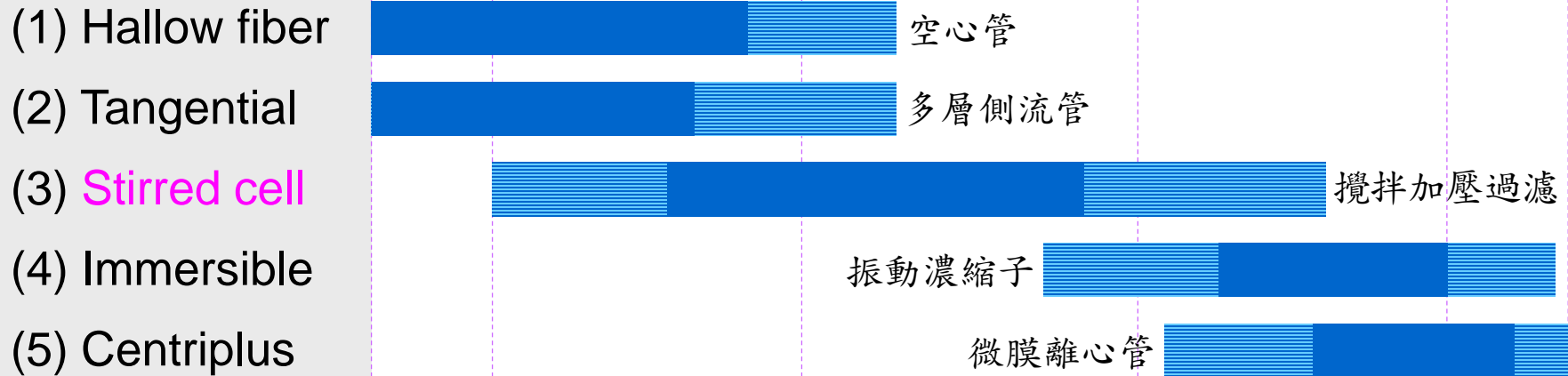


各種濃縮方法的使用範圍 Useful ranges

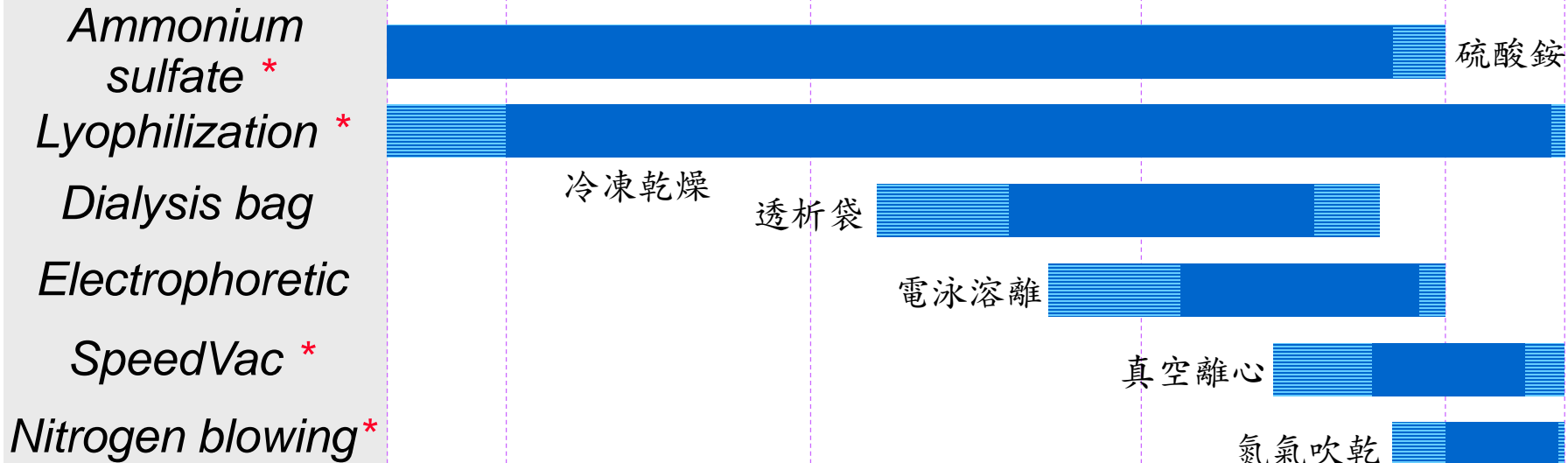
Applications

10 L - 1 L - 500 mL - 100 mL - 50 mL - 10 mL - 5 mL - 1 mL

Ultrafiltration

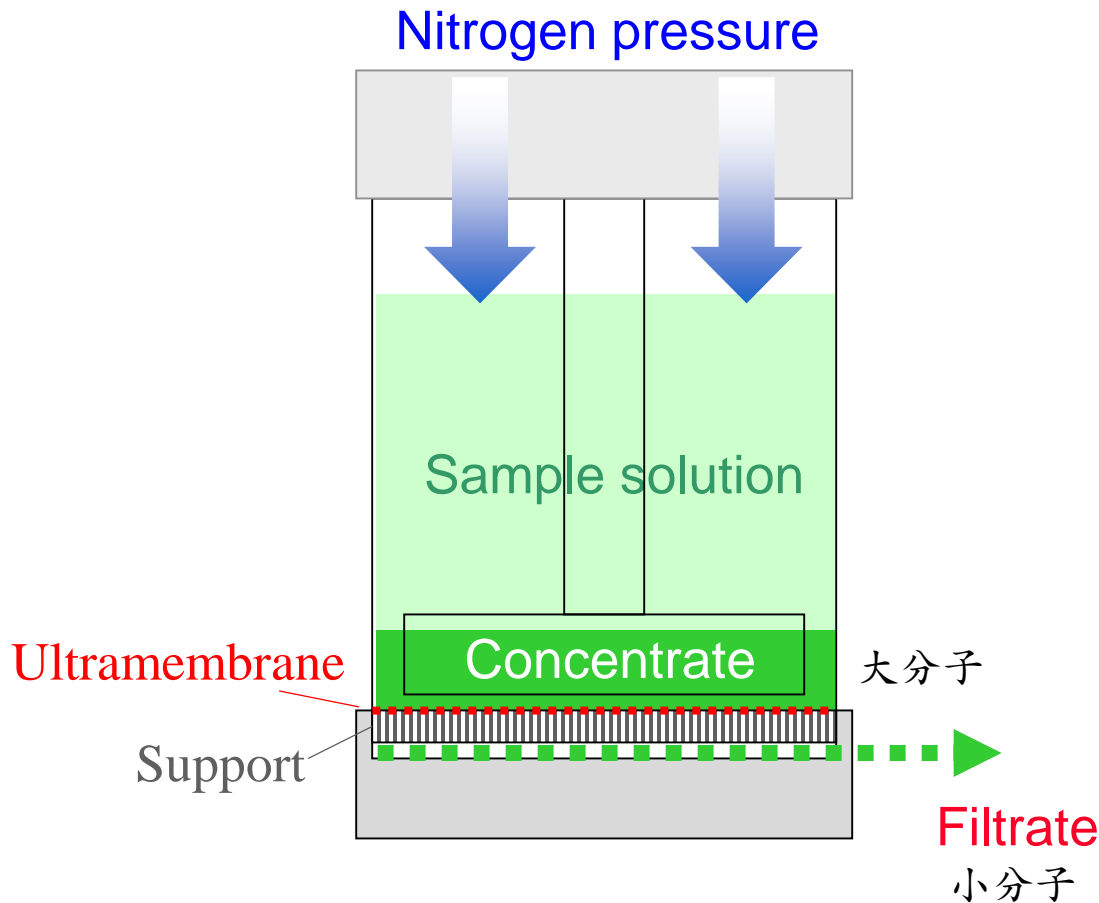


Other methods



* The salt concentration increases in the sample

超微薄膜濃縮裝置 - Stirred cell



Amicon Stirred Cells