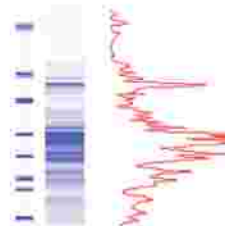
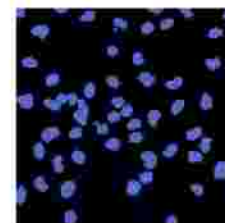


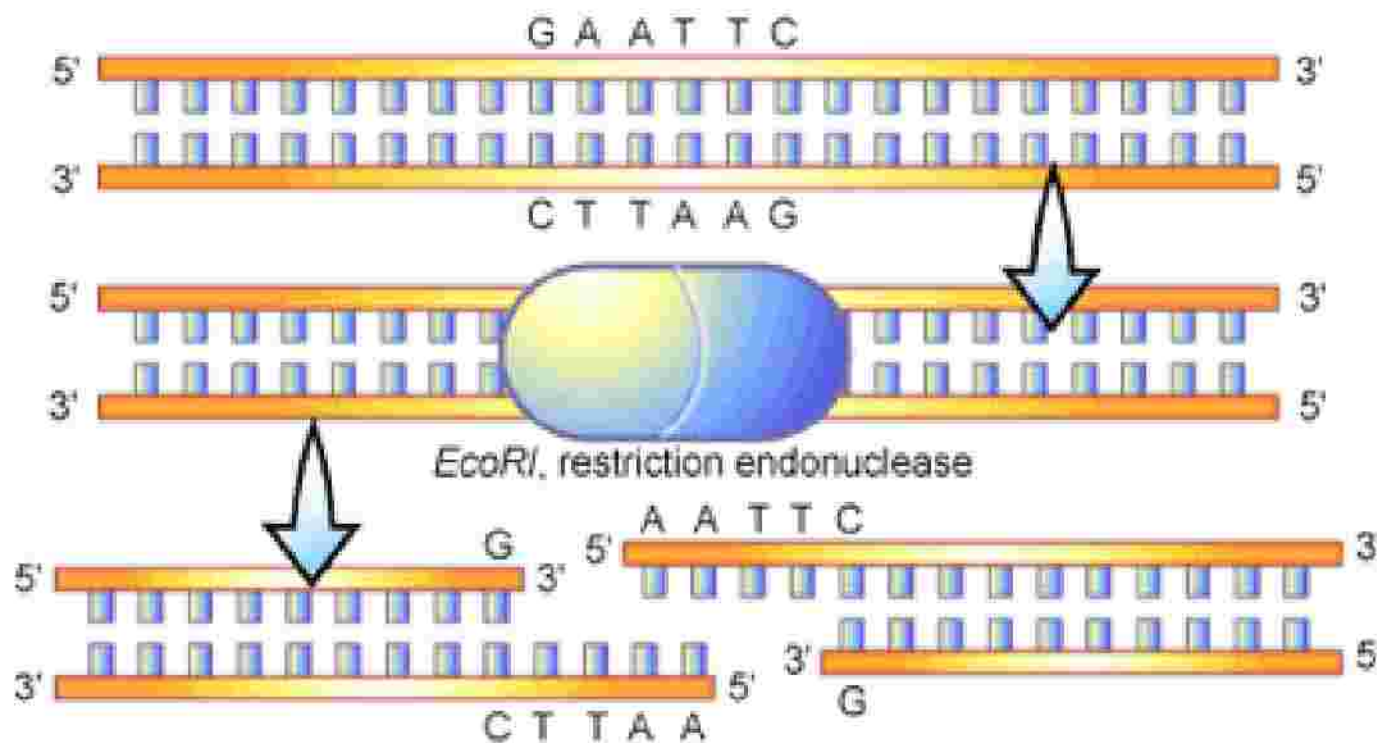
限制？處理及跑膠





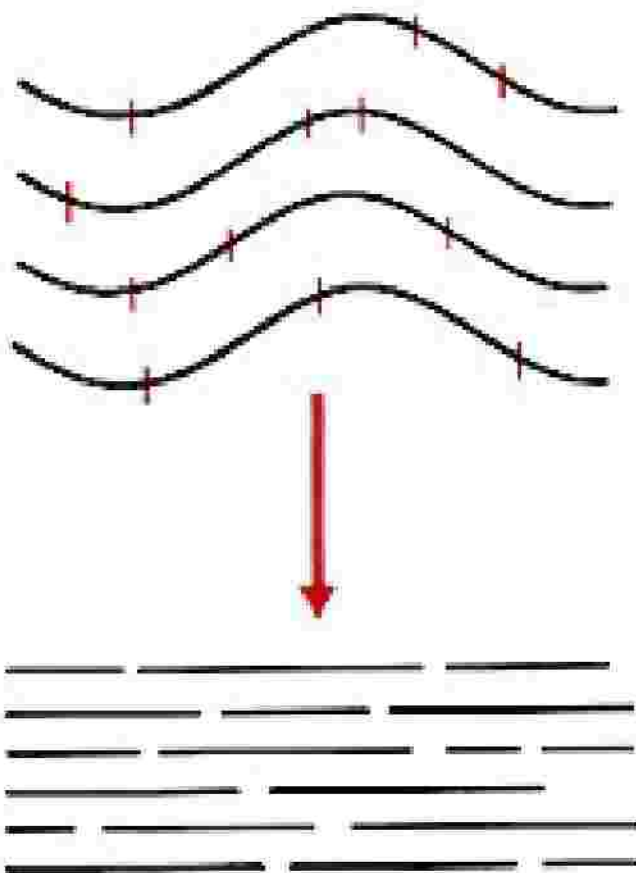
限制?

Restriction enzymes **DNA-cutting enzymes**





Genome digest with Restriction enzymes



Separate fragments with PFGE



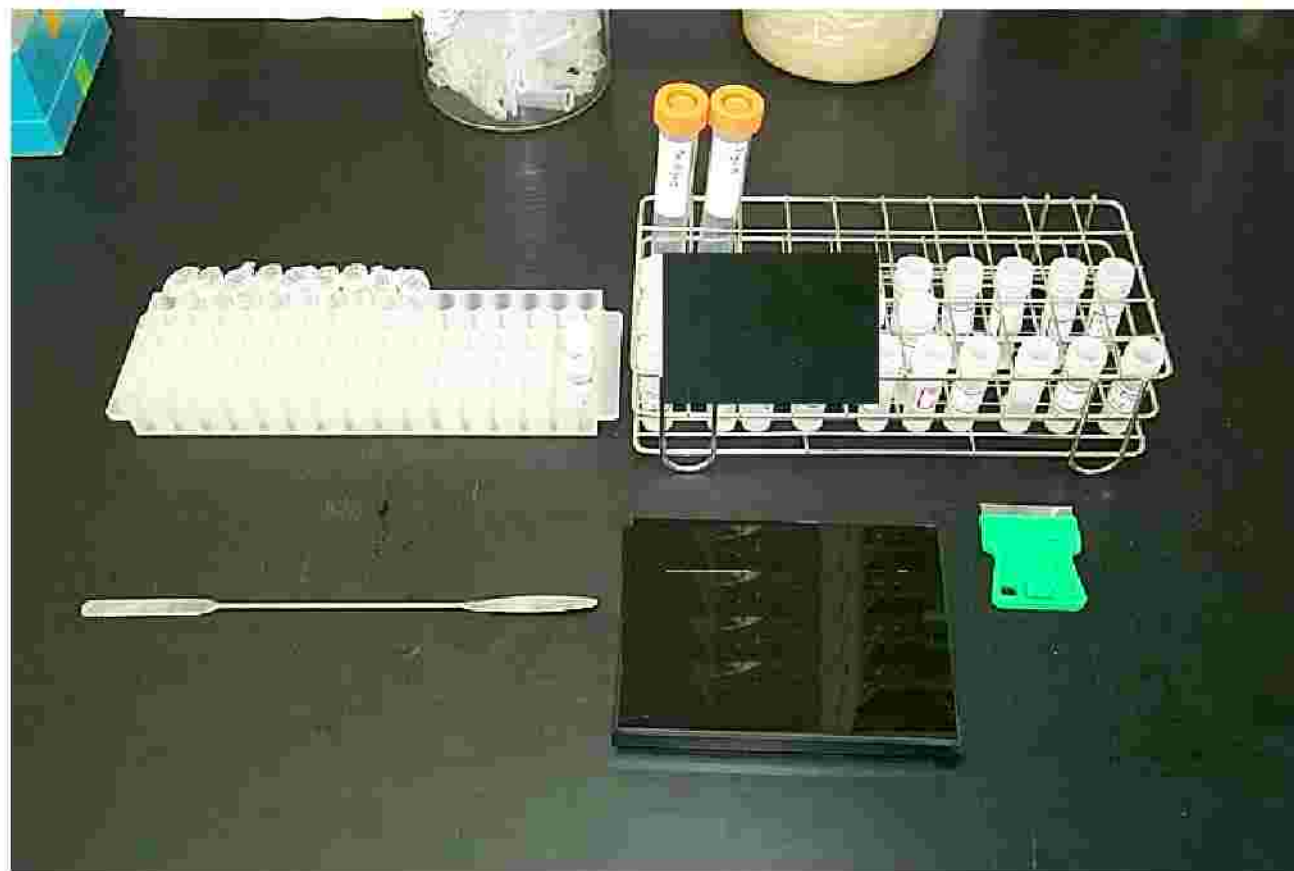


PFGE protocol

步驟		Shigella	Salmonella	Neisseria
培養	Media	TSA	TSA	5% sheep blood agar
	incubation	37 °C	37 °C	37 °C, 5 % CO ₂
包埋	Turbidity ^a	0.48-0.52	0.68-0.72	0.68-0.72
膠片製作	RE ^b (sample)	Not I	Xba I	Nhe I
	Marker	H9812	H9812	M413
	RE ^b (marker)	Xba I	Xba I	Nhe I
電泳條件	Initial switch time	2.16 s	2.16 s	2.2 s
	Final switch time	54.17 s	63.8 s	35 s
	Run time	19 hs	19hs	18 hs
	Angle	120 degrees	120 degrees	120 degrees
	voltage Gradient	6.0 volts/cm	6.0 volts/cm	6.0 volts/cm
	Temperature	14 °C	14 °C	14 °C
	Ramping factor	Linear	Linear	Linear



Plugs切割,切下約 2 mm寬度





限制? 處理





限制? 緩衝液配製: predigest

ddH ₂ O	178
--------------------	-----

BSA (10 mg/ml)	2
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10 X multicore buffer	20
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Total volume	200
--------------	-----

RT , 5 min



限制? 緩衝液配製: digest

ddH ₂ O	177
--------------------	-----

BSA (10 mg/ml)	2
----------------	---

10 X multicore buffer	20
-----------------------	----

Xba I (10 U/ml)	1
-----------------	---

Total volume	200
--------------	-----

37 °C , 2 h



電泳緩衝液配製

- 60 ml 10 X TBE + 2340 ml ddH₂O



10x Tris/Boric Acid/EDTA (TBE)
161-0733EDU



膠體配製

1% PFGE agarose:

1.5 g PFGE agarose

+

162-0137

Pulsed Field Certified Agarose

150 ml 0.5 X TBE Buffer

微波溶解,靜置於56 ℃約30分





將限制？處理之plugs置於
comb上,以吸水紙吸去多餘水份



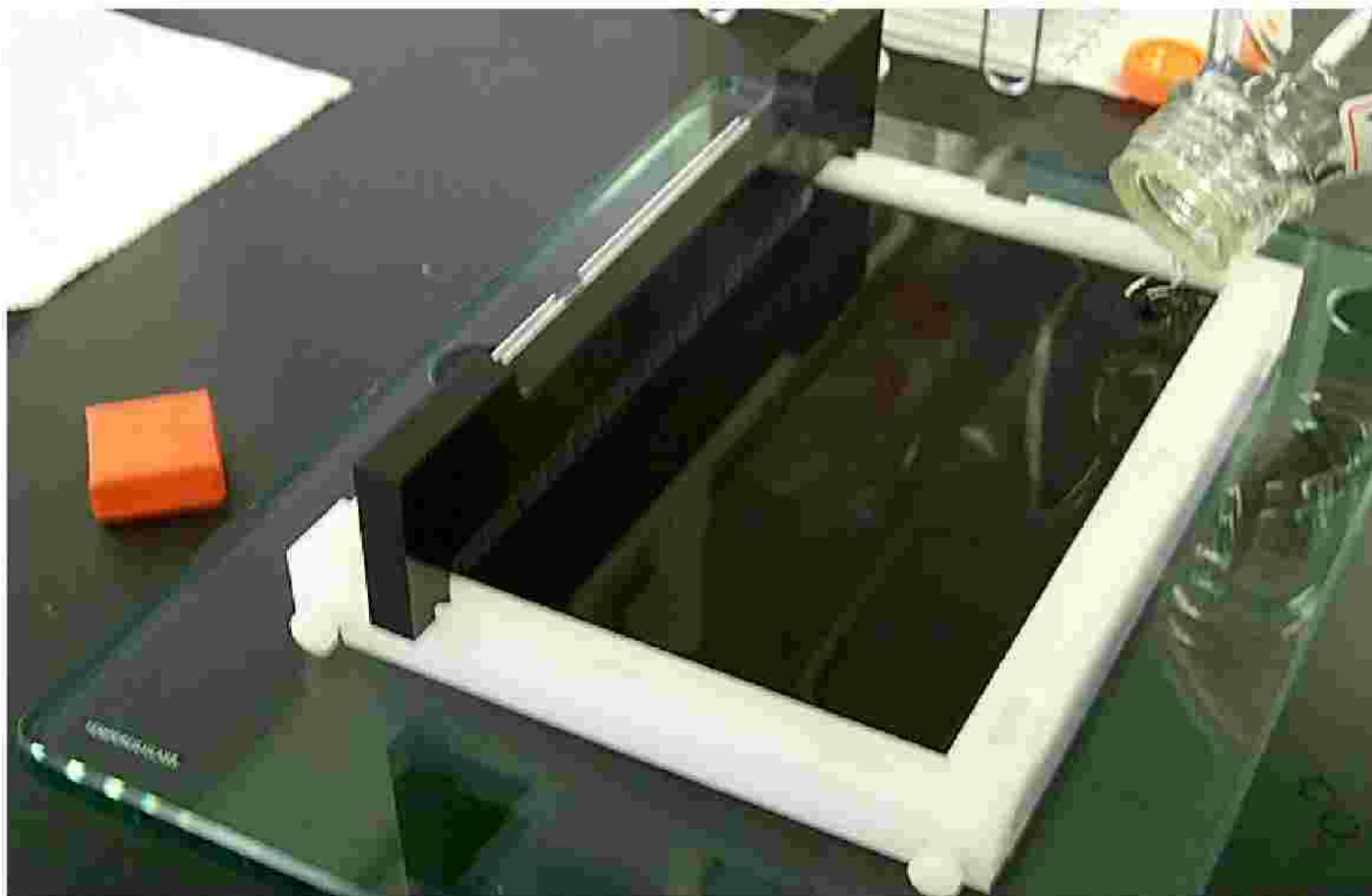


膠台設置及調水平





製膠:倒膠,避免氣泡產生.





凝固: 約20分



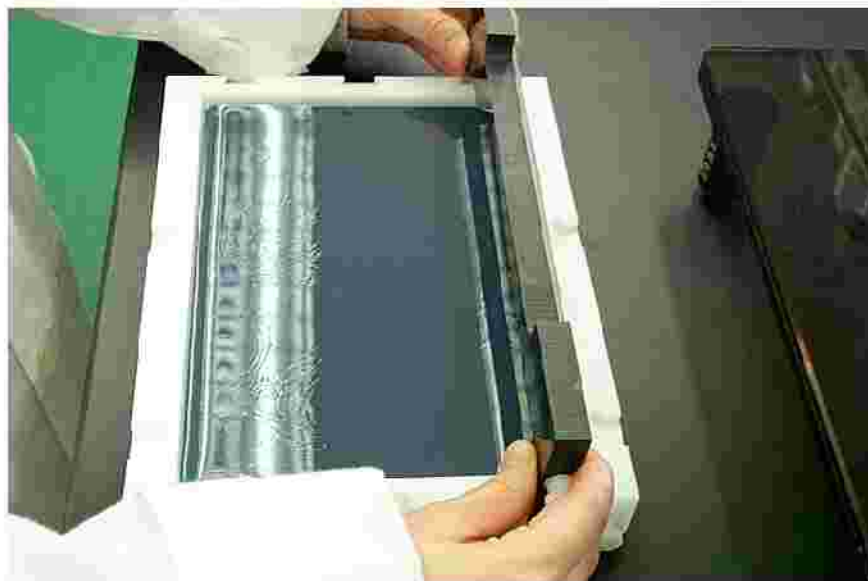


電泳槽準備

- 0.5X TBE 倒入電泳槽
- ? 度設定14 °C
- CHEF- Mapper 設定
 - Switch time: 2.16~ 63.8 s
 - Voltage: 6.0 volts/cm
 - Angle: 120

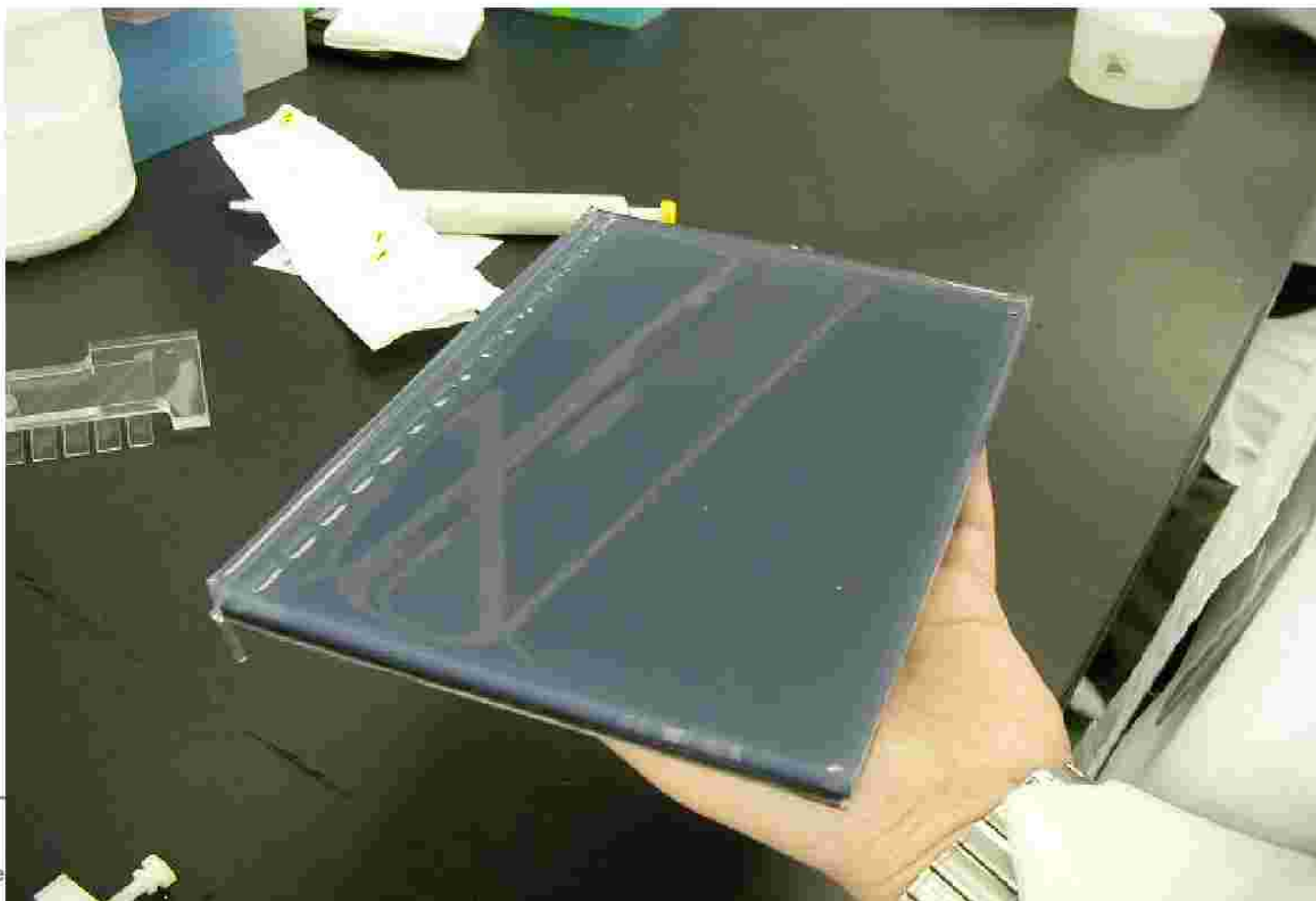


取出comb:避免plugs被抽出





清除製膠盤背面殘膠





電泳



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