DNA Ristriction Enzyme Mapping Flow Chart

Xun Zhao, xz2827

Step 1: Setting up the Restriction Digests

Whats	How	Why
Label EP tubes	E, H, P, EH, EP, HP, EHP, L, pBR322	Specify enzymes
$3\mu l$ pBR322	to all tubes except L	Plasmid to cut
$6\mu l$ λ DNA	to tube L	For ladder
Water	to total volume $10\mu l$	Same reaction volume
$1\mu l$ $10\times$ buffer	to all tubes	Reaction buffer
Enzymes	each $1\mu l$ as labels	Restriction enzymes
Place in floater	Incubate, 37°C, 60 min	Reaction starts

Step 2: Casting a 1.0% Agarose Gel

What	How	Why
Pour agarose	into flask with stir bar	To make the gel
Add buffer	into flask	DNA negative in solution
Heat	until agarose fully dissolve	Mix agarose and buffer
Cool	until cool enough to touch	
Add EtBr	$4\mu l$, use gloves	Make DNA visible under UV
Pour into gel mold	after gently swirling, set 20min	Make gel solid
Take out comb		
Lift, turn 90°, reinsert		Gel open to buffer chamber

What	How	Why
Pour running buffer	to each end of gel box	Connect anode and cathode

Step 3: Load Gel and Electrophorese

What	How	Why
Remove tubes from 37°C		Stop reaction
Add Loading dye	$2\mu l$ each tube	let digests sink, tell the location
Close tops, vortex, microfuge		Mix the dye and digests
Load	$12\mu l$ per well, new tip	
Connect, turn on the power	137V, to positive red	Let DNA run
Wait	until leading pigment run to #4	Let digests fully separate
Turn off current		

Step 4: View and Photograph

What	How	Why
Write name on weigh boat	with gloves	
Take out gel mold	slide gel into weigh boat, then transilluminator	
Turn on UV	with safty cover down	Show the DNA digests
Take photo		

Step 5: Cleanup

- Put used tips and tubes into red Sharps container
- Put used glassware on the tray, with stir bar, after rinsing

- Empty ice buckets, put container on the chart
- Put unused tips and tubes on the front bench
- Rinse off casting tray, with comb, leave them on paper towel
- Pour used buffer, leave it on paper towel upside down
- Discard gloves
- Make everything in order, wash hand