

III. Purification of PCR products.

A. Solid Phase Reversible Immobilization (SPRI) Purification of PCR Products

1. Sample Sheet

Plate name:

Gene	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	89
B	2	10	18	26	34	42	50	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

2. Preparation of magnetic beads

- Dilute beads (Eastapor SuperParMagnetic Microspheres [ME03N, Bangs Laboratories, INC, 317-5707020, Fax 317-5707034]) from bottle at 1:5 in 0.5M EDTA (pH8.0): 2 tubes 10ml each using 2ml beads, 8ml EDTA.
- Wash beads 3 times with 0.5M EDTA, using magnetic separation plate.
- Thoroughly resuspend beads prior to use.

3. Protocol

- Transfer PCR samples to plate (Falcon 353911).
- Add 50µl hybridization buffer (2.5M NaCl, 20% PEG8000) and 10µl washed beads to each 50µl PCR reaction, resuspend mixture well.
- Incubate 10 minutes at room temperature. Resuspend mixture well again.
- Place tubes on magnetic separation plate, allow to separate for 3 minutes or until beads have completely separated.
- Remove supernatant from tubes using aspirator and discard.
- Wash bead pellet with 150µl 70% EtOH while still on magnetic plate, repeat wash once.

- g. Allow beads to air dry for 1 hour. (starting time: _____, finish time: _____). (it helps to invert plates)
- h. Add 50 μ l elution buffer (10mM Tris) to each well, thoroughly resuspend bead pellet. Save tips.
- i. Incubate 3 minutes at room temperature. Resuspend mixture well, place tubes on magnetic separator.
- j. Allow beads to separate 5 minutes, remove supernatant (contains PCR product) to a new plate. Store at -20 degrees C.
- k. Prepare 4 μ l each sample to a plate for gel electrophoresis.

B. QIAquick Gel Extraction Kit Protocol

- Cat. No. 28704 (\$61.20 for 50 samples), Qiagen, Made in Germany
 - Turn the water bath to 50°C, make 1% agarose gel.
1. Excise the DNA fragment from the agarose gel with a clean, sharp scalpel.
 2. Weigh the gel slice in a colorless tube. Our 1.5ml tube is 1.1 g/each. Add 3 volumes of Buffer QG to 1 volume of gel (Usually 500ul).
 3. Incubate at 50°C for 10 min. To help dissolve gel, mix by vortexing the tube every 2-3 min during the incubation.
 4. Add 1 gel volume of isopropanol to the sample and mix (If DNA<500bp or >4kb).
 5. Place a QIAquick spin column in a provided 2ml collection tube.
 6. To bind DNA, apply the sample to the QIAquick column, and centrifuge for 1 min.
 7. Discard flow-through and place QIAquick column back in the same collection tube.
 8. Add 0.5ml of Buffer QG to QIAquick column and centrifuge for 1 min.
 9. To wash, add 0.75ml of Buffer PE to QIAquick column, let the column stand 3 min and centrifuge for 1 min.
 10. Discard the flow-through and centrifuge the QIAquick column for an additional 1 min at 13,000rpm.
 11. Place QIAquick column into a clean 1.5ml microfuge tube.
 12. To elute DNA, add 20µl water to the center of the QIAquick membrane, let the column stand 3 min and centrifuge the column for 1 min at 13,000rpm.
 13. Store the sample at -20°C freezer.

Example sample Sheet:

No.	PCR#	Sample#	Species	bp	ng/µl	Result
1						
2						
3						
4						
5						
6						
7						
8						