

FREEZE SQUEEZE FOR PURIFYING PCR PRODUCTS FOR SEQUENCING

REAGENTS 1X Low EDTA TAE Buffer Low melting point (LMP) agarose	Low EDTA TAE: 40mM Tris-acetate +0.1mM EDTA (50X : 242g Tris Base 57.1 glacial acetic acid 10ml 0.5M EDTA pH8)
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1. (If 2/50ul PCR reaction does not give strong band, concentrate sample to 25ul in speed vac. **CARE: too concentrated DNA will not run in gel**).
2. Run 25ul PCR reaction on 1.2% LMP -agarose /TAE gel.
CARE: products run faster than on TBE gel.
3. Samples should be loaded in alternate wells.
4. Cut out band, using low intensity UV, with as little agarose as possible.
5. Freeze band in 0.5ml tube for 5 mins. at -80°C .
6. Remove from freezer for 15 mins.
7. Repeat steps 4 & 5 three times.
8. Centrifuge at max. for 15 mins.
9. Remove all supernatant (10-30ul) to new tube.
10. Run 2ul on 1.5% normal TBE gel to check for yield for sequencing.