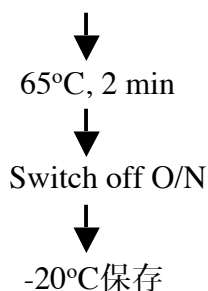


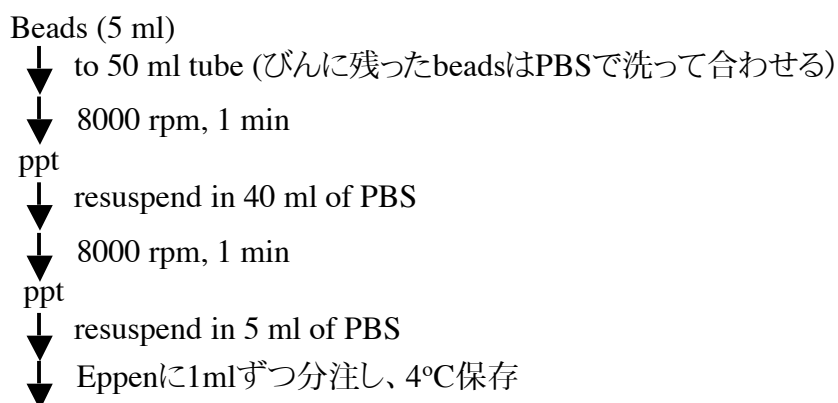
<DNAPプロトコール>

1. Oligo DNA annealing

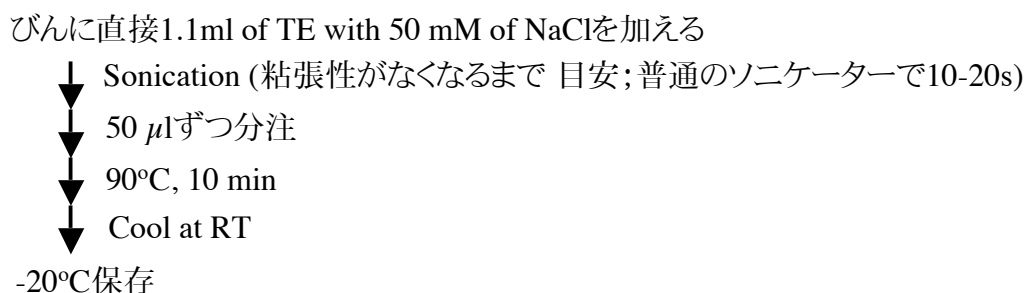
5' biotinated oligo A (50 μ M)	24 μ l	oligoはHPLC精製グレード
oligo B (50 μ M)	24 μ l	
0.5M MgCl ₂	1 μ l	
5M NaCl	1 μ l	
		50 μ l



2. Preparation of streptavidin-agarose (Sigma S-1638, 5 ml)



3. Preparation of Poly dI-dC (Roche 1 219 847, 50 A260 Units)



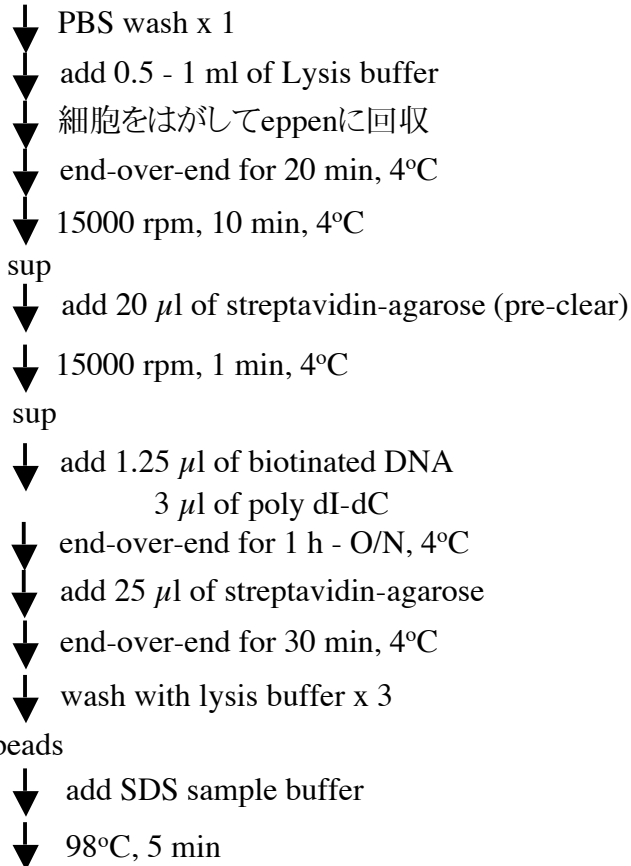
4. Lysis buffer

1% NP-40 20 mM Tris-HCl (pH 7.5) 150 mM NaCl
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使用直前にtrasyol (apolotinin)を加える

5. Procedure

Cells (10 cm dish, 6well plate etc...)



SDS-PAGE

6. 抗体 (7のプロープで、Smad2/3, Smad4の結合を見る場合)

抗Smad2/3 (BD transduction)

抗Smad4 (B4; Santa Cruz)

7. 文献

(Oligoの配列)

3xCAGA (JBC 274, 28716-)

3xTIE (JBC 277, 854-) : c-myc promoter のTGF-beta inhibitory element