DNA ladder radiolabelling

AIM: Preparation of a radiolabelled DNA ladder used in gels.

Two different protocols are proposed, each using a different approach. Both protocols work, so it is your choice.

PROTOCOL 1 : LABELLING USING T4 KINASE AND gamma-ATP

Materials:

- DNA ladder of your choice (eg. 50bp DNA ladder from Invitrogen cat no. 10416-014)
- T4 polynucleotide kinase (T4 PNK) (NEB cat no. M0201S)
- gamma-ATP^{32}
- TE 1x solution
- T4 PNK buffer 10x
- Sephadex G-50 columns
- Scintillation vials and counter
- 3M blotting paper
- PhosphorImager screens and Storm machine

Solutions:

TE 1x solution	100ml
10mM Tris pH8	1ml of 1M
1mM EDTA pH8	200ul of 0.5M
H2O	99ml

Autoclave
Store at RT

T4 PNK buffer 10x	1ml
700mM Tris pH7.5	700ul of 1M
100mM MgCl2	100ul of 1M
50mM DTT	50ul of 1M
1mM Spermidine	1ul of 1M
1mM EDTA pH7.5	2ul of 0.5M
H2O	147ul

Aliquot in 50ul.
Store at -20°C.
A precipitate can appear over repeated freeze/thaw cycles. Discard in this case.

Method:

Dephosphorylation (optional)

This step is optional; T4 PNK can exchange the existing 5'Ps from the DNA fragments with the radiolabelled phosphates. However, prior removal of the 5'phosphates with a phosphatase will greatly enhance the efficiency of the labelling reaction. To do so, refer to the protocol "Dephosphorylation of DNA or RNA".

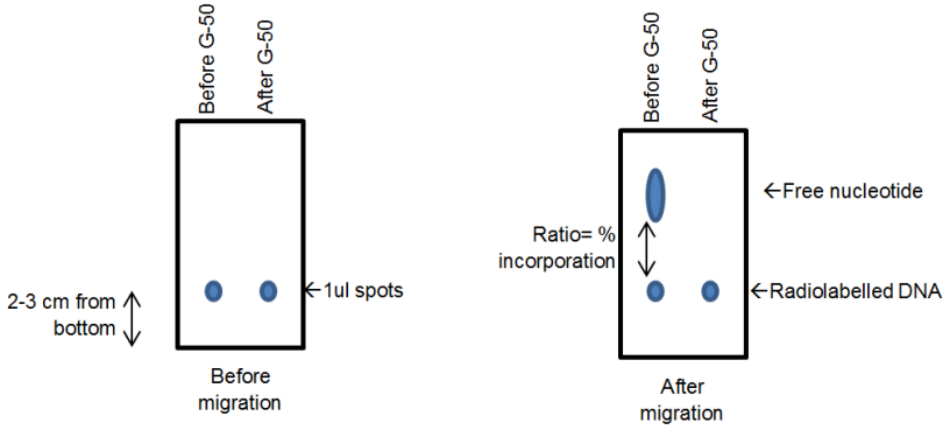
Labelling

1. Combine:
    - 3ul water
    - 1ul T4 PNK buffer 10x
    - 1ul ladder (0,5ug final) (ideally dephosphorylated)
    - 3ul gamma-ATP
    - 1ul T4 PNK
2. Incubate 45min-1h at 37°C.
3. Stop by adding 30ul TE 1x.
4. Incubate 5 min at 65°C
5. (Optional) Use 1ul to spot on a 3M paper for verification by chromatography.
6. Purify on a G-50 column. Collect flowthough.
7. (Optional) Use 1ul of the flowthought for verification by chromatography.
8. Verify 1ul in a scintillation vial (filled with 5ml Scintillation solution) in a scintillation counter.

Optionnal verification by chromatography

This step is to evaluate the % incorporation and G-50 column efficiency.

9. See steps 14 and 16. Spot 1 ul on a ~8cm long 3M paper →
10. Dip in beaker containing 2mm water at the bottom.
11. Let the water raise by capillarity
12. After ~3-4 cm separation, quickly wrap in saran and expose on phosphoImager screen.
13. Evaluate incorporation from the "Before G-50 lane" and G-50 purification from the "After G-50.



PROTOCOL 2 : LABELLING BY END-REPAIR USING T4 DNA POL

Materials:

- T4 DNA pol
- T4 DNA pol buffer 10x
- dATP 5mM solution
- dGTP 5mM solution
- dCTP 5mM solution
- dTTP 5mM solution
- alpha-dCTP^{32}
- SDS 1% solution
- Sephadex G-50 columns

Method:

1. Mix:
    - 2ul T4 Polymerase 10X Buffer
    - 10ul DNA Ladder(1mg/ml)
    - 4ul T4 DNA Pol 40 U
    - 4ul H2O

    Final=20ul

2. Incubate 5min at room temp.
3. Put on ice and add:
    - 10ul H2O
    - 3ul T4 Polymerase 10X Buffer
    - 2ul dATP (5 mM)
    - 2ul dGTP (5 mM)
    - 2ul dTTP (5 mM)
    - 3ul gamma-dCTP*32

    Final=50ul

4. Incubate 5 min at 30°C
5. Add 2ul dCTP (5mM)
6. Incubate 2 min at 37°C
7. Add 100ul SDS 1%. Let on ice. Total Volume= 150ul
8. (Optional) Use 1ul to spot on a 3M paper for verification by chromatography (see above).
9. Purify on a G-50 column. Collect flowthough.
10. (Optional) Use 1ul of the flowthought for verification by chromatography (see above)
11. Verify 1ul in a scintillation vial (filled with 5ml Scintillation solution) in a scintillation counter.