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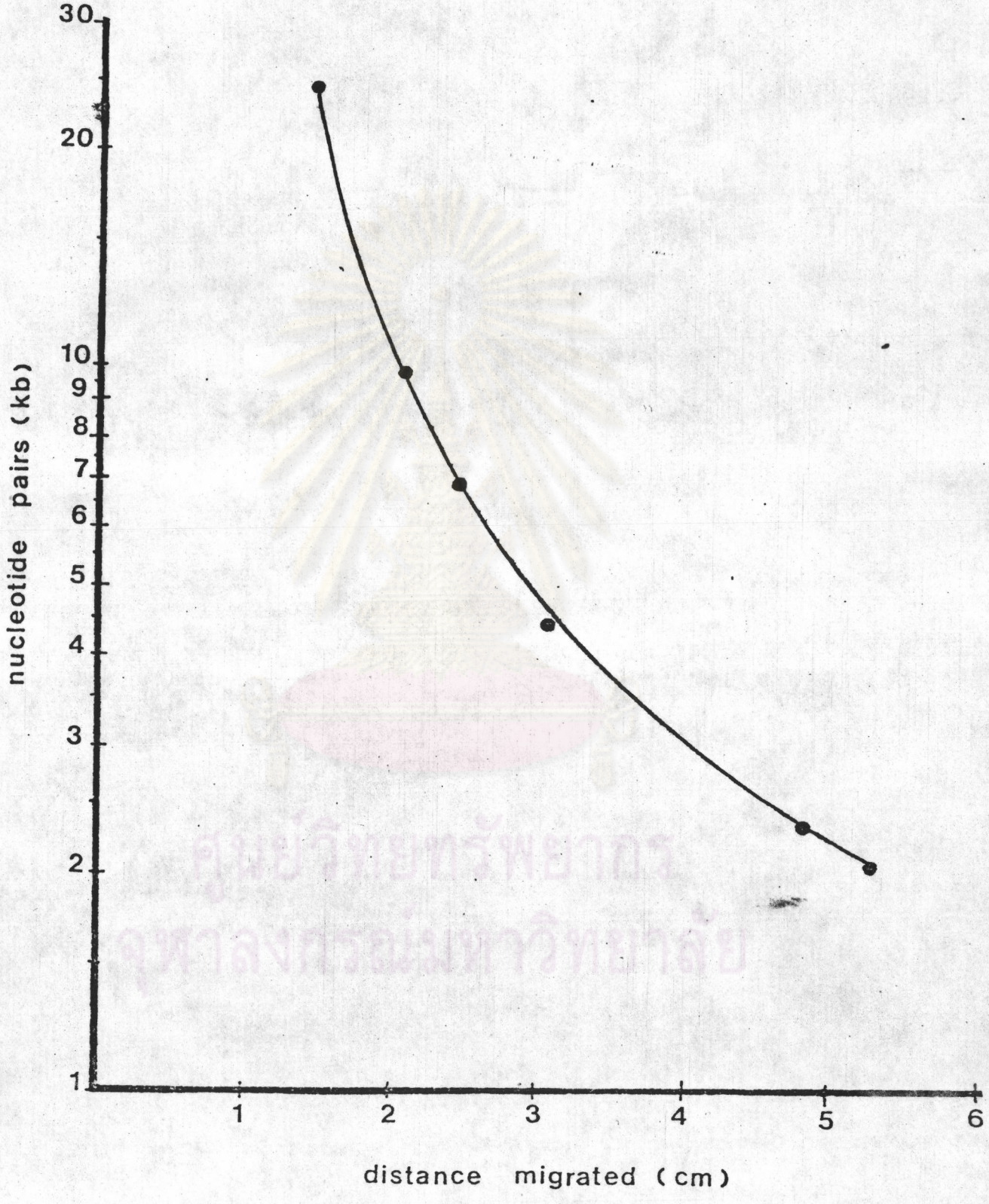
APPENDIX

1. Standard mobility curve of linear DNA fragments.



Appendix 2 Hybridization activity (cpm.)

Bacterial strains	Probes					
	nif fragemtn A1 5.76 Kb $2.9X10^7$ cpm/ug		*nif* fragment A2 1.44 Kb $1.5X10^8$ cpm/ug		*nif* fragment A3 3.70 Kb $7.2X10^7$ cpm/ug	
ng of chromosomal DNA	250	500	250	500	250	500
A. vinelandii KT1	65	61	110	120	95	97
A. vinelandii KT2	89	149	520	780	290	674
A. chroococcum KT	118	148	398	402	206	366
A. paspali B	104	110	182	190	100	110
A. chroococcum NP	61	65	193	200	114	129
A. brasilense Sp7	18	20	350	600	223	294
A. brasilense A2	20	20	104	115	103	118
A. lipoferum SpMRA1	17	20	180	191	93	131
*Azospirillum spp.*A12	20	22	220	350	141	157
K.pneumoniae M5a1 25ng	149		281		330	

Appendix 3 Calculation of homology

Calculation of relative homology of nif structural genes fragments between K.pneumoniae and Asotobecter spp. or Azospirillum spp.

Example of calculation between A.vinelandii KT1 and nif fragment A1 were shown following :

Slope of nif fragment A1 hybridized with A.vinelandii KT1 from figure 35 =0.36 cpm./ng DNA . Slope of self hybridization between K.pneumoniae M5a1 and nif fragment A1 =5.84 cpm./ng .

So,the percent homology of A.vinelandii KT1 with nif fragment A1 compare to K.pneumoniae = 0.36 x 100/5.84 = 5.0%

The result was shown in table 8.

The relative of homology values can compare within same nif fragment, in order to compare among nif fragment (table 9) ,thus the factor of specific activity and size of probes (Kb) should be eliminated by the calculation as the following:

From appendix 2, the hybridization activity of 25 ng of K. pneumoniae DNA hybridized with nif fragment A1 (5.76Kb , specific activity = 2.9×10^{7} cpm/ug) =149cpm . So,complete hybridization between K.pneumoniae DNA and nif fragment A1 was $149 / 5.76 \times 2.9 \times 10^{7} = 8.9 \times 10^{-7}$ ug of probe / Kb of probe .

A.vinilandii KT1 hybridized with nif fragment A1 that show reletive homology of 5.0 % . It define the relative homology in term ug /Kb of probe $= 8.9 \times 10^{-7} \times 5.0 / 100 = 4.4 \times 10^{-8}$.

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