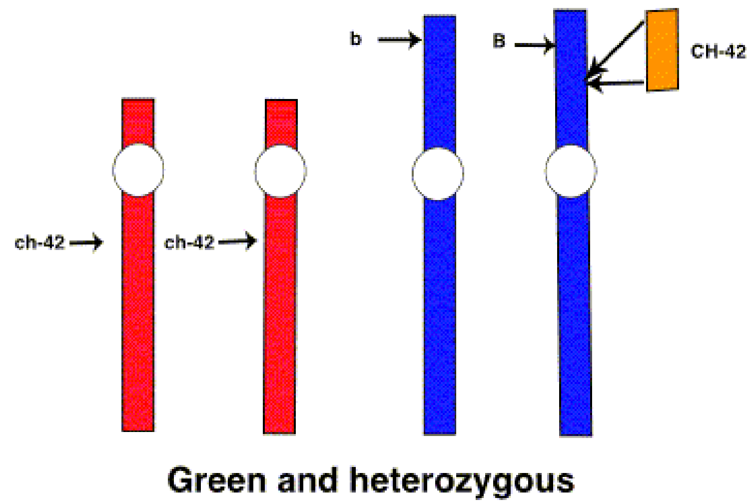
The cell-autonomy (CAUT) lines of *Arabidopsis*

Background and theory. Cell-autonomy is a property of particular genotypes and is useful to examine developmental and/or signalling interactions between cells and tissues. Cell-autonomy is studied by producing genetic mosaics and chimeras containing tissue of differing genotypes and analysing the resulting phenotypes. A trait is completely cell-autonomous if the genotype and phenotype of the tissue always correspond irrespective of the genotype of the adjacent tissue. Conversely a trait is non cell-autonomous if the phenotype of either tissue is affected by the genotype of the adjacent tissue.

Cell-autonomy has been studied in plants using a variety of methods to generate the chimeric or mosaic plants. Such methods include; grafting, site-specific recombination, transposon excision and radiation induced deletion. The latter method has been used for many elegant studies in maize but in only one study of *Arabidopsi*s (Furner et al 1996. Development 122; 1041-1050). In these studies recessive cell-autonomous colour markers are included in the experimental design to allow the routine identification of the tissue containing the appropriate deletion. This is comparatively easy in maize as many markers at different locations are available. In *Arabidopsis* there is a shortage of colour markers and finding a good cell-autonomous colour marker near a gene of interest is not usually possible.

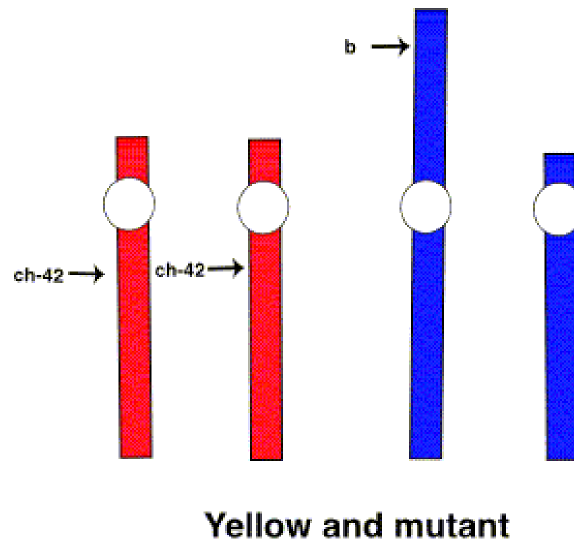
The CAUT lines are an attempt to overcome the shortage of useful markers by artificially generating markers using genetic complementation and *Agrobacterium* – mediated transgenesis. The idea is simple; the recessive yellow *ch-42* mutant can be complemented by transformation with the dominant wild-type *CH-42* gene on a T-DNA. This has the net effect of translocating the *CH-42* gene and the ability to synthesise chlorophyll to a novel and unique location in each transgenic plant. A recessive mutant trait of interest can be crossed into the yellow *ch-42* mutant background. Subsequent crosses to an appropriate green T-DNA transformant can generate a situation with the recessive mutant trait on one chromosome and on the homologous chromosome the wild-type gene and the dominant green *CH-42* marker. (This situation is illustrated below).

Generation of marked mutant tissue using the CAUT lines



Green and heterozygous

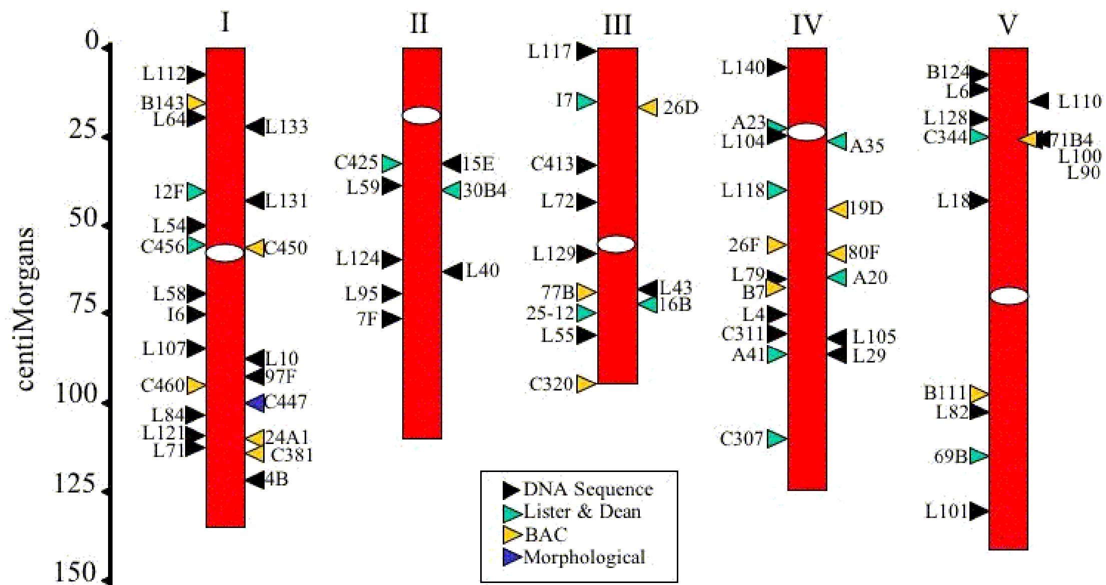
Irradiation and deletion



Yellow and mutant

The *ch-42* allele on chromosome 4 (red) is inactive but the wild-type *CH-42* function is provided by the T-DNA insert (yellow) on another chromosome (blue). The plant is initially heterozygous for the recessive gene b with the wild-type copy (B) linked in cis to the T-DNA. After irradiation (lower panel) phenotypically yellow sectors are selected and these have lost both the *CH-42* copy on the T-DNA and the adjacent B gene. The recessive b mutant is the only copy and the tissue is both yellow and genotypically mutant. The analysis of phenotype of the sector and adjacent wild-type tissue can be used to look for autonomous and non-autonomous interactions.

Generation of the CAUT lines. The yellow *ch-42* mutant was transformed with a construct containing the wild type *CH-42* gene. Green corrected plants were backcrossed to the yellow *ch-42* parent until a 1:1 segregation of yellow to green plants was observed. Sequences flanking the T-DNA inserts were recovered by plasmid rescue and mapped on one of the five *Arabidopsis* chromosomes. The mapping technique changed over the course of the work; the earliest lines were mapped using the Lister and Dean recombinant inbred lines. Later lines were mapped using gridded BACs from the ABRC. Finally, sequencing using a left border primer and BLAST searches of the *Arabidopsis* genome sequence at TAIR was used to position the last inserts. The results of the project are shown in graphical and tabular form (below). The lines containing these inserts were made homozygous and sent to NASC.

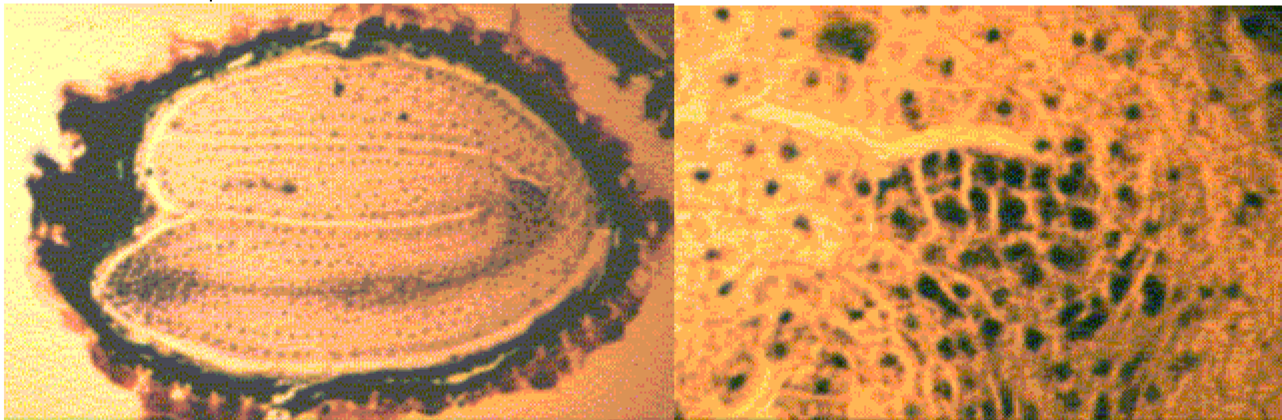


Line/Marker	clone name	method	BAC	position	
				cM	bp x 10-6
chromosome 1					
L112	L112-e	sequencing	F13M7	7	1.4
B143		BAC	F25P2	15	4.7
L64	L64-b	sequencing	F28G4	20	6.0
L133	L133-3	sequencing	T10F20	22	6.3
12F	FUR12F12	L&D		40	
L131	L131-h	sequencing	F3M18	43	10.0
L54	L54-d	sequencing	F3C3	50	11.6
C456	C456-6	L&D		56	
C450		BAC	F17K3	57	12.9
centromere		sequencing		58	
L58	L58-e	sequencing	F11A17	69	17.4
I6	I6-1	sequencing	F12M16	75	19.8
L107	L107-d	sequencing	T30E16	84	21.6
L10	L10-3	sequencing	F16M19	87	23.0
97F		sequencing	F22C12	92	22.9
C460		BAC	F9L18	95	24.2
C447		MM		100	
L84	L84-2	sequencing	F23O10	103	25.7
L121	L121-b	sequencing	F5A18	109	26.3
24A1	24-A1-17	BAC	F28P22	110	26.6
L71	L71-i	sequencing	F10I7	112	27.6
C381		BAC	F14G6	114	27.8
4B		sequencing	F23A5	121	29.1

Line/Marker	clone name	method	BAC	position	
				cM	bp x 10-6
chromosome 2					
centromere		sequencing		19	
C425	C425	L&D; seq	T24I21	33	7.2
15E		sequencing	MJB20	33	7.5
L59	L59-b	sequencing	F5H14	39	8.9
30B4	FUR30-2	L&D; seq	T16B14	40	9.3
L124	L124-f	sequencing	F23F1	59	12.7
L40	L40-14	sequencing	T9H9	63	13.4
L95	L95-d	sequencing	F3G5	70	15.7
7F		sequencing	MHK10	77	17.5
chromosome 3					
L117	L117-8	sequencing	F28J7	1	0.3
I7	I7-x	L&D; seq	F10A16	15	1.7
26D	26D2	BAC	F27D23	16	3.3
C413		sequencing	MLM24	33	8.3
L72	L72-4	sequencing	MFE16	40	9.7
centromere		sequencing		55	
L129	L129-af	sequencing	F7K15	58	15.2
L43	L43-2	sequencing	T20E23	68	18.8
77B		BAC	F7E13	70	18.5
16B	F16b	L&D; seq	T20E23	72	18.7
25-12	F25-12.2	L&D;seq	F1I16	75	20.6
L55	L55-a	sequencing	F21F14	81	22.9
C320		BAC	F17L17	95	21.1

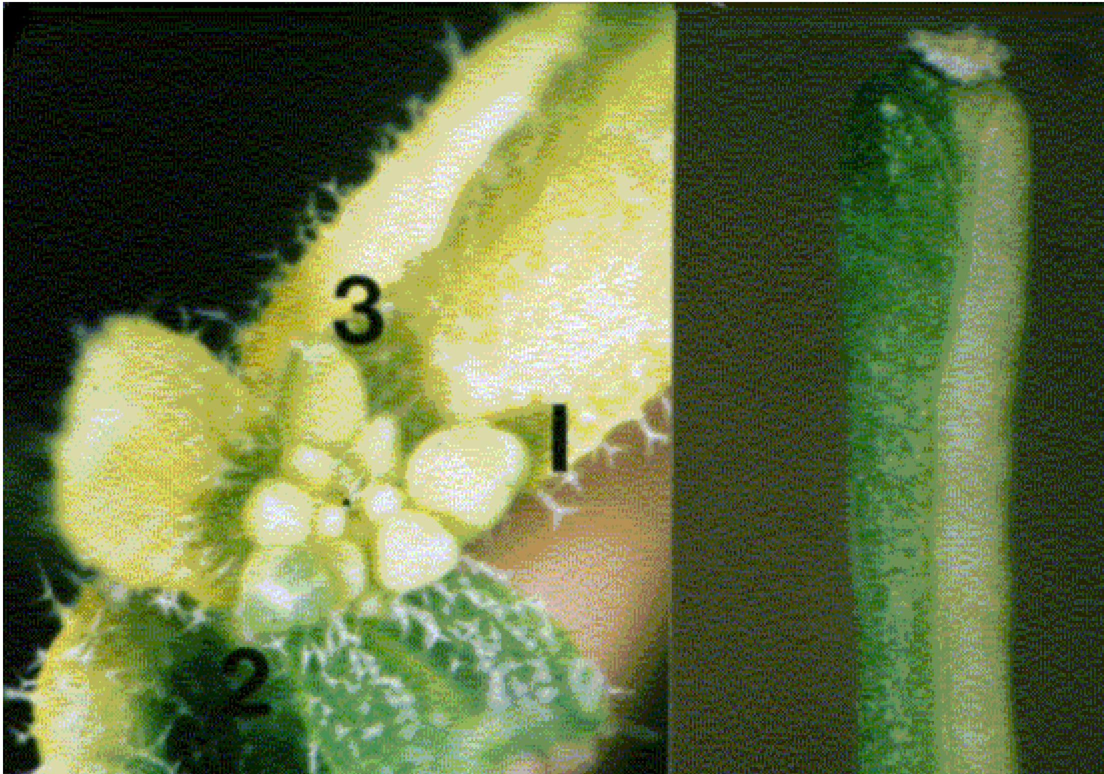
Line/Marker	clone name	method		BAC	position
				cM	bp x 10-6
chromosome 4					
L140	L140-c	sequencing	T18A10	5	0.3
A23	FurA23-7	L&D;seq	F5K24	23	3.5
centromere		sequencing		24	
L104	L104-a	sequencing	T15G18	25	4.9
A35		L&D;seq	T15F16	26	4.3
L118	L118-2	L&D;seq	T1P17	40	6.4
19D		BAC	F21E15	46	10.7
26F		BAC	F16D17	55	11.9
80F	FUR80F3	L&D		58	
L79	L79-1	sequencing	F17L22	63	10.4
A20	a20-4	L&D		65	
B7		BAC	F18C17	67	12.3
L4	L4-e	sequencing	M3E9	75	12.4
C311	C311-6	sequencing	F26P21	80	14.8
L105	L105-d	sequencing	F10M10	82	15.4
A41		L&D;seq	F15J1	86	15.8
L29	L29-q	sequencing	T19K4	86	16.0
C307	C307-1	L&D;seq	T5J17	110	17.5
chromosome 5					
B124	B124-1	sequencing	F17C15	7	0.9
L6	L6-1	sequencing	F32M21	11	1.3
L110	L110-1	sequencing	F15M7	15	2.0
L128	L128-6	sequencing	MBK20	20	2.4
C344	FurC344-7	L&D; seq	F17I14	25	2.9
71B4		BAC	F5H2	26	
L100	L100-3	sequencing	T5K6	26	3.5
L90	L90-2	sequencing	F2I11	26	3.6
L18	L18-6	sequencing	MQM1	43	7.9
centromere		sequencing		70	
B111	B111-5	BAC	F25B22	98	18.4
L82	L82-u	sequencing	K21P3	103	19.7
69B	Fur69B12	L&D;seq	K14B18	115	23.6
L101	L101-d	sequencing	MUD21	131	26.4

Working with the CAUT lines. The CAUT lines were designed for studies of cell-autonomy in the *Arabidopsis* shoot, leaves and flowers but in order to use them effectively you have to have some background in the development of the shoot apical meristem (SAM) and its derivatives**.** The *Arabidopsis* SAM consists of a group of about one hundred cells set up in three layers (L_1, L_2 and L_3). It is set up late in embryogenesis and remains about the same size throughout vegetative and reproductive growth. Once set up the layers are clonally distinct and cells within them have characteristic fates. The L_1 layer contributes to a one cell thick epidermis covering the plant. As it contains little chlorophyll sectors deficient in chlorophyll are not seen in this layer. The L_2 layer contributes most of the green tissue in the leaves and flowers and a layer in the stem. The L_3 layer contributes a core of tissue in the leaves and flowers and the centre of the stem. Chlorophyll deficient sectors can be visualised in green tissues derived from L_2 and/or L_3 but a colourless genetically wild-type epidermis derived from L_1 overlies such sectors.



The dry seed (sectioned) the dry seed SAM

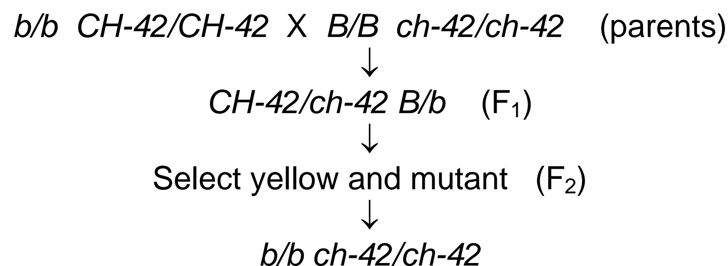
Cells at the periphery of the seed SAM typically make small contributions to the early leaves and cells nearer the centre of the structure make larger contributions to one or more leaves. Only cells at the centre of the seed SAM make large contributions to the late leaves and flowers (Furner and Pumfrey, 1992.Development 115; 755-764). Seed irradiation produces a large number of sectors affecting the early leaves and relatively few affecting the flowers. The axilliary meristems are clonally related to the L_2 derived tissue at the centre of the leaf beneath them. By cutting back the bolting stem it is possible to encourage the development of the axilliary meristem above sectored leaves and get a bolting stem containing the chlorophyll deficient tissue. This process can be repeated several times until periclinal chimeras of the type; L_1 wild-type, L_2 and L_3 yellow are obtained. Such plants can be used to study traits expressed only in flowers and to generate seeds to test sector genotype.



Large *ch-42* sectors affecting L_2derived tissue of the inflorescence and silique

Setting up the experiment

1. Genetics. The first step to using the CAUT lines is to cross your trait into the yellow *ch-42* background. This is illustrated for a monogenic recessive trait (*b*). It is possible to modify the crosses to cope with dominant, lethal and/or infertile traits.



b/b CH-42/CH-42 X *B/B ch-42/ch-42* (parents)

↓

CH-42/ch-42 B/b (F_1)

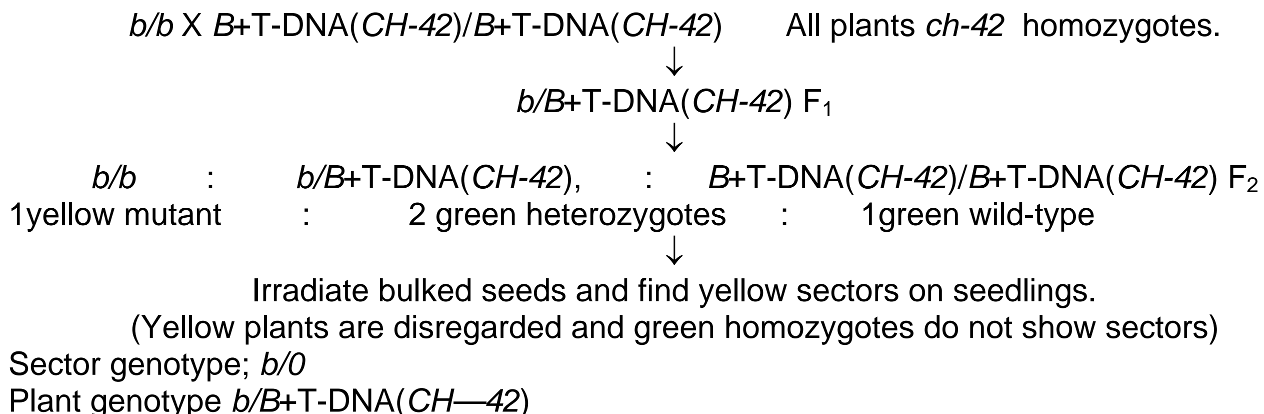
↓

Select yellow and mutant (F_2)

↓

b/b ch-42/ch-42

Choose one or two CAUT lines with inserts near your mutant of interest using the map and tables above and order them from NASC. You only need the map position of your mutation on either the Lister-Dean recombinant inbred map or a sequence position on the TAIR map. Grow up the mutant-*ch-42* and the chosen CAUT line(s) and make several crosses between them to generate an F_1. Grow this generation up and allow it to self-fertilise to generate a large F_2 for irradiation. The genetics involved is illustrated bellow for the hypothetical recessive gene *b*.



As the gametes are usually set in tissue derived from the L_2 layer of the floral primordium seeds set in the sector and the adjacent wild type tissue can be used to asses the genotype of L_2. Seeds set in the yellow sectors should give rise to 100% yellow and phenotypicaly mutant plants. Seeds set in the wild type tissue should segregate and give rise to3 green wild type plants to one yellow mutant plants. If large samples are planted a few recombinant types are typically found. Sometimes seeds which give rise to green plants are found in samples from yellow sectors, these may be due to contributions to the gametes from cells in the L_1 layer of the floral primordium.

2. Seed irradiation and sector analysis. In a typical experiment 10 batches each of 20 Mg of seeds are irradiated with unfiltered X-rays. Various doses can be used in these experiments but the highest level that can routinely be used is 16 kilorad. This dose delays leaf emergence by 3 days. Each batch of seed is broadcast to a filled seed tray in 10 gm of dry sand. It is important to keep the humidity high until the true leaves emerge, as the irradiated plants tend to dry out and die. Sectors are found on up to 1% of plants and sectored plants are picked off to new trays.

The *ch-42* sectors are a characteristic yellow colour and sectors with other phenotypes are usually the result of unrelated events and should be discarded. The sectors tend to green up over time but this effect can be reduced by increasing the light intensity. Once branches with yellow $L_2$and L_3 tissue have been generated by cutting back they can be marked with waterproof ink. This allows the seeds to be collected from the sector even though the dry silique has no chlorophyll and the sector cannot be seen. Sector phenotypes can be scored at whatever time and/or stage they normally appear. It is a good idea to retain a few yellow mutant homozygotes and a few green non-sectored plants as controls

3. Verification of the CAUT lines. The CAUT lines are phenotypically identical and all are full green and kanamycin resistant. So it is fairly easy to mix them up and ruin your cell-autonomy experiment by using the wrong one. As such errors do happen in any lab a simple and robust method of line verification has been developed. The method is based on Southern blots, T-DNA probes and comparisons to blots run in the Furner lab (see thumbnails below).

The parental *ch-42* mutant is tagged with a T-DNA containing a promoter less and silent neomycin phosphotransferase and an active hygromycin phosphotransferase conferring hygromycin resistance. The T-DNA also contains a pBR322 copy. Homozygous *ch-42* plants

are yellow in soil and grow to maturity. Southern blots of DNA from this line prepared with *ECORI* and probed with pUC18 have a single 9kb fragment corresponding to the tag.

The pCV002GC plasmid used to generate the CAUT lines also has a pBR322 region near the left border (Koncz et al, 1990 The EMBO Journal 9; 1337-1346.). There is one *ECORI* site in the T-DNA to the right of the pBR322 region. In the transformed plants a second *ECORI* site is found in the adjacent flanking plant DNA. The second site varies between integration events and therefore between lines. All of the lines are homozygous for a single correction but there can be multiple T-DNA copies at that site. The CAUT lines were made by introducing the correcting T-DNA into the *ch-42* mutant. After recurrent back crosses, the single correcting insertions were made homozygous. Southern blots of these lines made with genomic DNA digested with *ECORI* and probed with pUC18 show a characteristic pattern. All lines have a 9kb band corresponding to the tag at *ch-42* on chromosome 4. In addition all lines have one or more bands of varying sizes corresponding to the correcting *CH-42* insert at the new location. The fragment pattern is unique to each line and can be used as a distinctive fingerprint to verify the identity of a line.

Below are 7 blots corresponding to the inserts of the 5 chromosomes (chromosomes 1 and 4 are on two blots). The arrows are the positions of lambda *HindIII* fragments and the sizes (from the top) are: 23.1, 9.4, 6.6, 4.4, 2.3 and 2.0 KB. All lines have the 9kb fragment but there is slight variation in the migration of the fragment.

Help and advice. The CAUT lines were developed by Ian Furner and co-workers, if you run into any problems or need advice on their use please feel free to contact him at; ijf@mole.bio.cam.ac.uk or by surface mail at : The Department of Genetics university of Cambridge, Cambridge, CB2 3EH UK.