AN ULTRA-HIGH DENSITY GENETIC LINKAGE MAP OF POTATO AS A PLATFORM FOR TARGETED PHYSICAL MAPPING AND MAP-BASED CLONING

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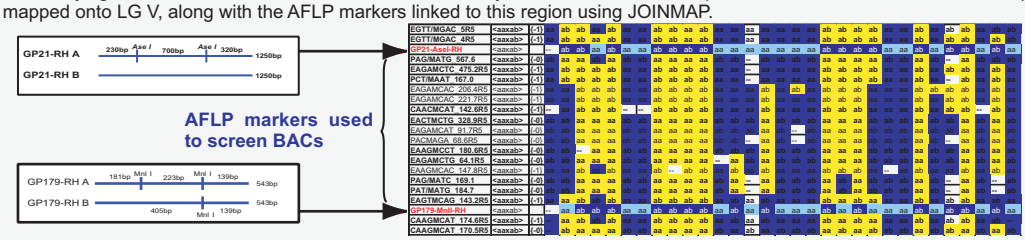
Introduction

An EU funded collaborative project is constructing an Ultra High Density (UHD) map of potato containing ~10,000 markers. This map, the densest yet made in a crop plant, is being generated from a cross between two heterozygous diploid clones, and will become a vital tool for potato genetics and genomics projects. To demonstrate the utility of the UHD map, we have started to construct a physical map of the region between two RFLP markers on potato linkage group V, which is known to harbour many important genes, several of which confer resistances to major pests and diseases. Moreover, genes affecting traits such as maturity, dormancy and earliness also reside in this region. The mapping strategy uses AFLP markers mapped to the region to seed a 'genetically anchored' physical map. We have developed a multi-dimensional pooling strategy, whereby a BAC library can be screened using AFLP in 200 PCR reactions. Multiple BACs obtained by AFLP screening are then used to initiate 'walks' into the surrounding DNA. BACs are 'skim' sequenced to identify gene content and to facilitate marker development (SSRs and SNPs) for validation of BAC location, fine-scale mapping, map-based gene cloning and marker-trait association studies.

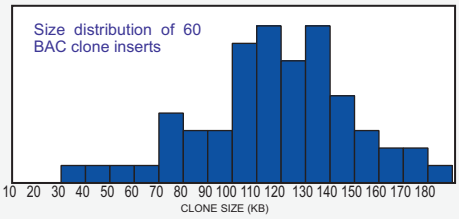
Mapping GP21 and GP179 on UHD cross

RFLP markers GP21 and GP179 have been mapped on the UHD cross using a targeted CAPS assay approach, which involves sequencing PCR products from mapping parents and identifying SNPs that remove restriction sites. Such assays have been used to map GP21 and GP179 in RH, one of the parents of the UHD cross. The data has been mapped onto LG V, along with the AFLP markers linked to this region using JOINMAP.



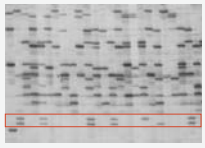
Construction of an RH BAC library

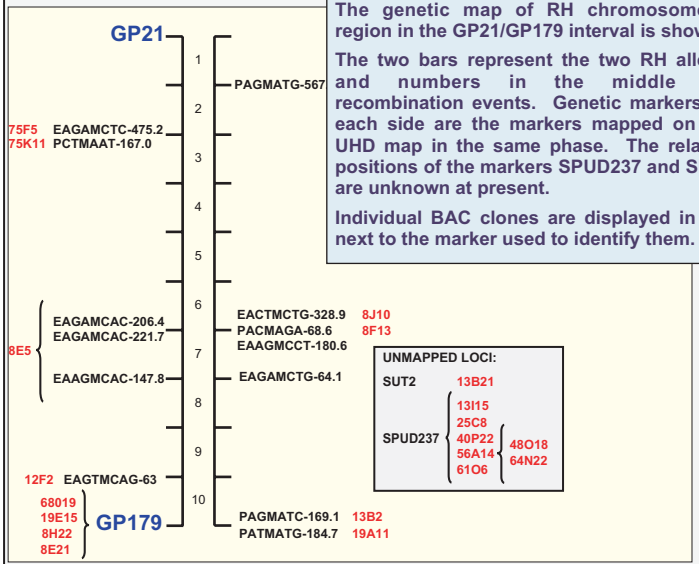
A BAC library of RH has been constructed in the vector pBELOBAC11. This library comprises ~35,000 clones of average size 100kb (~4 genome equivalents). This library is arrayed in 93 384-well plates.



Towards a physical map of GP21-GP179 region

The RH BAC library has been screened with RFLP markers and gene sequences known to map to the GP21-GP179 region, as well as the AFLP markers mapped to the locus, using the pooling scheme described. An example of an AFLP screening of the library is shown.





The genetic map of RH chromosome V region in the GP21/GP179 interval is shown.

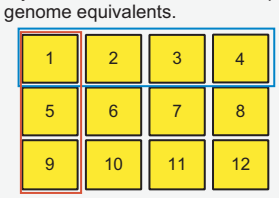
The two bars represent the two RH alleles and numbers in the middle are recombination events. Genetic markers on each side are the markers mapped on the UHD map in the same phase. The relative positions of the markers SPUD237 and SUT2 are unknown at present.

Individual BAC clones are displayed in red next to the marker used to identify them.

Pooling of RH BAC library

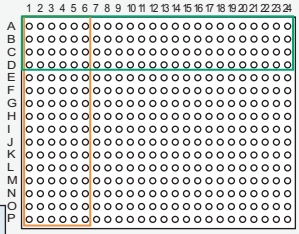
The RH BAC library has been pooled using a novel 6-dimensional pooling strategy, which permits AFLP or PCR-based screening of the library in 200 reactions. The pooling strategy we have used is outlined below.

Library is first sub-divided into 8 12-plate sub-libraries, each comprising roughly 0.5 genome equivalents.



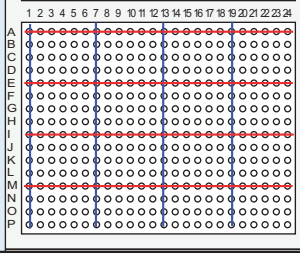
Dimension 1
Pool plates 1,2,3,4 = Pool#1
Pool plates 5,6,7,8 = Pool#2
Pool plates 9,10,11,12 = Pool#3

Dimension 2
Pool plates 1,5,9 = Pool#4
Pool plates 2,6,10 = Pool#5
Pool plates 3,7,11 = Pool#6
Pool plates 4,8,12 = Pool#7



Dimension 3
Pool rows A-D = Pool#8
Pool rows E-H = Pool#9
Pool rows I-L = Pool#10
Pool rows M-P = Pool#11

Dimension 4
Pool columns 1-6 = Pool#12
Pool columns 7-12 = Pool#13
Pool columns 13-18 = Pool#14
Pool columns 19-24 = Pool#15



Dimension 5
Pool rows A,E,I,M = Pool#16
Pool rows B,F,J,N = Pool#17
Pool rows C,G,K,O = Pool#18
Pool rows D,H,L,P = Pool#19

Dimension 6
Pool columns 1,7,13,19 = Pool#20
Pool columns 2,8,14,20 = Pool#21
Pool columns 3,9,15,21 = Pool#22
Pool columns 4,10,16,22 = Pool#23
Pool columns 5,11,17,23 = Pool#24
Pool columns 6,12,18,24 = Pool#25

Conclusions

We have been able to place 15 AFLP loci in the interval between GP21 and GP179 on potato LGV in the UHD map.

These markers have been used to screen a BAC library constructed from one of the parents of the UHD cross. This has involved the use of a novel pooling method which allows us to detect AFLP markers in the pools.

To date we have identified BAC clones from 10 of the AFLPs in the region. These BACs are being sample sequenced, in order to identify their gene content and to provide STS landmarks for initiating BAC contiging of the region.

Acknowledgements

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