



# Towards the Optimization of Resistance Gene Analog Discovery in *Solanum* Species

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## The Problem (Table 1):

- Low recovery of RGAs from *S. bulbocastanum* genomic DNA using degenerate PCR primers
- High recovery of highly repetitive elements from *S. bulbocastanum* genomic DNA using degenerate PCR primers

## Introduction:

Resistance gene analogs (RGA) are potential gene fragments that contain structural motifs, such as the NBS and LRR regions, that are common to most cloned R genes. Recovery of RGAs using degenerate primer PCR was first described for cultivated potato (Leister et al. 1996) and has become a popular method for recovering RGAs from many dicot and monocot plant species.

Towards the development of an integrated system of genetic and physical maps for potato (see poster P493), we used degenerate PCR in an attempt to isolate RGAs from genomic DNA of the disease resistant, primitive wild potato, *Solanum bulbocastanum*. We recovered few RGAs and most of our fragments were repetitive in nature. Recovery of repetitive fragments using degenerate PCR was previously reported for cultivated potato (Leister et al. 1996) but not for closely related tomato (Pan et al. 2000, Zhang et al. 2002).

BAC library construction enriches for low copy, euchromatic genome regions. Here we describe efforts to optimize RGA recovery for primitive potato using pooled BAC DNA, instead of genomic DNA, as a template for degenerate PCR.

Table 1: Sequence Statistics

Sequence Type	% of Sequences Recovered
Resistance Gene Analogs	7%
Non-RGA Plant Related Sequences	17%
Plant Related, Highly Repetitive Sequences	48%
Unassigned Sequences	28%

Table 2: BAC pooling strategy

# of plates	Total # of clones	% genome equivalents (g.e.)
¼	96	2.1%
1	384	8.3%
3	1152	25%
6	2304	50%
12	4608	100%

Table 3: Degenerate primer sequences

NAME	5'-SEQUENCE-3'	MOTIF	SPECIES	REFERENCE
s1	GGT GGG GTT GGG AAG ACA ACG	NBS- GGVGKTT	<i>S. tuberosum</i>	Nat. Genetics 14:421
s2	GGN GGN GTN GGN AAN ACN AC	NBS- GGBGKTT	<i>S. tuberosum</i>	Nat. Genetics 14:421
as2	IAA IGC IAG IGG IAA ICC	NBS- GLPAL	<i>S. tuberosum</i>	Nat. Genetics 14:421
motif 1	GAA GCA IGC GAT GTC IAG GAA	NBS- GLPAL	<i>P. vulgaris</i>	Phytopath. 93(1):88

## Materials and Methods:

- BAC pools were created to represent different percentages of the entire genome (Table 2)

-PCR was performed using two primer pairs (Table 3) with *S. bulbocastanum* genomic DNA and BAC pooled DNA as templates (Figure 1, 2a, and 3a). These PCR products were used to make blots for Southern hybridization.

- Probes for hybridization were made using RGA and highly repetitive (intergenic spacer region) sequences recovered from PCR of genomic DNA, and *E. coli* and BAC vector DNA.

- Sequence analysis was done using SeqMan™II (DNASar), BLAST-n, and ClustalW computer programs.

Figure 1: PCR of BAC pools- 2.1% and 8.3% genomic equivalents (g.e.)

- a) PCR with primer pair s1+as2  
b) PCR with primer pair s2+motif1

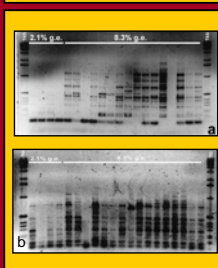


Figure 2: PCR and Southern blot analysis of primer pair s1+as2

- a) PCR; b) Southern blot with RGA probe; c) Southern blot with HRS probe; d) Southern blot with *E. coli* probe  
Lane1: genomic DNA; 2: 1kb ladder; 3: empty lane; 4-7: 2.1%g.e.; 8-11: 8.3% g.e.; 12-15: 25% g.e.; 16-19: 50% g.e.; 20:100% g.e.

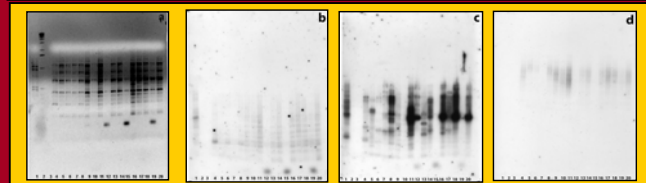
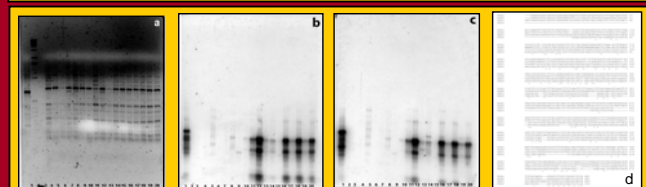


Figure 2: PCR and Southern blot analysis of primer pair s1+as2

- a) PCR; b) Southern blot with RGA probe; c) Southern blot with HRS probe; d) alignment of RGA and HRS sequences  
Lane1: genomic DNA; 2: 1kb ladder; 3: empty lane; 4-7: 2.1%g.e.; 8-11: 8.3% g.e.; 12-15: 25% g.e.; 16-19: 50% g.e.; 20:100% g.e.



## Results and Discussion:

Low incidence of RGA recovery in barley has been reported by Leister et al. (1998) and Brueggeman et al. (2001). Leister et al. (1998) found that degenerate PCR of barley genomic DNA resulted in non-RGA amplification. They hypothesized this was due to its large genome size. Brueggeman et al. (2001) stated species with large genomes often produce PCR fragments with no apparent homology to R genes. The use of pools of BAC DNA as a template for degenerate PCR resulted in improved recovery of RGAs in barley (Brueggeman et al., 2001).

Using genomic DNA from the wild potato *S. bulbocastanum* as a template, we have demonstrated low incidence of RGA recovery. A significant percentage of recovered clones have originated from highly repetitive DNA. Leister et al. (1996) also reported amplification of non-RGA repetitive DNA in cultivated potato, a species closely related to *S. bulbocastanum*. Towards improved recovery of RGAs, we adapted a pooled BAC template DNA approach for *S. bulbocastanum*. We hypothesize that due to the enrichment of euchromatic, gene-rich regions of the genome through BAC library creation, highly repetitive sequences can potentially be reduced.

To determine the consistency of amplification between BAC pools vs. genomic DNA and between BAC pools representing varying percentages of the genome, several degenerate PCR primer combinations were tested with each template preparation. Banding patterns differed between genomic DNA and BAC pools. Some similarly sized fragments were recovered from both template DNA sources but we cannot yet confirm fragment homology. Sequencing analyses are ongoing.

Reactions using BAC DNA as a template reveal that 2.1% genomic equivalent (g.e.) was insufficient to allow generation of consistent banding patterns across independent replicates (Fig. 1, 2a, and 3a). Banding patterns were more consistent (nearly identical) when template DNA contained 8.3% g.e. Based on both PCR and Southern data, we suggest that a minimum of 25% g.e. be used for RGA studies. At 25, 50, and 100% g.e., we see a consistent banding pattern regardless of the clone constitution of the pools. Although any specific RGA fragment may not be generated from only 25% g.e., representatives of each RGA homolog class may be generated.

Southern blot analysis using RGA and highly repetitive sequence (HRS) probes show the presence of similar products in the BAC pools and genomic DNA (Figures 2b&c and 3b&c). Labeled *E. coli* genomic DNA revealed only weak hybridization (Figure 2d) for both primer sets. Labeled BAC vector showed no hybridization to the amplification products of either primer combination (data not shown). Of particular interest is the nearly identical hybridization patterns of the RGA and HRS probes for the s2+motif1 primer pairs (Figure 3b&c).

Sequence analysis of the RGAs and HRS sequences generated with this primer pair revealed significant homology between these sequences (Figure 3d). BLAST results indicate high homology of the HRS to ribosomal intergenic spacer (IGS) and 2D8 rDNA repeat elements of *S. bulbocastanum* ( $e = 3e-20$  and  $1e-06$ ) as well as to other rDNA repeat and IGS sequences of *S. tuberosum* ( $e = 9e-08$  and  $3e-04$ ). There was no significant homology between HRS sequence and IGS sequences from related species, such as tomato (Figure 4). Non-significant ( $e = 1.3 - 5.0$ ) homology was detected between HRS and RGA sequences of *Manihot esculenta*, *Lycopersicon esculentum*, and *L. hirsutum* (Figure 5). This suggests that amplification of HRS and other repeat sequences via degenerate PCR may be a problem for potato species but not for closely related species such as tomato. Consistently, amplification of repeat sequence has been reported for potato (Leister et al. 1996) but not for tomato (Pan et al. 2000; Zhang et al. 2002).

Figure 4: cp Phylogeny of the Solanaceae

(Adapted from D. Spooner, USDA—ARS)

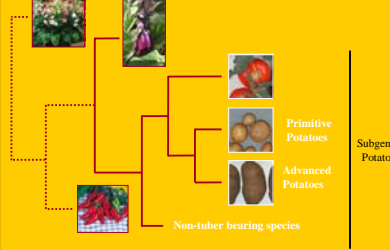


Figure 5: Alignment of HRS and non-potato species homologs

M.e. = *Manihot esculenta*  
L.e. = *Lycopersicon esculentum*  
L.h. = *L. hirsutum*

Brueggeman R, Druka A, Kudrna D, Kleinbols A. (2001) Efficient cloning of resistance gene analogs from barley. *Barley Genetics Newsletter*, 31:24-27.  
Leister D, Bailvora A, Salamini F, Gebhardt C. (1996) A PCR-based approach for isolating pathogen resistance genes from potato with potential for wide application in plants. *Nature Genetics*, 14: 421-425.  
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