

GENERATION AND CHARACTERIZATION OF cDNA CLONES FROM *SARCOPTES SCABIEI* VAR. *HOMINIS* FOR AN EXPRESSED SEQUENCE TAG LIBRARY: IDENTIFICATION OF HOMOLOGUES OF HOUSE DUST MITE ALLERGENS

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Abstract. Molecular studies on scabies, a disease of considerable human and veterinary significance, have been limited because of the difficulty of obtaining the causative organism *Sarcoptes scabiei*, the “itch mite.” We have used skin from the bedding of crusted scabies patients as a source of mites for the construction of libraries of cDNAs from *S. scabiei* var. *hominis* in the bacteriophage λ vector λ ZAP express. Sequences of 145 clones established that the libraries predominantly contain sequences from *S. scabiei*, enabling a major sequencing program to begin. Among those sequenced to date, cDNAs encoding *S. scabiei* homologues of 3 house dust mite allergens—the M-177 apolipoprotein, glutathione S-transferase, and paramyosin—were identified. The availability of cDNA libraries from *S. scabiei* var. *hominis* and *S. scabiei* var. *vulpes* and the emerging public sequence databases from both opens up new possibilities in scabies research.

INTRODUCTION

Scabies is endemic among some disadvantaged people, such as those in remote northern and central Australian Aboriginal communities. It is estimated that about 300 million people in the developing world are affected,¹ and it is a significant veterinary problem. As well as the intense itching, there are serious sequelae resulting from bacterial colonization of the burrows in the epidermis formed by the causative organism, *Sarcoptes scabiei*, including septicemia, renal damage,² and, it now appears, rheumatic fever and as a consequence rheumatic heart disease.³ We have discussed the rationale for a scabies vaccine and the likely relevance of house dust mite allergen homologues in the accompanying article by Harumal et al.

Molecular studies on scabies have been limited to date because of the difficulty of obtaining sufficient mites. A system of growing dog-derived mites on the ears of New Zealand white rabbits, which provides access to *S. scabiei* var. *canis* and allows the testing of potential vaccines, has been developed.^{4,5} A library of cDNA clones from *S. scabiei* var. *vulpes* has been constructed from mites obtained from red foxes, and the sequence of a cDNA clone encoding paramyosin has been characterized.⁶ In the accompanying article by Harumal et al., we describe the construction of a cDNA expression library cloned in the vector pGEX4T-2 from *S. scabiei* var. *hominis*, the isolation of a homologue of the house dust mite allergen Mag/M-177^{7,8} by immunoscreening and a vaccination/challenge trial with recombinant fused polypeptide in the rabbit/*S. scabiei* var. *canis* system. We report here the construction of cDNA libraries from *S. scabiei* var. *hominis* cloned in a bacteriophage λ vector (λ ZAP express, Stratagene, La Jolla, CA). This has the advantage of using the λ packaging system to generate a large number of clones from small amounts of cDNA. Then they can be converted *en masse* into plasmids suitable for sequencing after library amplification.

S. scabiei var. *hominis* mites were collected, and mRNA was prepared from approximately 1,000 mites as described (see Harumal et al.). Approximately 65% of the purified mRNA was used to prepare oligo-dT primed, double-stranded cDNA, which was directionally cloned into the λ ZAP express vector using Stratagene's λ ZAP express cDNA

synthesis kit (catalogue No. 200403). cDNA was size fractionated on a Sepharose CL-2B column to yield 3 fractions. The fractions were ligated independently into the vector and packaged using Stratagene's Gigapack III gold packaging extracts (catalogue No. 200451). A total of approximately 3×10^5 independent clones were generated in the 3 primary libraries Yv4 (with the largest cDNA), Yv5, and Yv6. The primary libraries were amplified yielding between 3×10^9 and 2×10^{10} plaque forming units/ml. Clones were excised to phagemids as described by the manufacturer and used to infect *Escherichia coli* strain XL0LR before sequencing. Insert sizes ranged from 300 bp to at least 2.5 kb.

Twenty phagemid clones from each of the 3 libraries were sequenced by the dideoxy chain termination procedure from the T3 primer using ABI PRISM BigDye terminator (Applied Biosystems, Foster City, CA, part No. 4303153) and an ABI PRISM 377 DNA sequencer. These sequences were manually trimmed of vector and low-quality sequence. An additional 96 clones from the Yv4 library were sequenced in 96-well plate format from the T3 primer using BigDye terminator and an ABI PRISM 3700 capillary DNA analyzer, under contract by the Australian Genome Research Facility. The program phred⁹ was used for automatic base calling and trimming of vector and low-quality sequences (phred score <20).

All 145 successful sequences were compared with the GenBank nonredundant (nr) databases using the basic local alignment search tool (BLAST).¹⁰ Sequences also were compared with the GenBank expressed sequence tag (EST) database using BLASTn, to check for identity with ribosomal RNAs or any of the available *S. scabiei* var. *vulpes* anonymous ESTs. We had found previously (see Harumal et al.) that the cDNA was contaminated with human DNA, presumably from skin cells in the gut of the mites; it was important to ascertain the level of contamination in the λ ZAP express library before starting a major EST project. Sequences giving a best BLASTn result against a human DNA sequence with a score of ≥ 200 (chosen to minimize the number falsely assigned) against the GenBank nr database were considered to be human DNA contamination ($n = 3$), whereas those with a best BLAST result of $E > 0.01$ were not considered to have a statistically significant match ($n = 39$). For the remaining sequences ($n = 103$), the E value, accession number, and

TABLE 1
S. scabiei ESTs—Most significant BLAST result

Clone	Accession no.	BLAST type, E value, accession no., and description
Yv4-1	BM276587	n, e^{-121} , X90464— <i>Eurypelma californica</i> 28S rRNA gene
Yv4-3	BM276588	n, 0.007, AC013727— <i>Homo sapiens</i> clone RP11-451G1
Yv4-4	BM276589	x, $8e^{-10}$, AAC47445— <i>Cymodusa distincta</i> mariner transposase
Yv4-5	BM276590	x, $1e^{-39}$, AAF49332— <i>Drosophila melanogaster</i> CG7571 gene product
Yv4-6	BM276591	x, $4e^{-31}$, P91927— <i>D. melanogaster</i> calcium-binding mitochondrial protein
Yv4-8	BM276592	x, $4e^{-16}$, NP_034192— <i>Mus musculus</i> deoxyribonuclease II
Yv4-9	BM276593	x, $8e^{-19}$, T17121— <i>Chironomus thummi</i> CPY protein
Yv4-10	BM276594	x, $9e^{-68}$, BAA91939— <i>H. sapiens</i> unnamed protein product
Yv4-11	BM276595	x, $3e^{-23}$, NP_068807— <i>H. sapiens</i> sphingosine kinase 1
Yv4-12	AF462189	x, $2e^{-45}$, AAF61565— <i>Boophilus microplus</i> cathepsin L-like proteinase
Yv4-13*	BM276596	n, e^{-126} , AF022032— <i>Histiogomata</i> sp. 18S ribosomal RNA gene
Yv4-14	BM276597	n, $7e^{-4}$, AJ293903—Human adenovirus type 2 hexon gene
Yv4-15	BM276598	x, $2e^{-54}$, CAA65156— <i>Anopheles gambiae</i> immune factor 1
Yv4-17	BM276599	x, $1e^{-31}$, P52481— <i>Rattus norvegicus</i> adenylyl cyclase-associated protein
Yv4-19	BM276600	x, $2e^{-15}$, NP_037023— <i>R. norvegicus</i> very long chain Acyl-CoA dehydrogenase
Yv4-20	BM276601	n, $2e^{-13}$, AL109943—human DNA sequence from clone RP4-657D12
Yv5-2	BM276602	x, $1e^{-56}$, P07687— <i>R. norvegicus</i> epoxide hydrolase
Yv5-3	BM276603	x, $3e^{-33}$, AAF55390— <i>D. melanogaster</i> cher gene product
Yv5-7*	BM276604	x, $2e^{-13}$, T19130— <i>Caenorhabditis elegans</i> hypothetical protein
Yv5-8*	AF462192	x, $3e^{-7}$, CAC79251— <i>Adalia decempunctata</i> cytochrome oxidase subunit II
Yv5-9*	BM276606	x, $3e^{-6}$, T14306—carrot glycine-rich protein
Yv5-10	BM276607	x, $2e^{-9}$, NP_071888— <i>H. sapiens</i> myeloid leukemia factor 1
Yv5-11*	BM276608	x, $2e^{-10}$, T19130— <i>C. elegans</i> hypothetical protein C09F9.2
Yv5-13	BM276609	x, $3e^{-34}$, AAH07216— <i>H. sapiens</i> unknown (protein for MGC:14986)
Yv5-16	BM276610	n, 0, AF212167— <i>Limulus polyphemus</i> ITS1 partial sequence, 5.8S ribosomal RNA gene, ITS2 and 28S ribosomal RNA gene
Yv5-17	BM276611	x, $6e^{-96}$, T47225— <i>Xenopus laevis</i> replication licensing factor MCM2
Yv5-18*	BM276612	n, $2e^{-4}$, AC025721— <i>C. elegans</i> cosmid Y48G8AL
Yv5-19	BM276613	x, $1e^{-17}$, AAF58539— <i>D. melanogaster</i> CG13165 gene product
Yv5-20	BM276614	x, $6e^{-52}$, NP_110407— <i>H. sapiens</i> folate transporter/carrier
Yv6-1	BM276615	x, $3e^{-47}$, NP_110407— <i>H. sapiens</i> folate transporter/carrier
Yv6-2	BM276616	x, $2e^{-68}$, P21914— <i>D. melanogaster</i> succinate dehydrogenase
Yv6-4	BM276617	x, $1e^{-33}$, AAK84924— <i>D. melanogaster</i> SD01170p
Yv6-5	BM276618	x, $8e^{-49}$, BAB30367— <i>M. musculus</i> putative
Yv6-6	AF462196	x, $5e^{-54}$, AAF14270— <i>Euroglyphus maynei</i> high-molecular-weight allergen M-177 precursor
Yv6-7	BM276619	x, $4e^{-35}$, AAH08578— <i>H. sapiens</i> RIKEN cDNA C030006K11 gene
Yv6-8	BM276620	n, 0.01, AC019057— <i>H. sapiens</i> chromosome 2 clone RP11-121A1
Yv6-9	BM276621	n, $1e^{-4}$, AJ002015— <i>Propionigenium modestum</i> mmdD, mmdC, mmdB genes and partial mmdA gene
Yv6-11	BM276622	x, $6e^{-49}$, CAC38784— <i>Suberites domuncula</i> FK506-binding protein
Yv6-12*	BM276623	x, 0.001, T14306—carrot glycine-rich protein
Yv6-13*	BM276624	x, $2e^{-11}$, P02517— <i>D. melanogaster</i> heat-shock protein 26
Yv6-15*	BM276625	n, $1e^{-4}$, AC025721— <i>C. elegans</i> cosmid Y48G8AL
Yv6-16	BM276626	n, 0, AF212167— <i>L. polyphemus</i> ITS1 partial sequence, 5.8S ribosomal RNA gene, ITS2 and 28S ribosomal RNA gene
Yv6-17*	AF462191	x, $6e^{-78}$, AAD15716— <i>Colias eurytheme</i> cytochrome oxidase subunit I
Yv6-19	BM276627	x, $3e^{-44}$, AAF53005— <i>D. melanogaster</i> CG6729 gene product
Yv6-20	BM276628	x, $2e^{-18}$, CAC28026— <i>M. musculus</i> b,b-carotene-9',10'-dioxygenase
Yv4A03	BM276629	x, $2e^{-2}$, NP_005888— <i>H. sapiens</i> a particle-promoted polypeptide
Yv4A04	BM276630	x, $2e^{-17}$, NP_055079— <i>H. sapiens</i> serine/threonine kinase 18
Yv4A08	AF462190	x, $1e^{-82}$, P46419— <i>Dermatophagoides pteronyssinus</i> major allergen, glutathione S transferase
Yv4A09	BM276631	x, $2e^{-11}$, T24918— <i>C. elegans</i> 3'-phosphoadenosine-5'-phosphosulfate synthetase
Yv4A12	BM276632	x, $2e^{-49}$, AAF47787— <i>D. melanogaster</i> CG10860 gene product
Yv4B01	BM276633	x, $4e^{-32}$, AAF51107— <i>D. melanogaster</i> CG3248 gene product
Yv4B02	BM276634	x, $4e^{-41}$, NP_003744— <i>H. sapiens</i> translation initiation factor 3
Yv4B03	AF462195	n, $1e^{-18}$, AF317670— <i>S. scabiei</i> paramyosin mRNA
Yv4B04	BM276635	x, $2e^{-11}$, AAH12125— <i>H. sapiens</i> up-regulated by BCG-CWS
Yv4B07	BM276636	x, $1e^{-21}$, P31403— <i>Manduca sexta</i> vacuolar ATP synthase
Yv4B08	BM276637	x, 0.001, NP_032158— <i>M. musculus</i> glutamine repeat protein 1
Yv4B09	BM276638	x, $1e^{-53}$, CAC17590— <i>H. sapiens</i> bA101E13.3 (ubiquitin C-terminal hydrolase UCH37)
Yv4B10	BM276639	x, $1e^{-10}$, XP_051425— <i>H. sapiens</i> glycogenin
Yv4B11	BM276640	x, $7e^{-30}$, AAC97504— <i>Aedes aegypti</i> peroxidase
Yv4B12	BM276641	x, $4e^{-14}$, Q9VH95— <i>D. melanogaster</i> hypothetical protein CG16817
Yv4C01	BM276642	x, $3e^{-29}$, 1A9T—bovine purine nucleoside phosphorylase
Yv4C02	BM276643	x, $3e^{-9}$, S42919— <i>D. melanogaster</i> glutamate dehydrogenase
Yv4C03	BM276644	x, $2e^{-32}$, AAD55413— <i>D. melanogaster</i> BcDNA.GH04120
Yv4C04	BM276645	x, $2e^{-06}$, T28724— <i>C. elegans</i> hypothetical protein F10D2.10
Yv4C06*	BM276646	n, $2e^{-4}$, AC025721— <i>C. elegans</i> cosmid Y48G8AL
Yv4C07	BM276647	n, 0.003, AF109076— <i>H. sapiens</i> chromosome 7 map 7q36 BAC H6
Yv4C09	BM276648	x, $1e^{-25}$, BAA96953— <i>Arabidopsis thaliana</i> ubiquinone/menaquinone biosynthesis methyltransferase-like

TABLE 1
S. scabiei ESTs—Most significant BLAST result (Continued)

Clone	Accession No.	BLAST type, E value, accession no., and description
Yv4C10	BM276649	x, 9e ⁻⁶² , P51149—Human Ras-related protein Rab-7
Yv4D01	AF462196	x, 8e ⁻⁸⁰ , P39673— <i>Dermatophagoides farinae</i> allergen Mag
Yv4D03	BM276650	x, 2e ⁻⁵⁹ , AAF46507— <i>D. melanogaster</i> CG3001 gene product
Yv4D05	BM276651	x, 1e ⁻⁴⁵ , AAD09246— <i>R. norvegicus</i> lipoprotein-binding protein
Yv4D07	BM276652	x, 0.003, A45555— <i>Plasmodium falciparum</i> glutamate-rich protein
Yv4D08	BM276653	x, 6e ⁻⁵⁴ , AAF43421— <i>D. melanogaster</i> epsin-like protein
Yv4D09	AF462193	x, 8e ⁻¹⁸ , 1BCCB—chain B, cytochrome Bc1 complex from chicken
Yv4D11	BM276654	x, 4e ⁻³⁷ , CAA75690— <i>D. melanogaster</i> hormone receptor 38
Yv4E01	AF462194	x, 1e ⁻⁰⁶ , T30940— <i>Pimpla nipponica</i> vitellogenin
Yv4E02	BM276655	n, 1e ⁻¹¹⁹ , X90464— <i>E. californica</i> DNA for 28S ribosomal RNA gene
Yv4E03	BM276656	x, 2e ⁻⁶⁴ , AAF47569— <i>D. melanogaster</i> α -Spec gene product
Yv4E04	BM276657	x, 2e ⁻⁰⁸ , P12261— <i>Artemia salina</i> elongation factor 1- γ
Yv4E05	BM276658	n, 7e ⁻⁵⁴ , AF212167— <i>L. polyphemus</i> internal transcribed spacer 1
Yv4E06	BM276659	x, 8e ⁻⁹¹ , P19388—human DNA-directed RNA polymerase II
Yv4E07	BM276660	x, 4e ⁻⁸⁹ , NP_058701— <i>R. norvegicus</i> hydratase
Yv4E09	AF462196	x, 2e ⁻⁴⁵ , P39673— <i>D. farinae</i> allergen Mag
Yv4F01	BM276661	x, 5e ⁻⁴⁷ , XP_003399— <i>H. sapiens</i> mannosidase, β A, lysosomal
Yv4F03	BM276662	x, 2e ⁻⁷⁴ , Q9VLS1— <i>D. melanogaster</i> probable phosphorylase b kinase
Yv4F04*	BM276663	x, 7e ⁻⁹ , NP_004860— <i>H. sapiens</i> suppressor of K ⁺ transport defect 1
Yv4F06	BM276664	x, 6e ⁻⁸³ , NP_031866— <i>M. musculus</i> DEAD box polypeptide 5
Yv4F07	BM276665	x, 2e ⁻³³ , AAF57776— <i>D. melanogaster</i> CG5784 gene product [alt 2]
Yv4F08*	BM276666	n, 1e ⁻⁷⁰ , AY004293— <i>Tegeticula carnerosana</i> cytochrome oxidase subunit I gene
Yv4F10	BM276667	x, 3e ⁻⁰⁹ , BAB24275— <i>M. musculus</i> putative
Yv4F11	BM276668	x, 1e ⁻⁵⁴ , BAB28500— <i>M. musculus</i> putative
Yv4G02	BM276669	x, 5e ⁻⁰⁵ , BAB28612— <i>M. musculus</i> putative
Yv4G03	BM276670	x, 2e ⁻⁴⁷ , AAA83150— <i>D. melanogaster</i> fizzy
Yv4G04	BM276671	x, 2e ⁻¹³ , CAB57225— <i>D. melanogaster</i> inhibitor-2
Yv4G05	BM276672	x, 3e ⁻³² , AAF04459— <i>Heliothis virescens</i> dihydrofolate reductase
Yv4G06	BM276673	x, 1e ⁻⁰⁶ , AAF57951— <i>D. melanogaster</i> CG5522 gene product [alt 1]
Yv4G07	BM276674	x, 2e ⁻³⁴ , CAA69203— <i>Strongylocentrotus purpuratus</i> mitochondrial ATP synthase α subunit precursor
Yv4G09	BM276675	x, 0.0004, CAB63407— <i>C. elegans</i> Pfam domain: PF01826
Yv4G10	BM276676	x, 0.002, NP_079423— <i>Homo sapiens</i> protein FLJ13448
Yv4G11*	BM276677	n, 0.002, AL139405—human DNA clone RP11-393E10
Yv4H01*	BM276678	n, 6e ⁻⁵⁴ , T32749— <i>C. elegans</i> hypothetical protein F57B10.3
Yv4H05	BM276679	x, 1e ⁻¹⁶ , T17332—human hypothetical protein DKFZp434D245.1
Yv4H07	BM276680	x, 2e ⁻¹⁸ , AAF50139— <i>D. melanogaster</i> CG11811 gene product
Yv4H08	BM276681	x, 3e ⁻¹⁰ , AAG50053— <i>Danio rerio</i> eIF4E-binding protein 3

* Sequences giving a best BLASTn match against the GenBank EST database with a *S. scabiei* var. *vulpes* EST.

description of the most significant BLAST result (BLASTn or BLASTx) are shown in Table 1. These sequences have been deposited at GenBank, and their accession numbers are listed in Table 1. The 39 sequences that did not give a significant match have been deposited in GenBank EST database as probable *Sarcoptes scabiei* var. *hominis* sequences under accession numbers BM276682 to BM276719 (except for YV4B03; see later). The closest matches to 56 of the sequences are nonvertebrate metazoan sequences, principally *Drosophila melanogaster* (n = 23) and *Caenorhabditis elegans* (n = 9), for which most such data have been published. These are exemplified by BLASTn matches to ribosomal sequences (Yv4-1, Yv4-13, Yv5-16, Yv6-16, Yv4E02, and Yv4E05), which have strong homology to lower metazoa.

In 2001, 395 *S. scabiei* var. *vulpes* ESTs were placed in the GenBank EST database. Fifteen sequences had a more significant result, with one of these ESTs using BLASTn against the GenBank EST database, than against the GenBank nr databases using BLASTn or BLASTx (marked with an * in Table 1). An additional 3 sequences (Yv5-6, Yv5-15, and Yv4H03) that had no significant BLAST matches against the GenBank nr databases had significant matches with ≥ 1 of the var. *vulpes* ESTs (data not shown). This initial characterization confirms that the library described here consists predominantly of clones derived from *S. scabiei* sequences with

limited contamination by human sequences and is suitable for the start of an EST sequence library.

Clones Yv4D01 and Yv4E09 had a most significant match to the house dust mite *Dermatophagoides farinae* Mag allergen,⁷ whereas clone Yv6-6 matched with the *Euroglyphus maynei* homologue, the high-molecular-weight allergen precursor M-177⁸ (Table 1). These sequences, corresponding to an apolipoprotein in hemolymph, are some of the most frequent cDNA clones in our library (Table 1 and unpublished data). We previously have identified a cloned sequence designated *S. scabiei* antigen 1 (Ssag1—EST database accession No. BM176880) corresponding to M-177, by screening with antibodies from scabies-infested rabbits (see Harumal et al.). The combined contiguous sequence of Yv4D01, Yv4E09, and Yv6-6 was submitted to GenBank as *S. scabiei* antigen 1 (Ssag1) with the accession number AF462196.

A BLASTx search using Yv4A08 revealed a highly significant match with the *D. pteronyssinus* allergen glutathione S-transferase (GST)¹¹ (E = 1e⁻⁸²; accession No. AAB32224). The complete coding sequence of the scabies GST was submitted to GenBank with the accession number AF462789. The *S. scabiei* GST homologue is a potential candidate for a scabies allergen.

Clone Yv4B03 is included in Table 1 despite being classified as a technical failure (phred score <20), as a BLASTn

search using the low-quality data revealed homology with the *S. scabiei* var. *vulpes* paramyosin clone⁶ ($E = 1e^{-18}$; accession No. AF317670), the only scabies protein coding sequence previously published. Further sequencing of Yv4B03 (accession No. AF462195) allowed the comparison of the fox and human mite sequences over a 1,948-bp region, which showed that the fox mite sequence differed at 1 nucleotide position and had an insertion of 3 nucleotides.

Clone Yv4-12 displayed significant homology at the amino acid level with cysteine proteases, in particular the *Boophilus microplus* cathepsin L-like enzyme¹² ($E = 2e^{-45}$; accession No. AAF61565), a cysteine protease with optimal enzyme activity occurring at an acidic pH. We previously have discussed the hypothesis that hydrolytic enzymes of the scabies gut may be potential vaccine molecules (see Harumal et al.). It would be relevant to investigate whether this molecule is an enzyme secreted into the gut of the mite.

Clone Yv4E01 was homologous to vitellogenin from *Pimpla nipponica* ($E = 1e^{-06}$), and further sequencing has revealed much more extensive homology (unpublished data). Egg antigens are the targets of delayed-type hypersensitivity reactions in schistosomiasis and have been used successfully as vaccines that suppressed granuloma formation and reduced mortality in *Schistosoma mansoni* infected mice.¹³ Although nothing is known at the moment, it is possible that egg antigens play some role in the immunology of scabies. A new avenue of investigation will be opened by the identification of such clones.

Two clones (Yv5-9 and Yv6-12) encoded a protein with a substantial number of internal repeats. Because this is an abundant molecule (unpublished results), it would be relevant to investigate whether it is the target of antibody responses in humans or animals or both. If so, it could be a candidate immunodiagnostic molecule.

Many molecules identified here offer the potential to extend previous studies on the relationships of scabies mites from various mammalian hosts, including the clones Yv6-17 encoding cytochrome oxidase subunit I, Yv5-8 encoding cytochrome oxidase subunit II, and Yv4D09 encoding the cytochrome Bc1 complex. Although microsatellite studies led us to conclude that mites clustered by host rather than geographic origin,¹⁴ studies on a fragment of the mitochondrial 12S RNA gene agreed with the concept that all mites are the same species.¹⁵

Limitations on availability of experimental material from which to generate *S. scabiei* var. *hominis* cDNA libraries have been overcome here by using mites obtained from skin fragments in the bedding of crusted scabies patients and by taking advantage of the highly efficient λ ZAP express/packaging system. The 3 *S. scabiei* var. *hominis* cDNA libraries described here have allowed the start of a program to generate 50,000 scabies sequences. Although the current libraries will require normalization to remove abundant sequences¹⁶ when the rate of new sequence acquisition in this program declines, they are sufficiently large (3×10^5 clones) to allow this. The beginning of EST databases derived from mites from humans and foxes and our demonstration that it is now technically feasible to carry out vaccination/challenge studies with cloned scabies antigens (see Harumal et al.) should facilitate greatly research on scabies.

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REFERENCES

1. Taplin D, Meinking TL, Chen JA, Sanchez R, 1990. Comparison of crotamiton 10% cream (Eurax) and permethrin 5% cream (Elimite) for the treatment of scabies in children. *Pediatr Dermatol* 7: 67-73.
2. Hoy W, 1996. Renal disease in Australian Aborigines. *Med J Aust* 165: 126-127.
3. Currie B, Carapetis J, 2000. Skin infections and infestations in Aboriginal communities in northern Australia. *Australas J Dermatol* 41: 139-143.
4. Arlian LG, Rapp CM, Morgan MS, 1995. Resistance and immune response in scabies-infested hosts immunised with *Dermatophagoides* mites. *Am J Trop Med Hyg* 52: 539-545.
5. Arlian LG, Runyan RA, Estes SA, 1984. Cross infestivity of *Sarcoptes scabiei*. *J Am Acad Dermatol* 10: 979-986.
6. Mattsson JG, Ljunggren EL, Bergstrom K, 2001. Paramyosin from the parasitic mite *Sarcoptes scabiei*: cDNA cloning and heterologous expression. *Parasitology* 122: 555-562.
7. Aki T, Ono K, Paik SY, Wada T, Jyo T, Shigeta S, Murooka Y, Oka S, 1994. Cloning and characterization of cDNA coding for a new allergen from the house dust mite, *Dermatophagoides farinae*. *Int Arch Allergy Immunol* 103: 349-356.
8. Epton M, Dilworth R, Smith W, Hart B, Thomas W, 1999. High-molecular weight allergens of the house dust mite: an apolipoprotein-like cDNA has sequence identity with the major M-177 allergen and the IgE-binding peptide fragments Mag1 and Mag3. *Int Arch Allergy Immunol* 120: 185-191.
9. Ewing B, Hillier L, Wendl M, Green P, 1998. Base-calling of automated sequencer traces using phred: I. accuracy assessment. *Genome Res* 8: 175-185.
10. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ, 1990. Basic local alignment search tool. *J Mol Biol* 215: 403-410.
11. O'Neill GM, Donovan GR, Baldo BA, 1994. Cloning and characterization of a major allergen of the house dust mite, *Dermatophagoides pteronyssinus*, homologous with glutathione S-transferase. *Biochim Biophys Acta* 1219: 521-528.
12. Renard G, Garcia JF, Cardoso FC, Richter MF, Sakanari JA, Ozaki LS, Termignoni C, Masuda A, 2000. Cloning and functional expression of a *Boophilus microplus* cathepsin L-like enzyme. *Insect Biochem Mol Biol* 30: 1017-1026.
13. Sun J, Stadecker M, Mielcarek N, Lakew M, Li B, Hernandez H, Czerkinsky C, Holmgren J, 2001. Nasal administration of *Schistosoma mansoni* egg antigen-cholera B subunit conjugate suppresses hepatic granuloma formation and reduces mortality in *S. mansoni*-infected mice. *Scand J Immunol* 54: 440-447.
14. Walton S, Low Choy J, Bonson A, Valle A, McBroom J, Taplin D, Arlian L, Mathews J, Currie B, Kemp D, 1999. Genetically distinct dog-derived and human-derived *Sarcoptes scabiei* in scabies-endemic communities in northern Australia. *Am J Trop Med Hyg* 61: 542-547.
15. Skerratt LF, Campbell NJH, Walton S, Kemp D, Barker SC, 2002. The mitochondrial 12S gene is a suitable marker of populations of *Sarcoptes scabiei* from wombats, dogs and humans in Australia. *Parasitol Res* 88: 373-379.
16. Berry R, Stevens TJ, Walter NA, Wilcox AS, Rubano T, Hopkins JA, Weber J, Goold R, Soares MB, Sikela JM, 1995. Gene-based sequence-tagged sites (STSs) as the basis for a human gene map. *Nat Genet* 10: 415-423.