ATP-dependent chromatin remodeling complexes in the Asian citrus psyllid, *Diaphorina citri*

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Introduction

Chromatin remodeling and regulation is a fundamental process in eukaryotes and has been studied mostly in humans, mice, and fruit flies (Cowell et al 1997). Gene expression patterns and responses to the environment in aphids have been associated with chromatin structure and remodeling (Rider et al. 2010). Eukaryotes possess chromatin remodeling complexes that regulate gene expression using histone modifying enzymes and ATPases, which have ATPase domains with seven helicase motifs (Eisen et al. 1995). Chromatin remodeling ATPases have been the subject of RNAi treatment in *Diabrotica virgifera* and *Euschistus heros* (Fishelivech et al. 2016). Further RNAi experiments may prove that these ATPases are valuable targets for controlling agriculturally important insect pests, including the vector of citrus greening, *Diaphorina citri*. Studying the different chromatin complexes and proteins that play a role in gene expression patterns may allow for a better understanding of how *D. citri* responds epigenetically to environmental or developmental factors.

There are four classes of remodelers with different ATPases and accessory subunits, including SWI/SNF, CHD, ISWI, and INO80 (Reddy et al. 2010). The family of chromodomain helicase DNA-binding proteins (CHD) were first identified in the mouse, then related proteins were reported in other eukaryotes. In *Drosophila*, the CHD family has four members including dCHD1, dCHD3, dMI-2 and Kismet, also known as CHD7 in other organisms (Woodage 1997). CHD1 was the first chromodomain protein to be identified in eukaryotes. In *Drosophila*, it has been found to play a

role in elongation and localization of puffs of polytene chromosomes (Bouazoune 2006). CHD1 also was found to interact with histone H3 tails methylated at residue K4. Accordingly, it was deduced that CHD1 plays a role in mobilizing chromatin structure and therefore promoting transcription. Mi-2 is a CHD member that is known for its ATPase activity in histone remodeling complexes including nucleosome remodeling histone deacetylase (NuRD) (Kehle et. Al 1998). The specific mechanism by which Mi-2 works is still undefined (Rider 2010).

Since the discovery of the chromatin remodeling SWI/SNF complex in yeast, more ATP-dependent complexes have been identified in other organisms, notably three groups in *Drosophila* (Feng and Zhang, 2003). The first group of ATP-dependent remodelers are SWI/SNF related complexes containing SWI2/SNF2-like ATPases and include Brahma-associated proteins (BAP) and Polybromo-associated BAP (PBAP) complexes (Feng and Zhang, 2003). The second group uses ISWI or ISWI-like ATPases and includes chromatin accessibility complex (CHRAC), ATP-utilizing chromatin assembly and remodeling factor (ACF), and nucleosome remodeling histone deacetylase (NURF). The third group has Mi-2 as the ATPase and includes the nucleosome remodeling histone deacetylase (NURD) complex.

In *D. citri*, we identified and annotated 6 chromatin remodeling complexes, including 27 genes coding for SNF2 members, INO8 and the CHD family, as well as different subunits forming these complexes. These genes have an important role in regulating gene expression and chromatin assembly (Bouazoune et al 2006), which makes them a great target for potential RNAi treatment for *Diaphorina citri,* as it has been targeted in other insects (Fishelivech et al. 2016).

Results and Discussion

SNF2 Family of chromatin remodelers

Single orthologs for CHD1, CHD7 and INO80 have been identified in *D. citri.* An orthologous sequence for CHD3 (Mi2α) could not be located in the 2.0 version of the *D. citri* genome. In *Drosophila*, humans, and other vertebrates, CHD3 (Mi-2α) is a shorter, truncated version of CHD4 (Mi-2β) (Bouazoune et al. 2006). In addition, other closely related organisms including *A. pisum* lack a copy of CHD3 (Mi-2α) (Rider et al 2010). In *D. citri*, two copies of the Mi-2β gene were identified and annotated. It therefore appears that the duplication of the Mi-2β gene is unique to *D. citri* (Figure1). In contrast, *Drosophila* has only one single copy of each of Mi-2α and Mi-2β (Table 1). Annotation results also show that chromatin remodeling complexes in *D.citri* match to the Dipteran model, and contains all the subunits found in *D. melanogaster* (Tanigushi et al. 2014).

NURD Complex

Nurd complex in *D.citri* contains a remodeling chromodomain ATPase subunit Mi-2, a member of the CHD family, coupled with histone deacetylase Rpb3 (Figure3). Although there have been some variations in the Nurd complexes studied so far in vertebrates, the main proteins in the Nurd complex are Mi2β (ATPase), HDAC 1 and 2 (Rpd3), MTA2/3, P66, and MBD proteins (Reddy et al 2010). The *Drosophila* counterparts share high identity with the vertebrate proteins (Feng et al. 2003). Based on phylogenetic analysis, the *D. citri* Mi-2β ATPase is closely related to its *A.pisum* ortholog (Figure1) sharing an amino acid identity of 68%. All annotated *D. citri* orthologs of the Nurd proteins, with the exception of p55 and p66, contain homeobox-binding domains, which

supports the NuRD complex being involved in embryonic development by down regulating HOX genes as in *Drosophila* (Feng and Zhang, 2003).

ACF Complex

In the ATP-utilizing chromatin assembly and remodeling factor (ACF), the activity of the ATPase, ISWI, is regulated by Acf1 (Figure 3) (Ito et al., 1999). Acf1 is a protein related to WSTF in humans that when deleted it causes Williams' syndrome (Lu et al., 1998; Ito et al., 1999). ACF1 and ISWI are both single-copy genes in *D.citri* as well as other closely related insects (Table1).

CHRAC Complex

The chromatin assembly complex (CHRAC), has been identified in *Drosophila* as an ISWI-containing regulator of the initiation of replication (Alexiadis et al. 1998). CHRAC is also required for repositioning of the nucleosomes to activate ribosomal DNA promoters (Längst et al. 1998). This complex also contains ACF1, in addition to two subunits: CHRAC1 and CHRAC2 (Figure3). In *D.citri*, one copy of each of ACF1 and ISWI were identified, as well as the two subunits CHRAC1 and CHRAC2 (Table 1). BLAST results of annotated sequences of the CHRAC subunits show that CHRAC2 is a DNA polymerase epsilon subunit that contains a histone H4 domain. Whereas CHRAC1 contains a histone-like transcription factor domain (Pfam08008), and a transcription repressor. Minimal research is available on CHRAC1 and CHRAC2 in closely related insects.

NURF Complex

In addition to the ISWI ATPase, two ISWI-associated proteins have been characterized in *Drosophila* as subunits in the nucleosome remodeling factor (NURF) complex (Figure3), including a WD repeat protein called p55 (Martinez-Balbas et al. 1998). p55, and other WD repeat proteins,

are also associated with the NuRD complex. NURF-38 is an inorganic pyrophosphatase that has been shown to have a role in regulating the activity of the NURF complex (Gdula et al. 1998). Three out of the four annotated orthologous subunits of NURF- ISWI, NURF-38, and NURF301 are located in *D.citri* as single copy genes. p55 accessory protein is also a single copy gene in other closely related organism (Table 1). However, two copies of p55 were found in the 2.0 version of the *D.citri* genome.

BRM complexes

The BRM complexes include Brahma-associated proteins (BAP) and polybromo-associated BAP (PBAP). BAP and PBAP share most subunits (Figure 3). BAP, however, contains OSA subunit, whereas PBAP contains Polybromo and BAP170 (an AT-rich protein). Interactions between subunits have been studied with a far-western assay blots (Kal et al. 2000), which showed that MOR binds to BRM (Crosby et al. 1999), Bap111, Bap60, and Bap47 or Bap45. In the *D.citri* genome, orthologous sequences of all the common subunits, as well as OSA in BAP, Polybromo and BAP170, also known as AT-rich interactive domain containing protein 2, were located as single-copy genes. In addition, 3 copies of the actin subunit BAP47 (actin42A) were identified in *D.citri* (Table1). Phylogenetic analysis shows that *D.citri´*s actin42A groups with other Hemiptera (Figure 2). In *Drosophila*, beta-centractin, a paralog of actin42A, was also shown to bind to the BRM complexes (Zhao et al 1998). However, a beta-centractin ortholog could not be found in the 2.0 version of the genome.

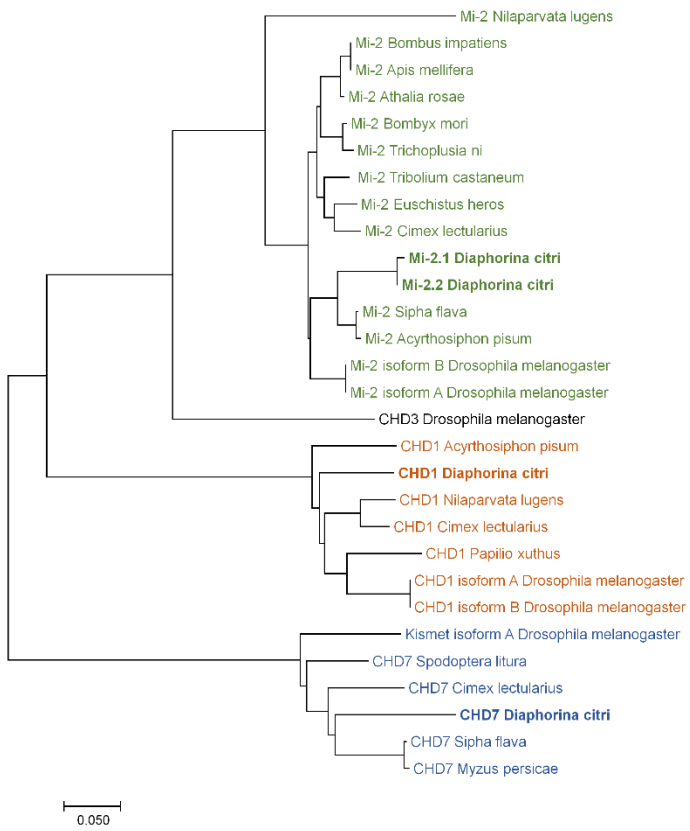

Figure 1: Phylogenetic relationships between proteins in the CHD family from different insects. *Drosophila melanogaster*, *Tribolium castaneum*, *Acyrthosiphon pisum, Sipha flava, Nilaparvata lugens, Bombyx moris*, *Myzus persicae*, *Euschiatus heros,* and *Diaphorina citri*, are represented in the phylogram. Multiple sequence alignment (ClustalX) for overlapping regions of the sequences were used to generate a rooted Neighbor-joining tree. Evolutionary analyses were conducted in MEGA7. Accession numbers for orthologous sequences used to generate the tree in sequential order from top to bottom: NP649154.2, NP001014591.1, XP004921642.1, XP026742775.1, XP 012269327.1, XP012247679.1, XP016768935.1, XP008197696.1, AML25531.1, XP024084974.1, ACYPI56610-PA, XP025415391.1, XP022201384.1, NP649111.1, XP008187602.1, XP008187604.1, NP 477197.1, NP 001245851.1, KPI94909.1, XP022197424.1, XP014258160.1, XP025425891.1, XP 022175648.1, NP 523441.1, XP 022820442.1, XP 014247633.1

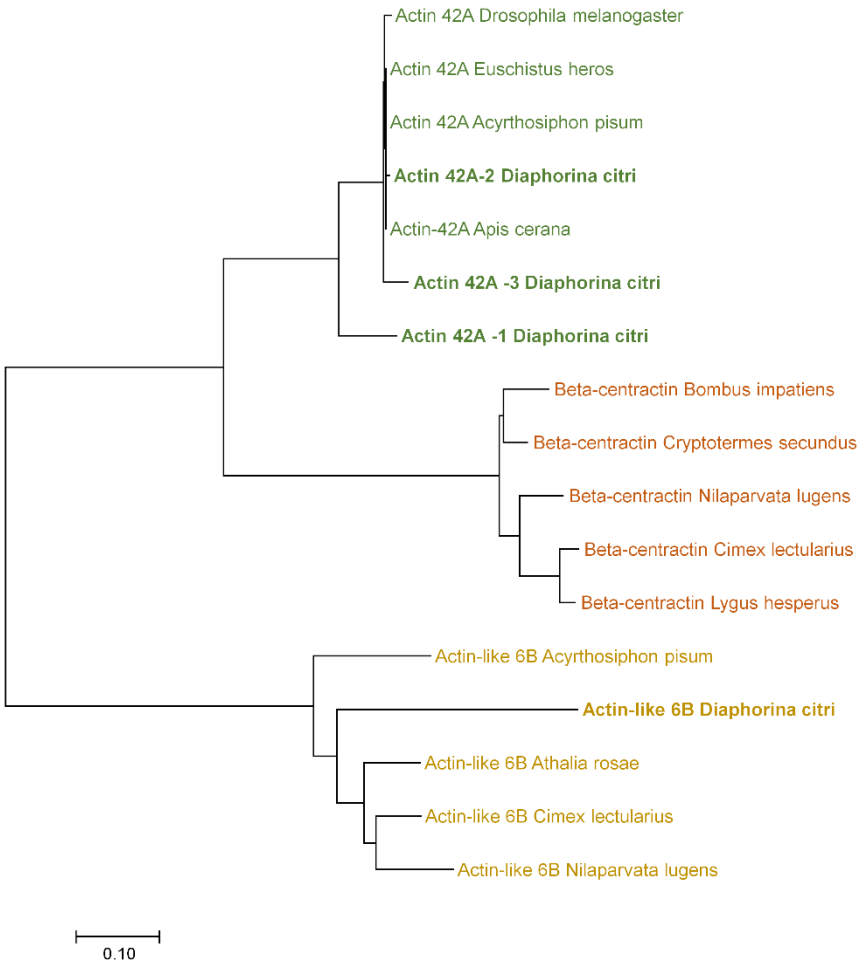

Figure 2: Phylogenetic relationships between actin proteins, Actin 42A and Actin-like protein 6B, Identified in *Drosophila* as BAP47 and BAP55 subunits of the BAP complex. Orthologous sequences from *Drosophila melanogaster (*XP_022187474.1*), Acyrthosiphon pisum (*XP012258146.1*), Cimex lectularius (actin42* XP022196053.1; actin6B XP001948128.1*), Acyrthosiphon pisum (*JAG04432.1*), Euschistus heros (*XP014245208.1*), Bombus impatiens (*XP001950512.1*), Cryptotermes secundus (*PNF40171.1), *Apis cerena (*XP001945563.1*), Lygus hesperus (*XP022196053.1), *Nilaparvata lungens (*XP024222562.1, XP 025410470.1), and *Diaphorina citri,* are represented in the phylogram. *Diaphorina citri* lacks a clear ortholog of Beta-centractin. Multiple sequence alignment (ClustalX) for overlapping regions of the sequences were used to generate a midpoint rooted Neighbor-joining tree. Evolutionary analyses were conducted in MEGA7.

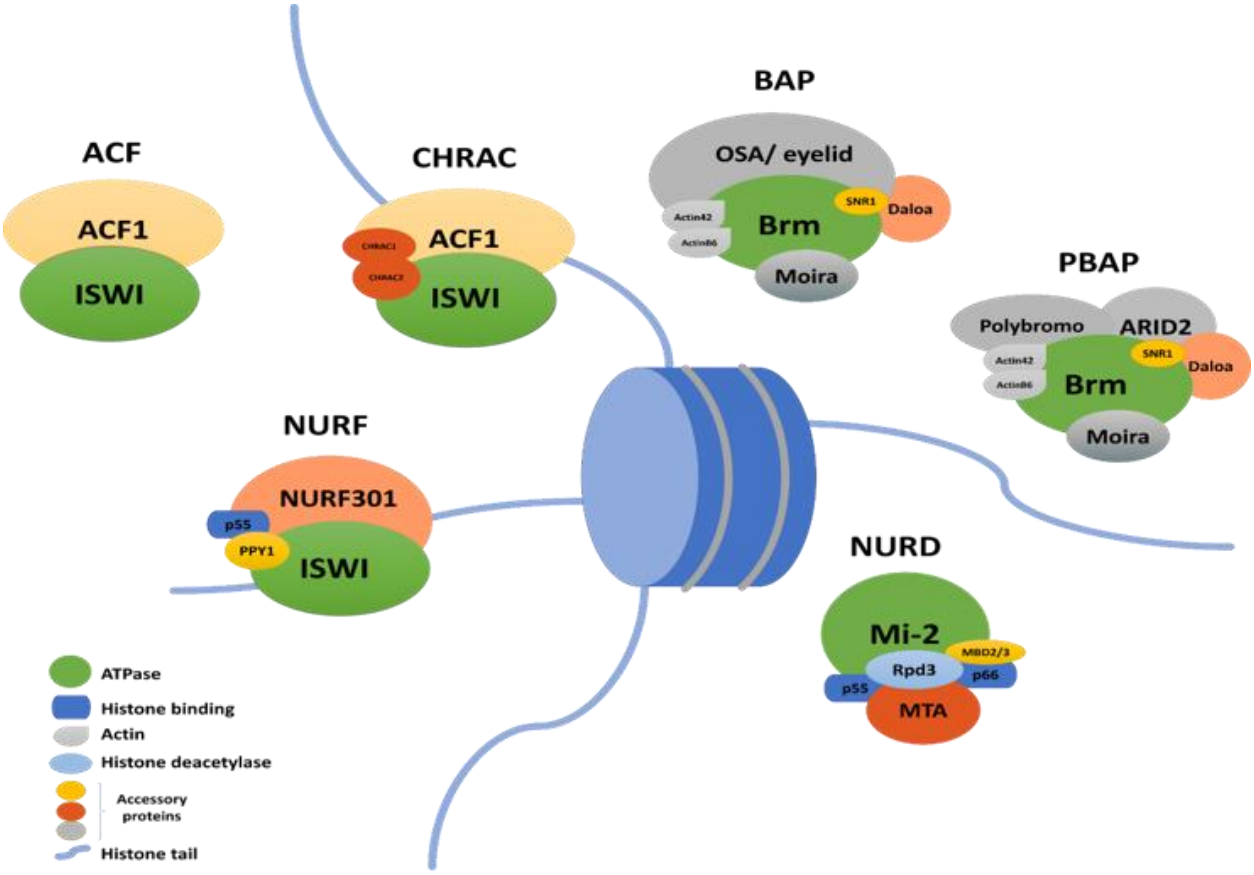

Figure 3: *Diaphorina citri* models for Mi-2, BRM, and ISWI containing complexes, based on the *Drosophila* models and results of annotation in genome 2.0. BAP: Brahma-associated proteins; PBAP: polybromo-associated BAP; CHRAC: chromatin accessibility complex; NURF: nucleosome remodeling factor; NuRD: nucleosome remodeling histone deacetylase;. ACF: ATP-utilizing chromatin assembly and remodeling factor.

Table 1: Gene copy number of ATP-dependent chromatin remodeling complexes proteins in *Diaphorina citri* and other insects from different orders

Complex	Gene	*Diaphorina citri*	*Drosophila melanogaster*	*Tribolium castaneum*	*Acyrthosiphon pisum*	*Apis mellifera*	*Cimex lectularius*
NuRD	Mi-2	2	1	1	1	1	1
NuRD / Nurf	p55	2	1	1	1	1	1
NuRD	p66 (simjang, serine protease	1	1	1	1	2	1
NuRD	MEP-1	1	1	1	2	1	1
NuRD	MTA3	1	1	1	1	1	1
NuRD	RPD3	1	1	1	4	1	2

NuRD	MBD2/3	1	1	1	1	1	1
ACF/CHRAC	ACF1	1	1	1	1	1	1
CHRAC	CHRAC 14 / DNA pol Epsilon 3	1	1	1	1	1	1
CHRAC	CHRAC 16	-	1	1	1	1	1
Nurf/ACF/ CHRAC	ISWI	1	1	1	2	1	1
Nurf	Nurf301 (Enhancer of bithorax)	1	1	1	1	1	1
Nurf	Inorganic pyrophophatase (Nurf38)	1	1	1	3	1	1
BAP	OSA/eyelid	1	1	1	1	1	1
BAP/ PBAP	BRM	1	1	1	1	1	1
BAP/ PBAP	SNR1/BAP45	1	1	1	1	1	1
BAP/ PBAP	BAP111/Dalao	1	1	1	1	1	2
BAP/ PBAP	Moira/Bap155	1	1	1	2	1	1
BAP/ PBAP	BAP55	1	1	2	3	2	1
BAP/ PBAP	BAP47	3	5	2	4	8	7
BAP/ PBAP	BAP60	1	1	1	1	1	1
PBAP	polybromo	1	1	1	1	1	1
SNF2	CHD1	1	1	1	1	1	2
SNF2	CHD3	1	2	1	1	1	1
SNF2	CHD7	1	1	1	1	1	1
SNF2	Ino80	1	1	1	1	1	1

Table 2: Manually annotated chromatin remodeling genes in *Diaphorina citri*. There are 27 genes in total. Each gene has been assigned an identifier and denoted as complete or partial based on available evidence. MCOT: a de novo Oases or Trinity model from an independent transcriptome was identified and the sequence from that transcript was used to validate or modify our model; IsoSeq: single, long RNAseq reads generated with Pacific Biosciences technology were available and were used to help validate the exon structure of the model; RNASeq: individually mapped Illumina RNASeq reads were used to help validate or modify our model; Ortholog: ortholog sequences from other insects and information about conserved motifs and domains had to be used to help determine the final annotation.

			Gene Model		Evidence Suppo				D.citri Identifier

					rting Annotation				
Complex	Gene	Location	Complete	Partial	MCOT	IsoSeq	RNASeq	Ortholog	
NuRD	Mi-2	ScVcwli_611:318..30865 (30.55 Kb) ScVcwli_2004:123820..160772 (36.95 Kb)	X		X	X	X		DcitrG014970.2.1
NuRD / Nurf	p55	ScVcwli_1637:201467..215369 (13.9 Kb)	X		X	X	X	X	DcitG040453.1.1
NuRD	p66	ScVcwli_113:470501..507650 (37.15 Kb)	X			X	X		DcitrG003180.1.1
NuRD	MEP-1	ScVcwli_2521:323118..338687 (15.57 Kb)	X		X		X		DcitrG062490.2.1
NuRD	MTA	ScVcwli_852:350758..392560 (41.8 Kb)	X		X	X	X	X	DcitrG020105.2.1
NuRD	RPD3		X		X	X	X	X	
NuRD	MBD2/3 c*	ScVcwli_2457:480679..483339 (2.66 Kb)	X				X		
ACF/CHRAC	ACF1	ScVcwli_255:317883..343328 (25.45 Kb)	X		X	X	X		
Nurf/ACF/CHRAC	ISWI	ScVcwli_3448:313307..331937 (18.63 Kb)	X		X	X	X	X	DcitrG088375.2.1
Nurf	Nurf301	ScVcwli_1433:319797..389437 (69.64 Kb)	X		X	X	X	X	
Nurf	Nurf38	ScVcwli_1137:524641..535821 (11.18 Kb)	X			X	X	X	
BAP	OSA/eyelid	ScVcwli_1928:1218191..1223805 (5.62 Kb)	X			X	X	X	
BAP/PBAP	BRM	ScVcwli_3318:498160..522991 (24.83 Kb)	-	X		X	X		DcitrG081172.1.1
BAP/PBAP	SNR1/BAP45	ScVcwli_950:1389619..1401669 (12.05 Kb)	X		X		X	X	

BAP/ PBAP	BAP111/Dalao	ScVcwli_2447:627010..648196 (21.19 Kb)	X			X	X	X	
BAP/ PBAP	Moira/ Bap155	ScVcwli_3640:358266..403789 (45.52 Kb)	X			X	X	X	
BAP/ PBAP	BAP55/actin 6B	ScVcwli_1951:1040580..1046472 (5.89 Kb)	X			X	X	X	
BAP/ PBAP	BAP47 Actin 5c	ScVcwli_742:821088..84669 (2.56 Kb)	X		X	X	X		
CHRAC	CHRAC 1	ScVcwli_3580:409677..414347 (4.67 Kb)	X		X		X		
CHRAC	DNA pol epsilon 3 / CHRAC 2	ScVcwli_3499:822905..825856 (2.95 Kb)	X		X	X	X		DcitG092762.1.1
BAP/ PBAP	BAP60	ScVcwli_2255:1703213..1726077 (22.87 Kb)	X		X		X		
PBAP	BAP170	ScVcwli_3644:131706..152842 (21.14 Kb)	X		X	X	X	X	
PBAP	polybromo	ScVcwli_910:37961..72967 (35.01 Kb)	X				x		DcitrG021720.1.1
SNF2	CHD1	ScVcwli_3542:57425..76801 (19.38 Kb)	X				x		DcitrM096525.1.1
SNF2	CHD3		-	-	-	-	-	-	
SNF2	CHD7	ScVcwli_1220:160131..201984 (41.85 Kb)	X		x		x		
SNF2	Ino80 helicase	ScVcwli_2859:933707..954390 (20.68 Kb) ScVcwli_3595:4078592..4107719 (29.13 Kb)	X		X	X	x		DcitrG070250.2.1DcitrG099735.1.1

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