



Genomic Studies of the Polyketide Synthase Genes :

Generation of a BAC-Based Physical Map of the Red Raspberry Genome



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ATPER2013-June 1-2, 2013 Stockholm,
Sweden.



OUTLINES :

Red Raspberry & Polyketide synthase (PKS)

- Red raspberry**
- Anthocyanins / health benefits**
- Polyketide Synthase (PKS)/Biosynthetic pathway**
- Molecular Plant Breeding and Applications**





I. Why Red Raspberry ?

- Small fruit / 37 countries world wild
- Powerful flavour and colour
- Commercially important fruit
- Food industrial manufacturing / fresh fruit market
- **Source of Anthocyanins**
- Red raspberry cultivars and improvement
 - disease resistances
 - trait qualities





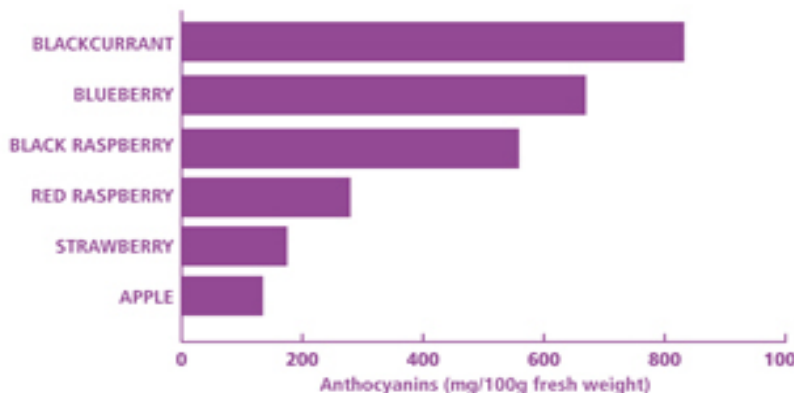
How important is anthocyanins ?

- **Responsible for fruit colour and aroma**
- **Having significant antioxidant properties :**
 - **promoting health**
 - **improving vision**
 - **preventing cancer**
 - **controlling diabetes**
 - **retarding the effects of aging**
 - **preventing the loss of memory and motor skills**



Anthocyanins in Red raspberry !!

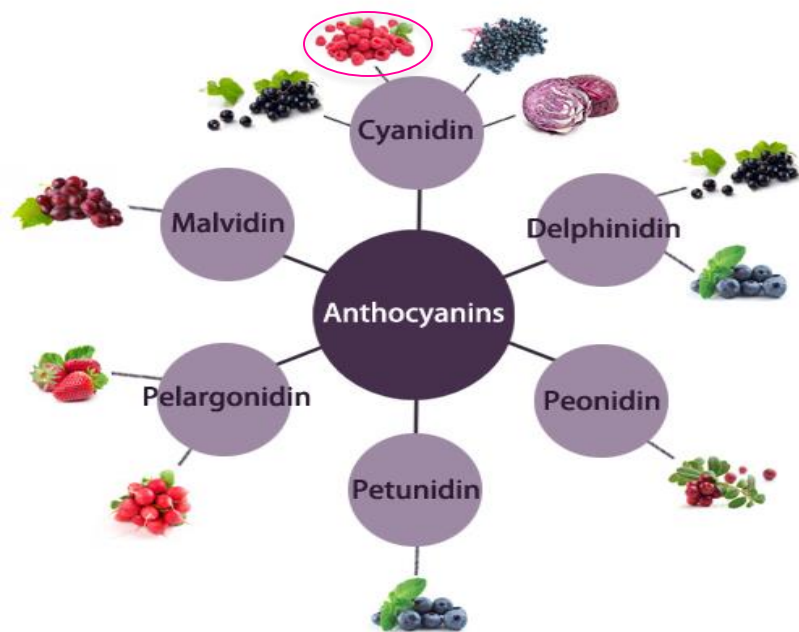
ANTHOCYANINS CONTENT OF FRUIT



Tab. 1 Concentrations of total anthocyanins (TA) (mg CGE/l)^a, total polyphenols (TP) (mg GAE/l)^a, antioxidant activity of red fruit juices ($\mu\text{mol TE/ml}$)^b, and TA/TP ratio

	Total anthocyanins [mg/l]	Total polyphenols [mg/l]	Antioxidant activity [$\mu\text{mol TE/ml}$]	TA/TP
Black currant	1543.89 \pm 5.5	2770.94 \pm 63.9	30.15	0.56
Red raspberry	217.39 \pm 5.2	1234.27 \pm 54.8	8.20	0.18
Blackberry	739.93 \pm 37.5	1831.21 \pm 111.6	8.75	0.40
Sour cherry	369.36 \pm 2.4	2054.43 \pm 140.2	12.52	0.18
Sweet cherry	256.60 \pm 2.5	1566.84 \pm 130.2	4.07	0.16
Strawberry	205.98 \pm 2.2	1271.85 \pm 106.9	4.39	0.16
Chokeberry	3042.20 \pm 196.3	9154.47 \pm 595.4	72.44	0.33
Elderberry	4188.63 \pm 257.0	6361.89 \pm 298.9	62.14	0.66

^a values are means \pm SD (n=3); ^b antioxidant activity determined after 15 min



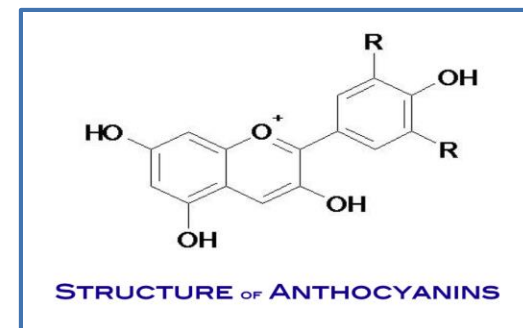
Selected anthocyanidins and their substitutions

Basic structure	Anthocyanidin	R ₃ '	R ₄ '	R ₅ '	R ₃	R ₅	R ₆	R ₇
	Aurantidin	-H	-OH	-H	-OH	-OH	-OH	-OH
	Cyanidin	-OH	-OH	-H	-OH	-OH	-H	-OH
	Delphinidin	-OH	-OH	-OH	-OH	-OH	-H	-OH
	Europinidin	-OCH ₃	-OH	-OH	-OH	-OCH ₃	-H	-OH
	Luteolinidin	-OH	-OH	-H	-H	-OH	-H	-OH
	Pelargonidin	-H	-OH	-H	-OH	-OH	-H	-OH
	Malvidin	-OCH ₃	-OH	-OCH ₃	-OH	-OH	-H	-OH
	Peonidin	-OCH ₃	-OH	-H	-OH	-OH	-H	-OH
	Petunidin	-OH	-OH	-OCH ₃	-OH	-OH	-H	-OH
	Rosinidin	-OCH ₃	-OH	-H	-OH	-OH	-H	-OCH ₃



What is Polyketide synthase (PKS) ?

- A large family of enzymes



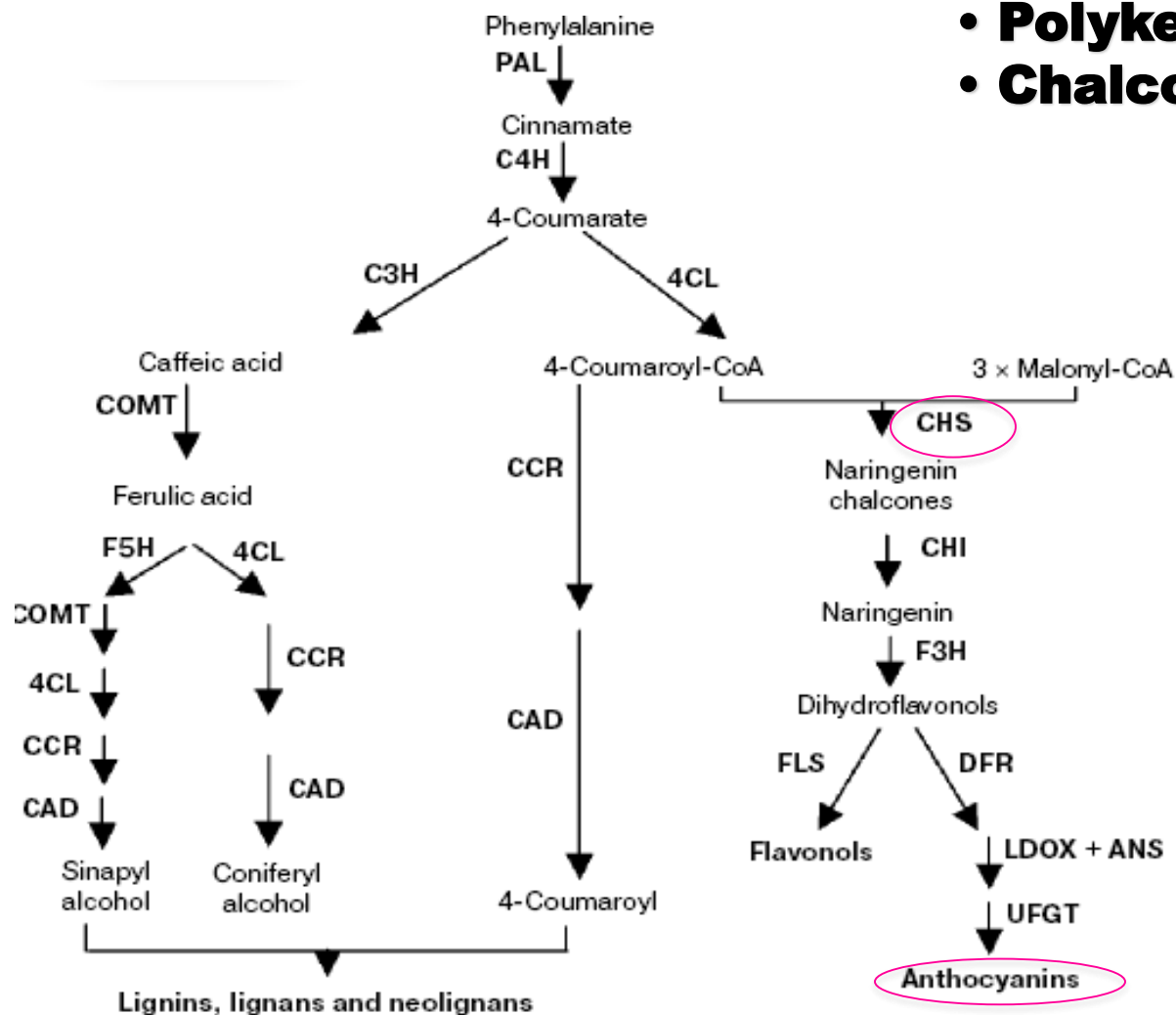
- Produce aromatic polyketides / flavour, colour
- Have been found in microbial, plants and animals
- Are precursor for the next steps
- There are three types of PKS ; I, II and III
Type III : higher plants





Polyketide biosynthesis pathway

- **Polyketide synthase (PKS)**
- **Chalcone synthase (CHS)**





Molecular plant breeding and applications

** Crossing 2 characteristically different cultivars

1. Markers / quality trait loci (QTL) / which chromosome / where on chromosome / linkage mapping



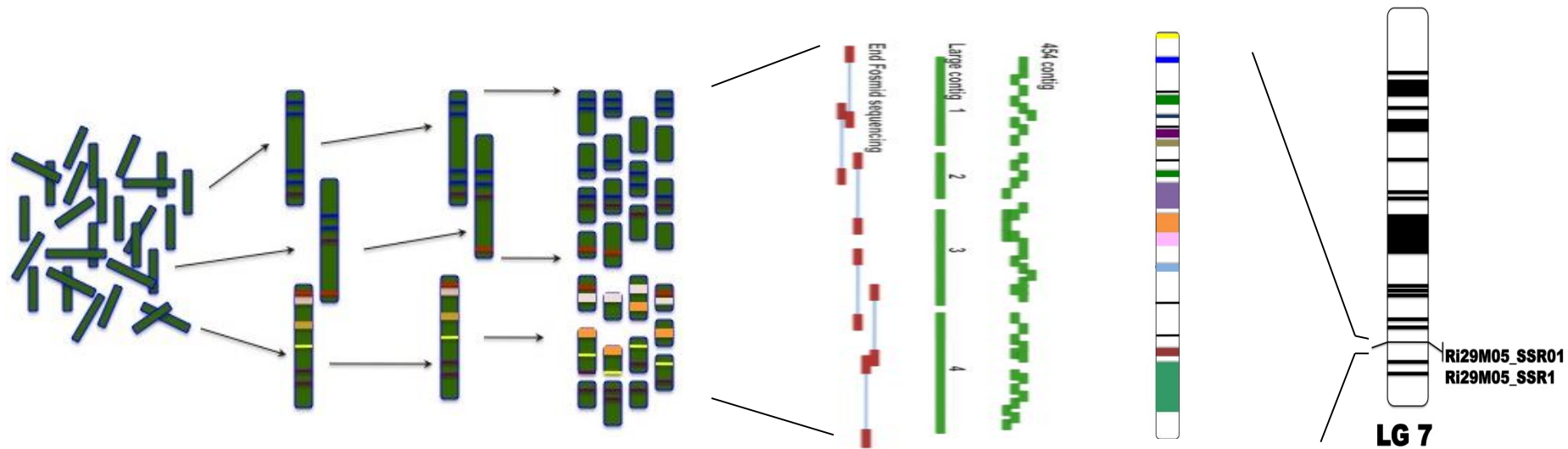
North America X European

2. Genome sequence studies / sequence of DNA / DNA variation / gene structure / Physical mapping / comparative mapping or integrated mapping / compare with the other related plants



3. Expression / Genetic engineering

II. Material & Methodology

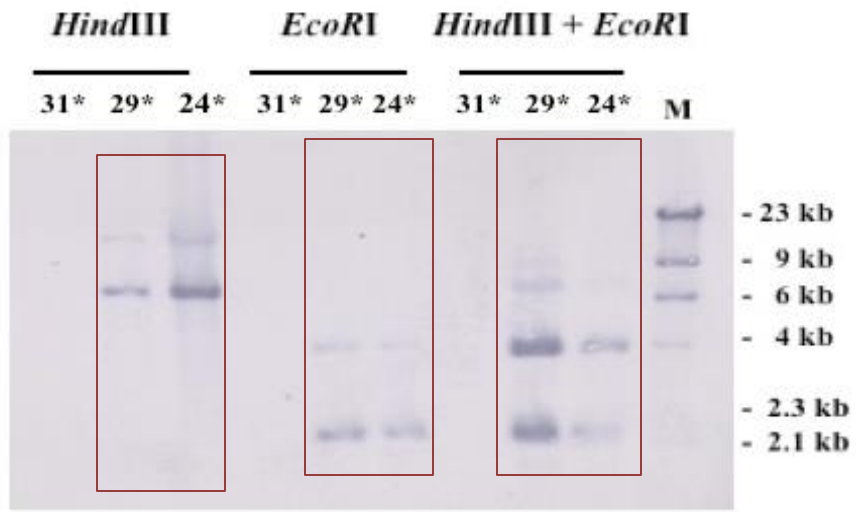
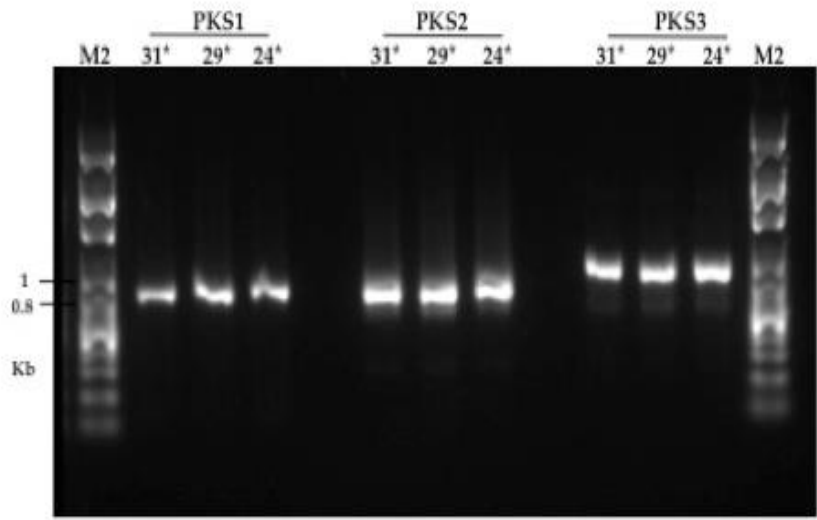
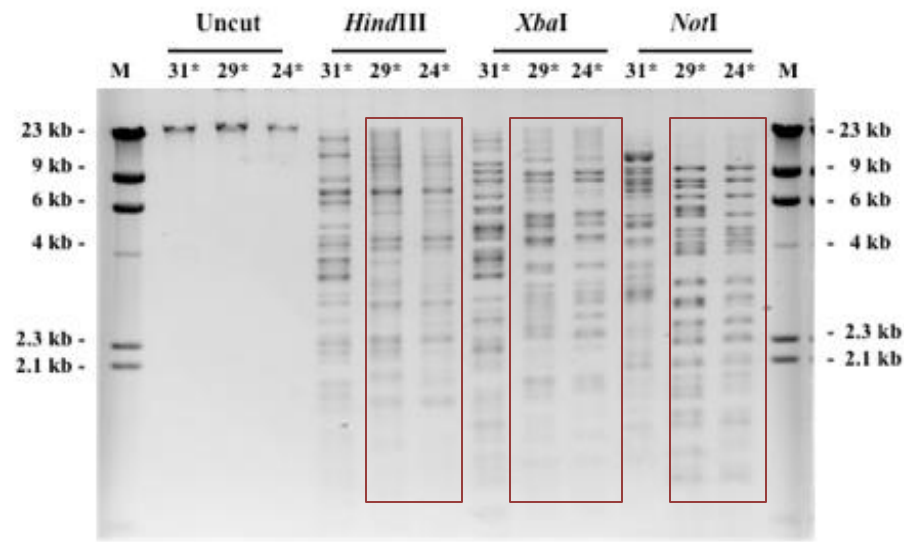


<p>1. Construction : A BAC library</p> <ul style="list-style-type: none"> - a BAC library from genomic DNA isolated from red raspberry cv. Glen Moy - screen positive BAC clones with CHS11 probe 	<p>2. Analysis BAC clones : positive BAC clones</p> <ul style="list-style-type: none"> - restriction digestion, - southern blotting, - PCR using primers derived from gene sequences for red raspberry cv. Royalty - carry out 454 sequencing for each BAC 	<p>3. Construction : fosmid library by partially mechanical shearing digestion</p> <ul style="list-style-type: none"> - screen by - colony hybridization, - PCR, - restriction digestion, - paired-end fosmid sequencing to help assemble 454 data for <u>BACs</u> 	<p>4. Generation : physical map</p> <ul style="list-style-type: none"> - detailed annotation - gene order - agarose fingerprinting by rare cutter enzyme digestion - MTP ** comparison to cut map of 454 assembly 	<p>5. Bioinformatics : Information of genes on the chromosomal region</p> <ul style="list-style-type: none"> - annotation - genetic variation - comparison to red raspberry cv. Latham and related fruits
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III. Results

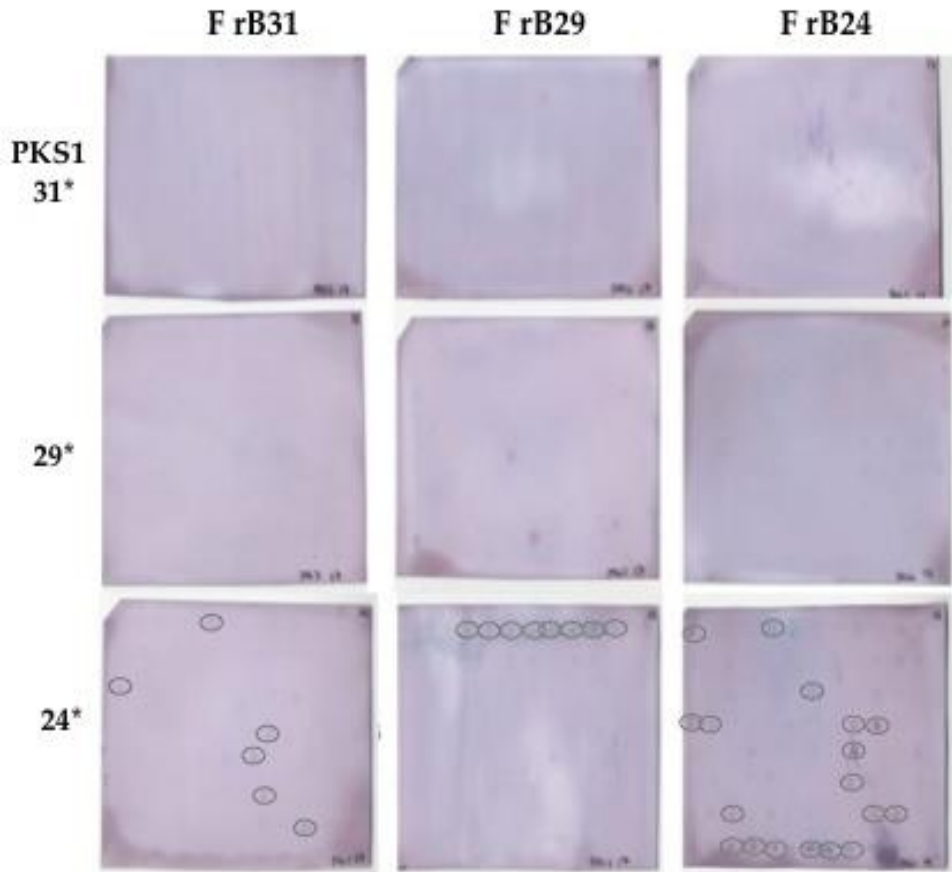
i Analysis : positive BAC clones



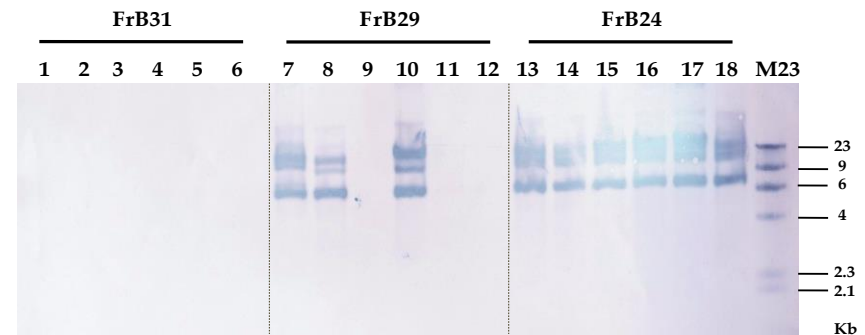
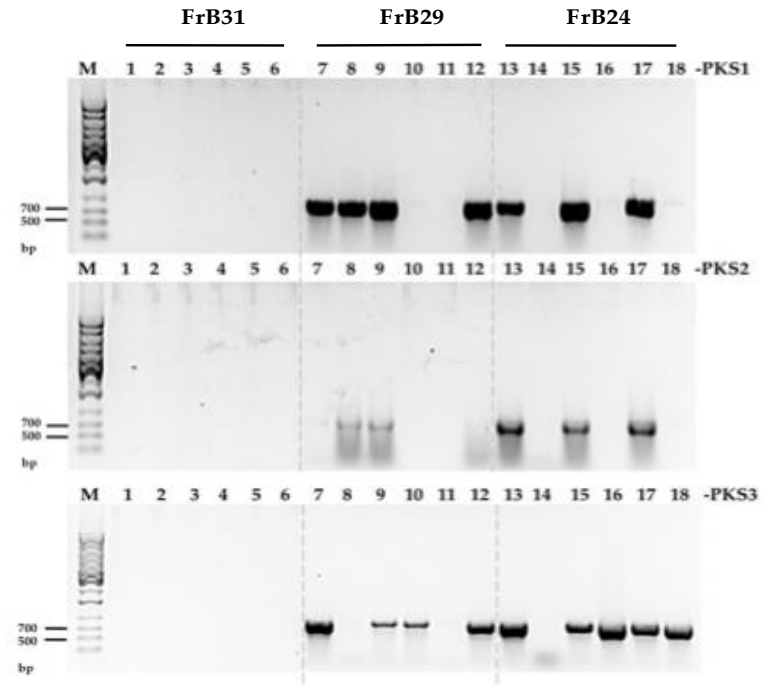
BAC clones contain ~ 130-200 kb of DNA



ii Construction of Fosmid library



A sub-library contains more manageable sizes ~ 36-40 kb of DNA





iii Assembly of BAC sequences

24P12-raw data assembly

Assembly Parameters: AbN Assemble Automatically Assemble Interactively Assemble to Reference

Parameters: (Dirty Data, With ReAligner, 3' gap placement): Min Overlap = 20, Min Match = 85%

Name	Size	Quality	Kind	Label	Modified
718000000489	266 BP	97.7%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:06
718000000490	202 BP	98.0%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:06
718000000494	274 BP	69.7%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:06
718000000495	437 BP	84.0%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:06
718000000496	253 BP	99.2%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:06
718000000497	386 BP	98.7%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:06
718000000498	339 BP	97.6%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:06
718000000499	444 BP	94.1%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:06
718000000500	331 BP	60.4%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:06
718000000501	163 BP	98.2%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:06
718000000502	154 BP	77.9%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:06
718000000503	210 BP	97.1%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:06
718000000506	157 BP	93.0%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:06
718000000508	228 BP	97.4%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:06
718000000509	429 BP	76.9%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:06
718000000510	222 BP	81.5%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:06
718000000513	357 BP	97.5%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:06
718000000514	347 BP	93.7%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:06
Contig[0001]	117033 BP	-	Contig of 252	-	Tue, Jun 14, 2011 18:29:58
Contig[0066]	312 BP	-	Contig of 2	-	Tue, Jun 14, 2011 18:28:58

29M05-1-raw data assembly

Assembly Parameters: AbN Assemble Automatically Assemble Interactively Assemble to Reference

Parameters: (Dirty Data, With ReAligner, 3' gap placement): Min Overlap = 20, Min Match = 85%

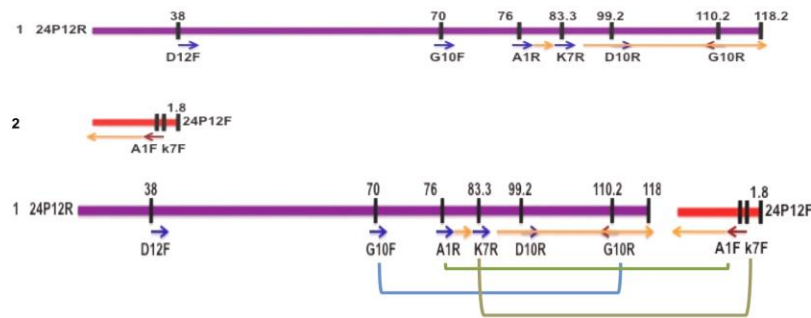
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718000000663	429 BP	74.1%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:08
718000000664	258 BP	55.8%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:08
718000000665	443 BP	72.9%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:08
718000000666	418 BP	80.9%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:08
718000000667	402 BP	85.8%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:08
718000000669	227 BP	87.7%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:08
718000000670	372 BP	84.7%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:08
718000000671	350 BP	83.4%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:08
718000000672	295 BP	96.6%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:08
718000000673	481 BP	87.3%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:08
718000000674	338 BP	70.4%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:08
718000000675	245 BP	86.9%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:08
718000000676	417 BP	77.5%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:08
Contig[0001]	24672 BP	-	Contig of 59	-	Tue, Jun 14, 2011 17:58:22
Contig[0002]	46085 BP	-	Contig of 65	-	Tue, Jun 14, 2011 17:58:29
Contig[0003]	95469 BP	-	Contig of 171	-	Tue, Jun 14, 2011 17:58:32
Contig[0006]	7298 BP	-	Contig of 19	-	Tue, Jun 14, 2011 17:58:24
Contig[0085]	357 BP	-	Contig of 2	-	Tue, Jun 14, 2011 17:56:46

31B12-1-raw data assembly .SPF

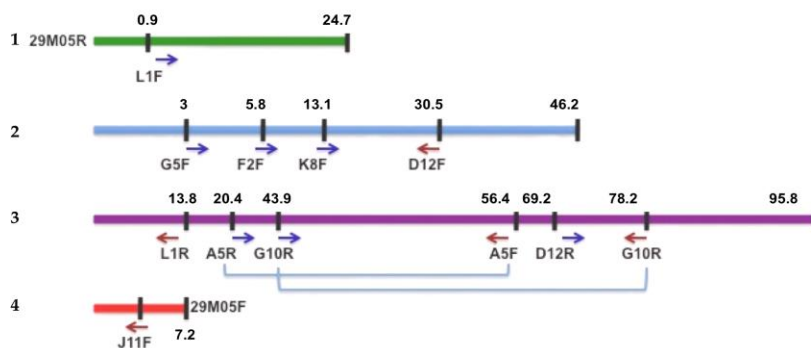
Assembly Parameters: AbN Assemble Automatically Assemble Interactively Assemble to Reference

Parameters: (Dirty Data, With ReAligner, 3' gap placement): Min Overlap = 20, Min Match = 85%

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718000000994	311 BP	99.7%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:10
718000000995	350 BP	85.4%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:10
718000000997	347 BP	93.4%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:10
718000000998	227 BP	86.3%	DNA Fragment	-	Wed, Aug 25, 2010 17:23:10
Contig[0001]	33478 BP	-	Contig of 140	-	Sat, Mar 9, 2013 20:42:56
Contig[0003]	4456 BP	-	Contig of 14	-	Sat, Mar 9, 2013 20:42:34
Contig[0004]	4795 BP	-	Contig of 15	-	Sat, Mar 9, 2013 20:42:34
Contig[0007]	12843 BP	-	Contig of 47	-	Sat, Mar 9, 2013 20:42:53
Contig[0008]	11760 BP	-	Contig of 29	-	Sat, Mar 9, 2013 20:42:44
Contig[0013]	22146 BP	-	Contig of 62	-	Sat, Mar 9, 2013 20:42:53
Contig[0016]	9420 BP	-	Contig of 16	-	Sat, Mar 9, 2013 20:42:44



A) 24P12



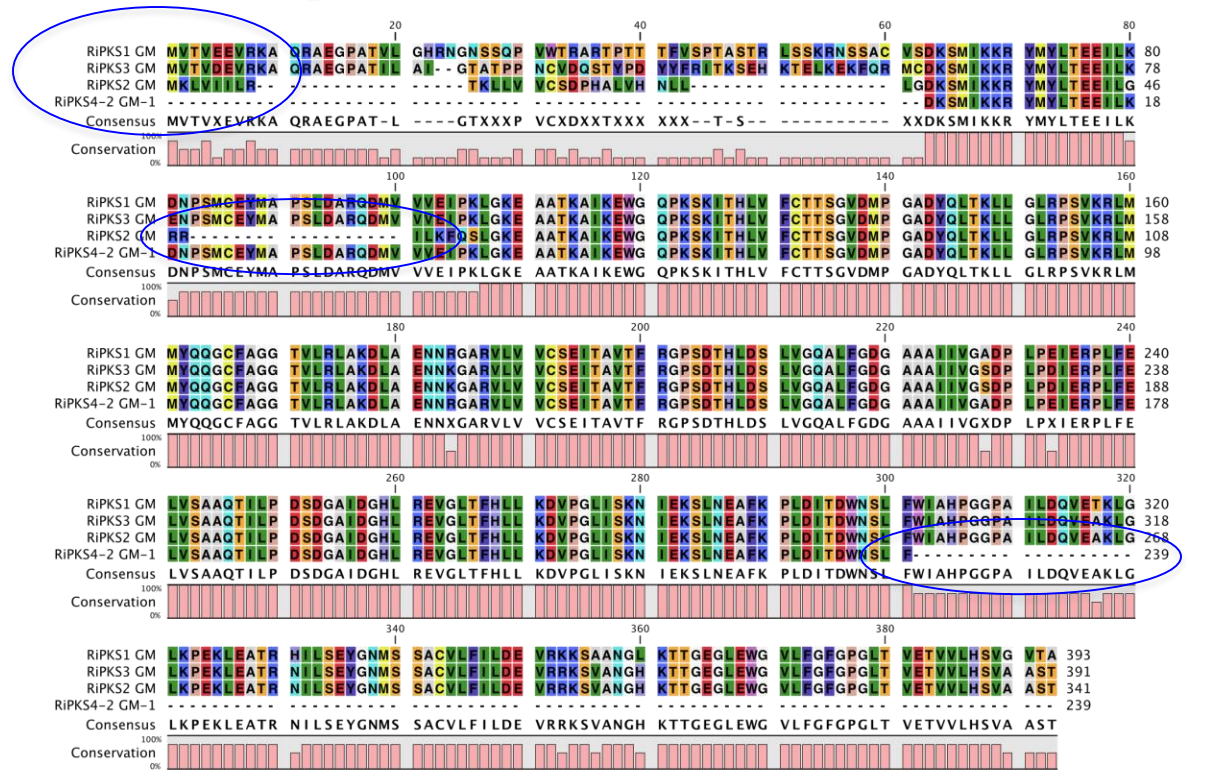
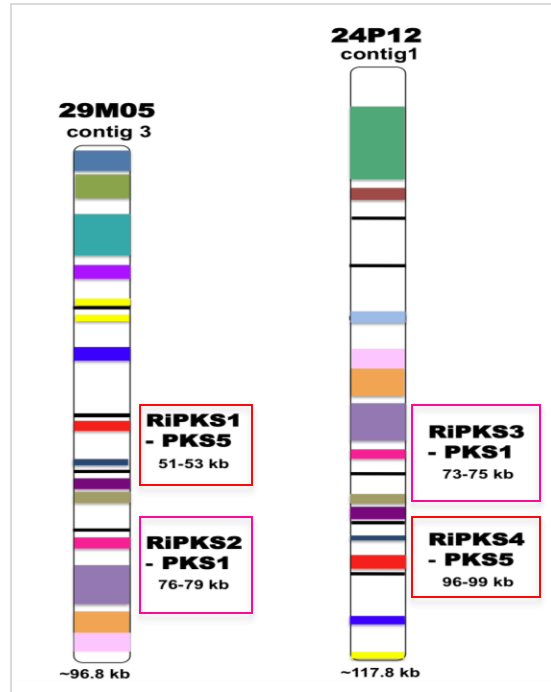
B) 29M05

454 sequencing and assembly :

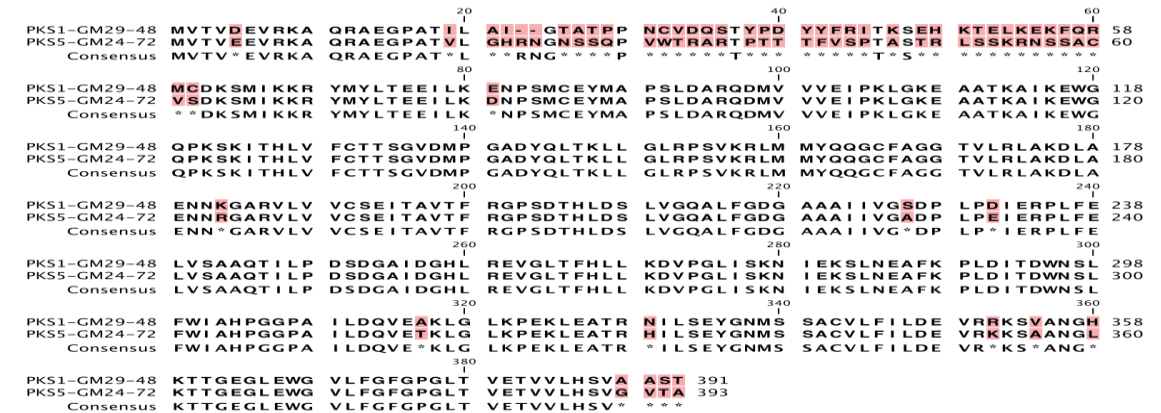
- 24P12 ~ 117 kb / 12 FES
- 29M05 ~ 98 – 120 kb / 18 FES



iv. Genetic organization and sequence variation



Each BAC carries 2 regions of PKS genes





Amino acid sequence

- PKS1

PKS1 R Royalty PKS1-GM29-48 PKS5-GM24-72	MVTVDEVRKA MVTVDEVRKA MVTVDEVRKA	GRAEGPATIL GRAEGPATIL GRAEGPATIL	AI - - GTATPP AI - - GTATPP GHRNGNSSQP	NCVDQSTYPD NCVDQSTYPD VWTRARTPTT	YYFRITKSEH YYFRITKSEH TFVSPATASTR	KTELKEKFFQR KTELKEKFFQR LSSKRNSAC	58 58 60
PKS1 R Royalty PKS1-GM29-48 PKS5-GM24-72	MCDKSMIKKR MCDKSMIKKR VSDKSMIKKR	MYMLTEEILK MYMLTEEILK MYMLTEEILK	ENPSMCEYMA ENPSMCEYMA DNPSMCEYMA	PSLDARQDMV PSLDARQDMV PSLDARQDMV	VVEI PKLGKE VVEI PKLGKE VVEI PKLGKE	AATKAIKEWG AATKAIKEWG AATKAIKEWG	118 118 120
PKS1 R Royalty PKS1-GM29-48 PKS5-GM24-72	QPKSKI THLV QPKSKI THLV QPKSKI THLV	FCTTSGVDMP FCTTSGVDMP FCTTSGVDMP	GADYQLTKLL GADYQLTKLL GADYQLTKLL	GLRPSVKRLM GLRPSVKRLM GLRPSVKRLM	MYQQGCFAGG MYQQGCFAGG MYQQGCFAGG	TVLRLAKDLA TVLRLAKDLA TVLRLAKDLA	178 178 180
PKS1 R Royalty PKS1-GM29-48 PKS5-GM24-72	ENNKGARVLV ENNKGARVLV ENNKGARVLV	VCSEITAVTF VCSEITAVTF VCSEITAVTF	RGPSDTHLDS RGPSDTHLDS RGPSDTHLDS	LVGQALFGDG LVGQALFGDG LVGQALFGDG	AAAI IVGSDP AAAI IVGSDP AAAI IVGSDP	LPDI ERPLFE LPDI ERPLFE LPDI ERPLFE	238 238 240
PKS1 R Royalty PKS1-GM29-48 PKS5-GM24-72	LVSAAQTI LP LVSAAQTI LP LVSAAQTI LP	DSDGAIDGHL DSDGAIDGHL DSDGAIDGHL	REVGLTFHLL REVGLTFHLL REVGLTFHLL	KDVPGLI SKN KDVPGLI SKN KDVPGLI SKN	IEKSLNEAFK IEKSLNEAFK IEKSLNEAFK	PLDITDWNLS PLDITDWNLS PLDITDWNLS	298 298 300
PKS1 R Royalty PKS1-GM29-48 PKS5-GM24-72	FWIAHPGGPA FWIAHPGGPA FWIAHPGGPA	ILDQVEAKLQ ILDQVEAKLQ ILDQVEAKLQ	LKPEKLEATR LKPEKLEATR LKPEKLEATR	NILSEYGNMS NILSEYGNMS NILSEYGNMS	SACVLFILDE SACVLFILDE SACVLFILDE	VRRKSVANGH VRRKSVANGH VRRKSVANGH	358 358 360
PKS1 R Royalty PKS1-GM29-48 PKS5-GM24-72	KTTGEGLEWG KTTGEGLEWG KTTGEGLEWG	VLFGFGPGLT VLFGFGPGLT VLFGFGPGLT	VETVVLHLSVA VETVVLHLSVA VETVVLHLSVG	AST 391 NCI 391 VTA 393			

- PKS5

PKS1-GM29-48 PKS5 R Royalty PKS5-GM24-72	MVTVDEVRKA MVTVDEVRKA MVTVDEVRKA	GRAEGPATIL GRAEGPATIL GRAEGPATIL	AI - - GTATPP AI - - GTATPP GHRNGNSSQP	NCVDQSTYPD NCVDQSTYPD VWTRARTPTT	YYFRITKSEH YYFRITKSEH TFVSPATASTR	KTELKEKFFQR KTELKEKFFQR LSSKRNSAC	58 58 60
PKS1-GM29-48 PKS5 R Royalty PKS5-GM24-72	MCDKSMIKKR MCDKSMIKKR VSDKSMIKKR	MYMLTEEILK MYMLTEEILK MYMLTEEILK	ENPSMCEYMA ENPSMCEYMA DNPSMCEYMA	PSLDARQDMV PSLDARQDMV PSLDARQDMV	VVEI PKLGKE VVEI PKLGKE VVEI PKLGKE	AATKAIKEWG AATKAIKEWG AATKAIKEWG	118 118 120
PKS1-GM29-48 PKS5 R Royalty PKS5-GM24-72	QPKSKI THLV QPKSKI THLV QPKSKI THLV	FCTTSGVDMP FCTTSGVDMP FCTTSGVDMP	GADYQLTKLL GADYQLTKLL GADYQLTKLL	GLRPSVKRLM GLRPSVKRLM GLRPSVKRLM	MYQQGCFAGG MYQQGCFAGG MYQQGCFAGG	TVLRLAKDLA TVLRLAKDLA TVLRLAKDLA	178 178 180
PKS1-GM29-48 PKS5 R Royalty PKS5-GM24-72	ENNKGARVLV ENNRGARVLV ENNRGARVLV	VCSEITAVTF VCSEIXAVTF VCSEITAVTF	RGPSDTHLDS RGPSDTHLDS RGPSDTHLDS	LVGQALFGDG LVGQALFGDG LVGQALFGDG	AAAI IVGSDP AAAI IVGSDP AAAI IVGSDP	LPDI ERPLFE LPDI ERPLFE LPDI ERPLFE	238 238 240
PKS1-GM29-48 PKS5 R Royalty PKS5-GM24-72	LVSAAQTI LP LVSAAQTI LP LVSAAQTI LP	DSDGAIDGHL DSDGAIDGHL DSDGAIDGHL	REVGLTFHLL REVGLTFHLL REVGLTFHLL	KDVPGLI SKN KDVPGLI SKN KDVPGLI SKN	IEKSLNEAFK IEKSLNEAFK IEKSLNEAFK	PLDITDWNLS PLDITDWNLS PLDITDWNLS	298 298 300
PKS1-GM29-48 PKS5 R Royalty PKS5-GM24-72	FWIAHPGGPA FWIAHPGGPA FWIAHPGGPA	ILDQVEAKLQ ILDQVETKLG ILDQVETKLG	LKPEKLEATR LKPEKLEATR LKPEKLEATR	NILSEYGNMS HILSEYGNMS HILSEYGNMS	SACVLFILDE SACVLFILDE SACVLFILDE	VRRKSVANGH VRRKSAANGL VRRKSAANGL	358 358 360
PKS1-GM29-48 PKS5 R Royalty PKS5-GM24-72	KTTGEGLEWG KTTGEGLEWG KTTGEGLEWG	VLFGFGPGLT VLFGFGPGLT VLFGFGPGLT	VETVVLHLSVA VETVVLHLSVG VETVVLHLSVG	AST 391 VTA 391 VTA 393			

- CHS5

PKS1-GM29-48 CHS5 R Meeker PKS5-GM24-72	MVTVDEVRKA MVTVDEVRKA MVTVDEVRKA	GRAEGPATIL GRAEGPATIL GRAEGPATIL	AI - - GTATPP AI - - GTATPP GHRNGNSSQP	NCVDQSTYPD NCVDQSTYPD VWTRARTPTT	YYFRITKSEH YYFRITKSEH TFVSPATASTR	KTELKEKFFQR KTELKEKFFQR LSSKRNSAC	58 58 60
PKS1-GM29-48 CHS5 R Meeker PKS5-GM24-72	MCDKSMIKKR MCDKSMIKKR VSDKSMIKKR	MYMLTEEILK MYMLTEEILK MYMLTEEILK	ENPSMCEYMA ENPSMCEYMA DNPSMCEYMA	PSLDARQDMV PSLDARQDMV PSLDARQDMV	VVEI PKLGKE VVEI PKLGKE VVEI PKLGKE	AATKAIKEWG AATKAIKEWG AATKAIKEWG	118 118 120
PKS1-GM29-48 CHS5 R Meeker PKS5-GM24-72	QPKSKI THLV QPKSKI THLV QPKSKI THLV	FCTTSGVDMP FCTTSGVDMP FCTTSGVDMP	GADYQLTKLL GADYQLTKLL GADYQLTKLL	GLRPSVKRLM GLRPSVKRLM GLRPSVKRLM	MYQQGCFAGG MYQQGCFAGG MYQQGCFAGG	TVLRLAKDLA TVLRLAKDLA TVLRLAKDLA	178 178 180
PKS1-GM29-48 CHS5 R Meeker PKS5-GM24-72	ENNKGARVLV ENNRGARVLV ENNRGARVLV	VCSEITAVTF VCSEITAVTF VCSEITAVTF	RGPSDTHLDS RGPSDTHLDS RGPSDTHLDS	LVGQALFGDG LVGQALFGDG LVGQALFGDG	AAAI IVGSDP AAAI IVGSDP AAAI IVGSDP	LPDI ERPLFE LPDI ERPLFE LPDI ERPLFE	238 238 240
PKS1-GM29-48 CHS5 R Meeker PKS5-GM24-72	LVSAAQTI LP LVSAAQTI LP LVSAAQTI LP	DSDGAIDGHL DSDGAIDGHL DSDGAIDGHL	REVGLTFHLL REVGLTFHLL REVGLTFHLL	KDVPGLI SKN KDVPGLI SKN KDVPGLI SKN	IEKSLNEAFK IEKSLNEAFK IEKSLNEAFK	PLDITDWNLS PLDITDWNLS PLDITDWNLS	298 298 300
PKS1-GM29-48 CHS5 R Meeker PKS5-GM24-72	FWIAHPGGPA FWIAHPGGPA FWIAHPGGPA	ILDQVEAKLQ ILDQVETKLG ILDQVETKLG	LKPEKLEATR LKPEKLEATR LKPEKLEATR	NILSEYGNMS HILSEYGNMS HILSEYGNMS	SACVLFILDE SACVLFILDE SACVLFILDE	VRRKSVANGH VRRKSAANGL VRRKSAANGL	358 358 360
PKS1-GM29-48 CHS5 R Meeker PKS5-GM24-72	KTTGEGLEWG KTTGEGLEWG KTTGEGLEWG	VLFGFGPGLT VLFGFGPGLT VLFGFGPGLT	VETVVLHLSVA VETVVLHLSVG VETVVLHLSVG	AST 391 VTA 391 VTA 393			

C



Amino acid sequence

CHS5 of strawberry (*Fragaria vesca* subsp. *Vesca*).

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                20                            40                            60
PKS1-GM29-48 MVTVD EVRKA QRAEGPAT I L AI - - GTATPP NCVDQSTYPP YFRITKSEH KTELKEKFOR 58
CHS-P. persical MVTVEEVRKA QRAEGPATVL A I - - GTATPP NCVDQATYPP YFRITNSEH KTELKEKFOR 58
PKS5-GM24-72 MVTVEEVRKA QRAEGPATVL GHRNGNSSQP VWTRARTPTT TFFVSPASTR LSSKRNSAC 60
Consensus MVTVEEVRKA QRAEGPATVL AI - - GTATPP NCVDQ*TYPP YFRIT*SEH KTELKEKFOR 120
                80                            100                            120
PKS1-GM29-48 MCDKSM I KKR YMYL TEE I LK ENPSMCEYMA PSLDARQDMV VVE I P KLGKE AATKA I KEWG 118
CHS-P. persical MCDKSM I KKR YMYL TEE I LK ENPSMCEYMA PSLDARQDMV VVE I P KLGKE AATKA I KEWG 118
PKS5-GM24-72 VSDKSM I KKR YMYL TEE I LK DNPSMCEYMA PSLDARQDMV VVE I P KLGKE AATKA I KEWG 120
Consensus MCDKSM I KKR YMYL TEE I LK ENPSMCEYMA PSLDARQDMV VVE I P KLGKE AATKA I KEWG 180
                140                            160                            180
PKS1-GM29-48 QPKSK I THLV FCTTSGVDMP GADYQLTKLL GLRPSVKRLM MYQQGCFAGG TVLRLAKDLA 178
CHS-P. persical QPKSK I THLV FCTTSGVDMP GADYQLTKLL GLRPSVKRLM MYQQGCFAGG TVLRLAKDLA 178
PKS5-GM24-72 QPKSK I THLV FCTTSGVDMP GADYQLTKLL GLRPSVKRLM MYQQGCFAGG TVLRLAKDLA 180
Consensus QPKSK I THLV FCTTSGVDMP GADYQLTKLL GLRPSVKRLM MYQQGCFAGG TVLRLAKDLA 240
                200                            220                            240
PKS1-GM29-48 ENNKGARVLV VCSEITAVTF RGPSTDH LDS LVGQALFGDG AAA I I VGS DP LPDI ERPLFE 238
CHS-P. persical ENNRGARVLV VCSEITAVTF RGPSTDH LDS LVGQALFGDG AAA I I VGS DP LPEI EKPLFE 238
PKS5-GM24-72 ENNRGARVLV VCSEITAVTF RGPSTDH LDS LVGQALFGDG AAA I I VGS DP LPEI ERPLFE 240
Consensus ENNRGARVLV VCSEITAVTF RGPSTDH LDS LVGQALFGDG AAA I I VGS DP LPEI ERPLFE 300
                260                            280                            300
PKS1-GM29-48 LVSA AQT I LP DSDG A I DGHL REVGLTFHLL KDVPGL I SKN I EKSLNEAFK PLDI TDWNSL 298
CHS-P. persical VVSA AQT I LP DSDG A I DGHL REVGLTFHLL KDVPGL I SKN I EKSLNEAFK PLDI TDWNSL 298
PKS5-GM24-72 LVSA AQT I LP DSDG A I DGHL REVGLTFHLL KDVPGL I SKN I EKSLNEAFK PLDI TDWNSL 300
Consensus LVSA AQT I LP DSDG A I DGHL REVGLTFHLL KDVPGL I SKN I EKSLNEAFK PLDI TDWNSL 360
                320                            340                            360
PKS1-GM29-48 FWI AHPPGGA I LDQVEAKLG LKPEKLEATR NILSEYGNMS SACVLFI LDE VRRKSA VANGH 358
CHS-P. persical FWI AHPPGGA I LDQVESKLA LKPEKLEATR HILSEYGNMS SACVLFI LDE VRKRATKKG L 358
PKS5-GM24-72 FWI AHPPGGA I LDQVETKLG LKPEKLEATR HILSEYGNMS SACVLFI LDE VRKSA ANGL 360
Consensus FWI AHPPGGA I LDQVE* KLG LKPEKLEATR HILSEYGNMS SACVLFI LDE VRRKSA ANGL 360
                380
PKS1-GM29-48 KTTGEGLEWG VLFGFGPGLT VETVVLH SV A AST 391
CHS-P. persical KTTGDGLDWG VLFGFGPGLT VETVVLH SV G LNA 391
PKS5-GM24-72 KTTGEGLEWG VLFGFGPGLT VETVVLH SV G VTA 393
Consensus KTTGEGLEWG VLFGFGPGLT VETVVLH SV G * * A

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CHS of prune (*Prunus persical*)

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                20                            40                            60
PKS1-GM29-48 MVTVD EVRKA QRAEGPAT I L AI - - GTATPP NCVDQSTYPP YFRITKSEH KTELKEKFOR 58
CHS5-Fr MVTVEEVRKA QRAEGPATVL A I - - GTATPP NCVDQSTYPP YFRITNSEH KTELKEKFOR 58
PKS5-GM24-72 MVTVEEVRKA QRAEGPATVL GHRNGNSSQP VWTRARTPTT TFFVSPASTR LSSKRNSAC 60
Consensus MVTVEEVRKA QRAEGPATVL AI - - GTATPP NC*VDQSTYPP YFRIT*SEH K*ELKEKFOR 120
                80                            100                            120
PKS1-GM29-48 MCDKSM I KKR YMYL TEE I LK ENPSMCEYMA PSLDARQDMV VVE I P KLGKE AATKA I KEWG 118
CHS5-Fr MCDKSM I KKR YMYL TEE I LK ENPSMCEYMA PSLDARQDMV VVE I P KLGKE AA V KA I KEWG 118
PKS5-GM24-72 VSDKSM I KKR YMYL TEE I LK DNPSMCEYMA PSLDARQDMV VVE I P KLGKE AATKA I KEWG 120
Consensus MCDKSM I KKR YMYL TEE I LK ENPSMCEYMA PSLDARQDMV VVE I P KLGKE AATKA I KEWG 180
                140                            160                            180
PKS1-GM29-48 QPKSK I THLV FCTTSGVDMP GADYQLTKLL GLRPSVKRLM MYQQGCFAGG TVLRLAKDLA 178
CHS5-Fr QPKSK I THLV FCTTSGVDMP GADYQLTKLL GLRPSVKRLM MYQQGCFAGG TVLRLAKDLA 178
PKS5-GM24-72 QPKSK I THLV FCTTSGVDMP GADYQLTKLL GLRPSVKRLM MYQQGCFAGG TVLRLAKDLA 180
Consensus QPKSK I THLV FCTTSGVDMP GADYQLTKLL GLRPSVKRLM MYQQGCFAGG TVLRLAKDLA 240
                200                            220                            240
PKS1-GM29-48 ENNKGARVLV VCSEITAVTF RGPSTDH LDS LVGQALFGDG AAA I I VGS DP LPDI ERPLFE 238
CHS5-Fr ENNRGARVLV VCSEITAVTF RGPSTDH LDS LVGQALFGDG AAA I I VGS DP LPEI ERPLFE 238
PKS5-GM24-72 ENNRGARVLV VCSEITAVTF RGPSTDH LDS LVGQALFGDG AAA I I VGS DP LPEI ERPLFE 240
Consensus ENNRGARVLV VCSEITAVTF RGPSTDH LDS LVGQALFGDG AAA I I VGS DP LPEI ERPLFE 300
                260                            280                            300
PKS1-GM29-48 LVSA AQT I LP DSDG A I DGHL REVGLTFHLL KDVPGL I SKN I EKSLNEAFK PLDI TDWNSL 298
CHS5-Fr LVSA AQT I LP DSDG A I DGHL REVGLTFHLL KDVPGL I SKN I EKSLNEAFK PLDI TDWNSL 298
PKS5-GM24-72 LVSA AQT I LP DSDG A I DGHL REVGLTFHLL KDVPGL I SKN I EKSLNEAFK PLDI TDWNSL 300
Consensus LVSA AQT I LP DSDG A I DGHL REVGLTFHLL KDVPGL I SKN I EKSLNEAFK PLDI TDWNSL 360
                320                            340                            360
PKS1-GM29-48 FWI AHPPGGA I LDQVEAKLG LKPEKLEATR NILSEYGNMS SACVLFI LDE VRRKSA VANGH 358
CHS5-Fr FWI AHPPGGA I LDQVEAKLA LKPEKLEATR HILSEYGNMS SACVLFI LDE VRRKSA ANGH 358
PKS5-GM24-72 FWI AHPPGGA I LDQVETKLG LKPEKLEATR HILSEYGNMS SACVLFI LDE VRKSA ANGL 360
Consensus FWI AHPPGGA I LDQVEAKLG LKPEKLEATR HILSEYGNMS SACVLFI LDE VRRKSA ANGH 360
                380
PKS1-GM29-48 KTTGEGLEWG VLFGFGPGLT VETVVLH SV A AST 391
CHS5-Fr KTTGEGLEWG VLFGFGPGLT VETVVLH SV S A - 389
PKS5-GM24-72 KTTGEGLEWG VLFGFGPGLT VETVVLH SV G VTA 393
Consensus KTTGEGLEWG VLFGFGPGLT VETVVLH SV * A * *

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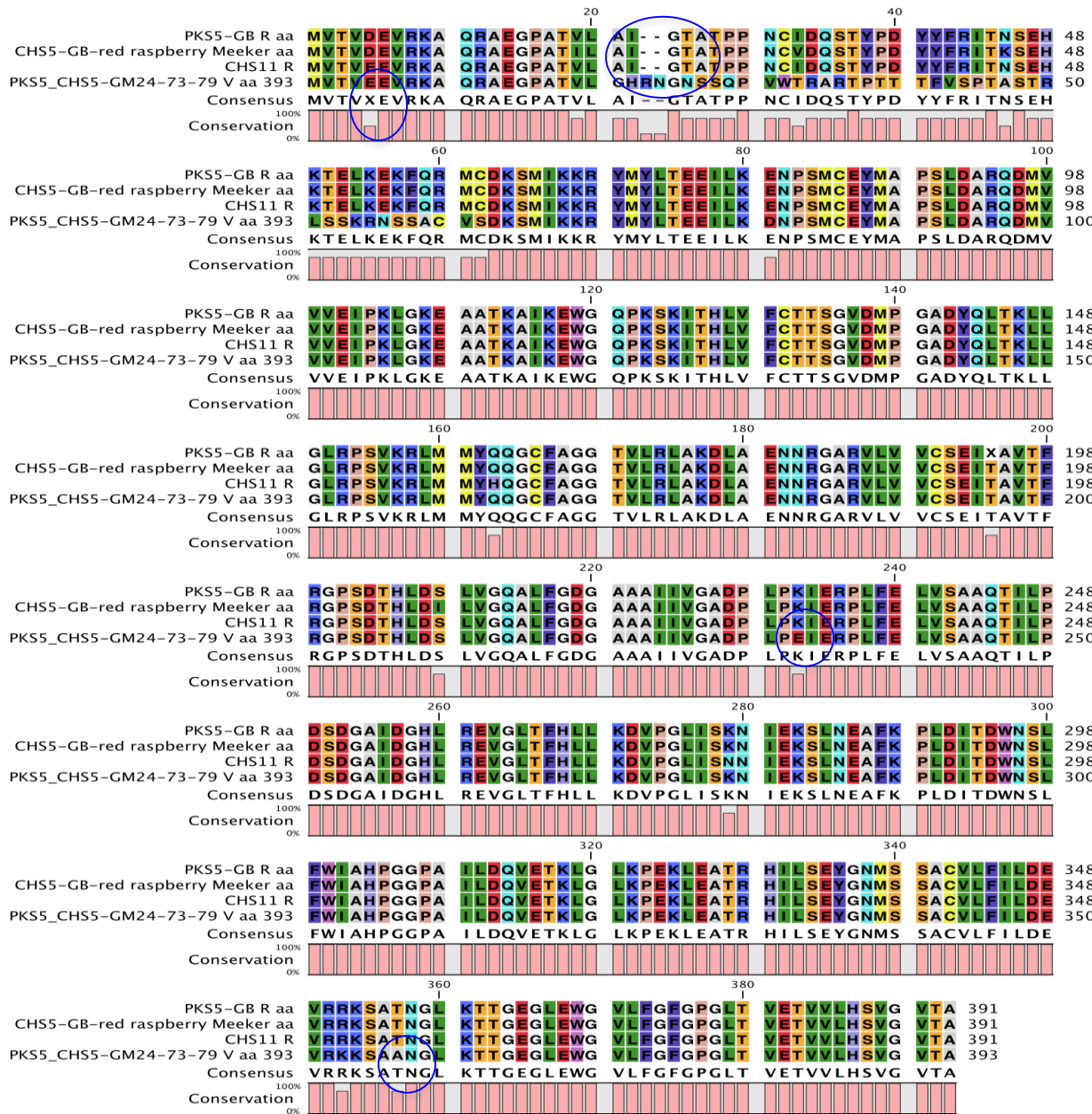
There are more differences



RiPKS1 of BAC24p12

Comparison at Amino acid sequence level with red raspberry PKS cv. - Royalty - Meeker

RiPKS1 : PKS5/CHS5/CHS11



96% identity with PKS5

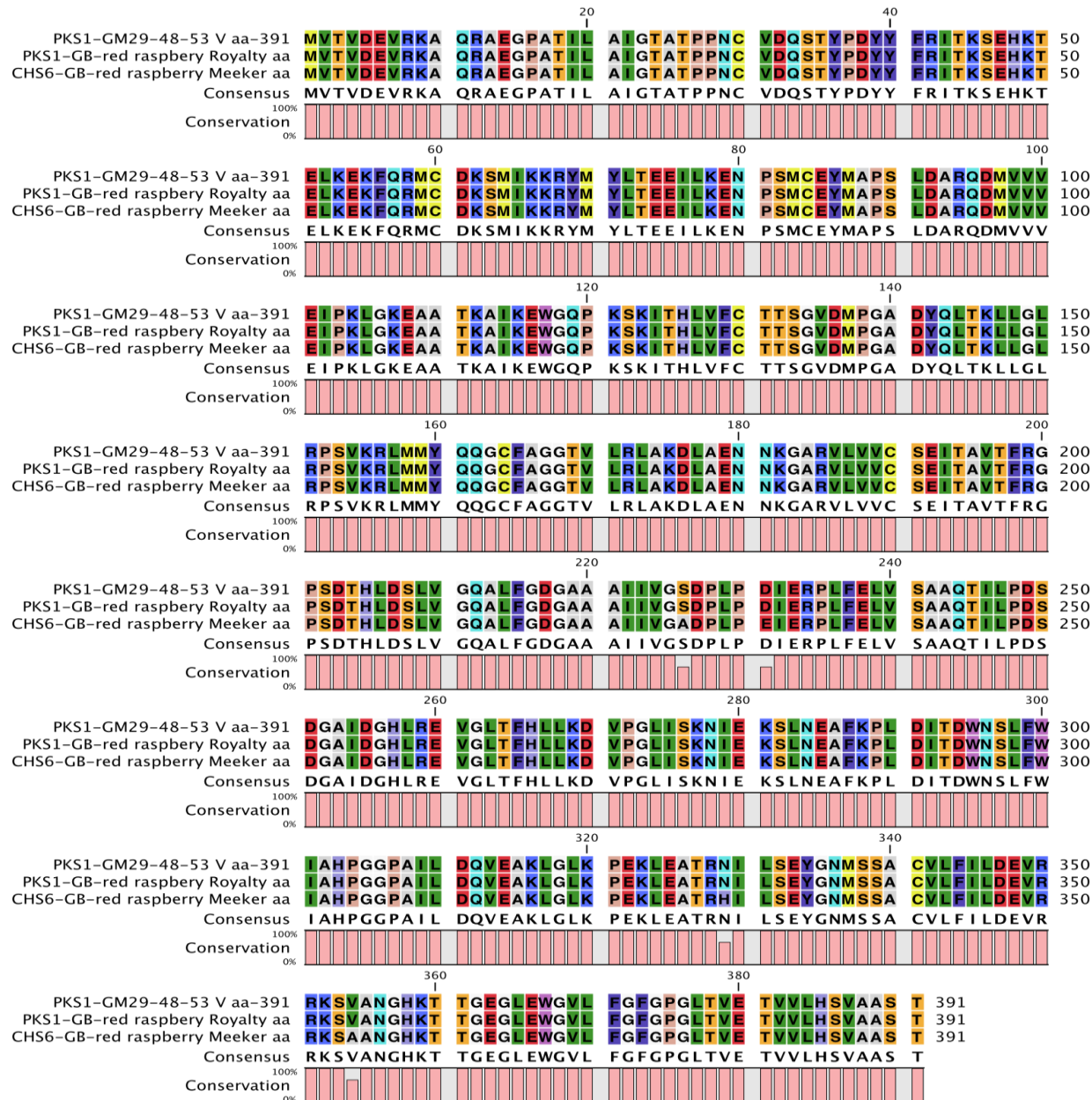


RiPKS3 of BAC29M05

Comparison at Amino acid sequence level with red raspberry PKS cv. - Royalty - Meeker

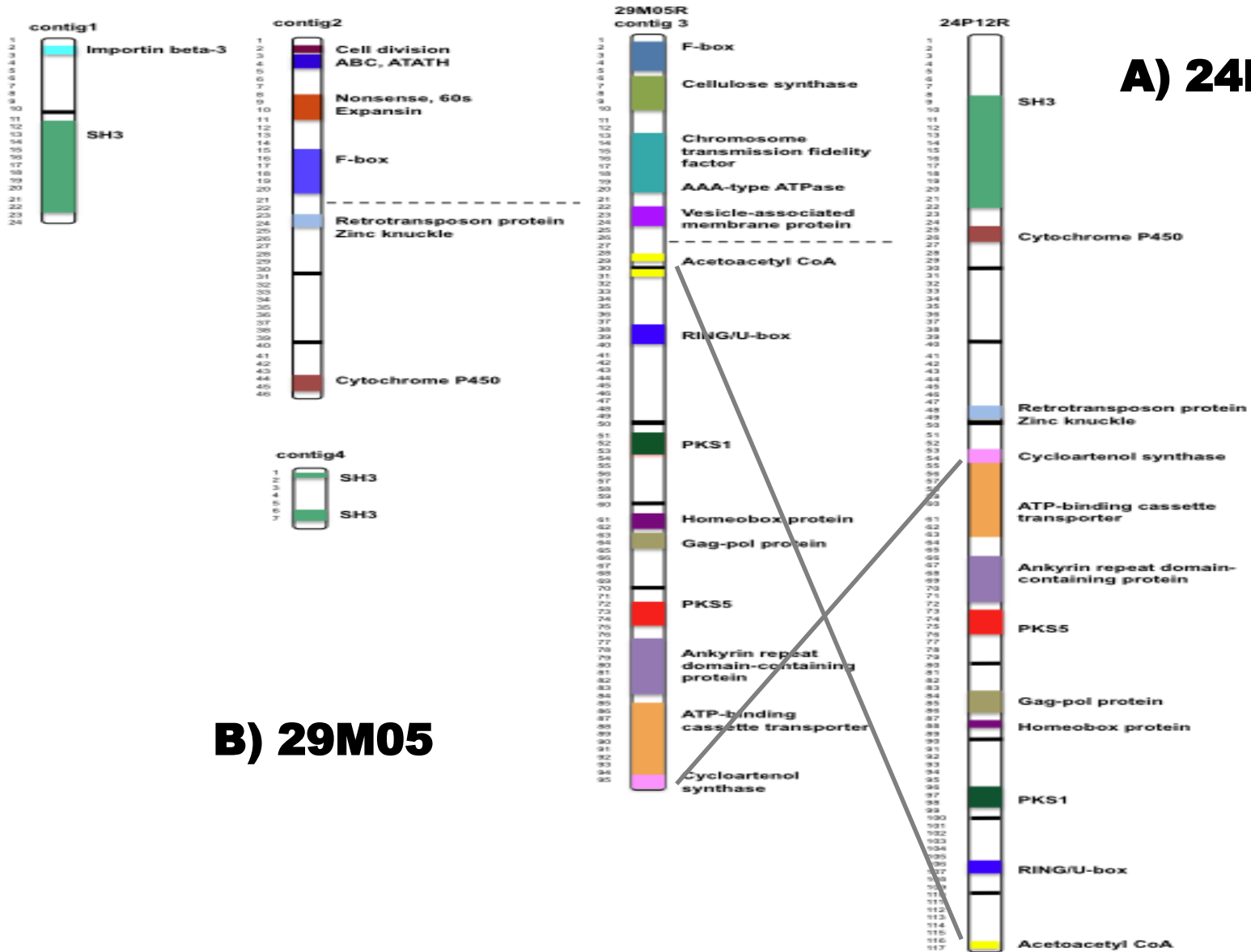
RiPKS1 : PKS5/CHS5/CHS11

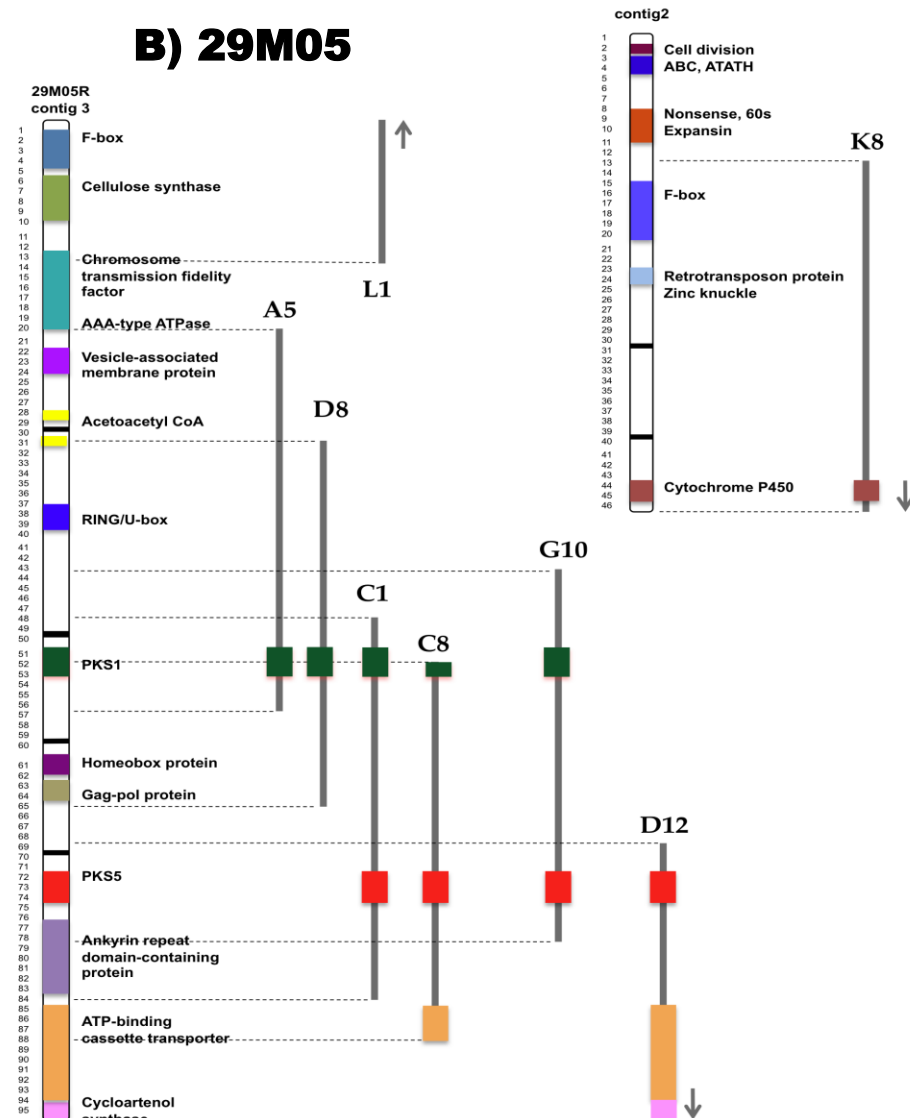
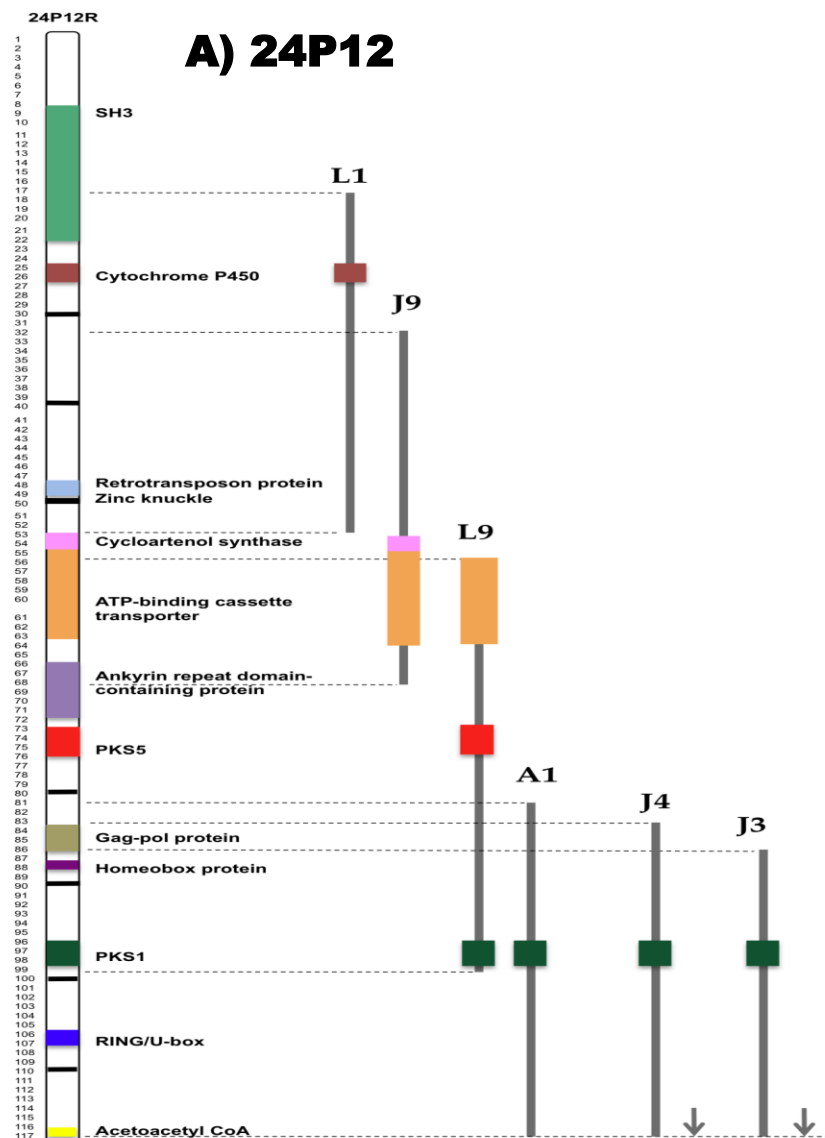
100% identity with PKS1



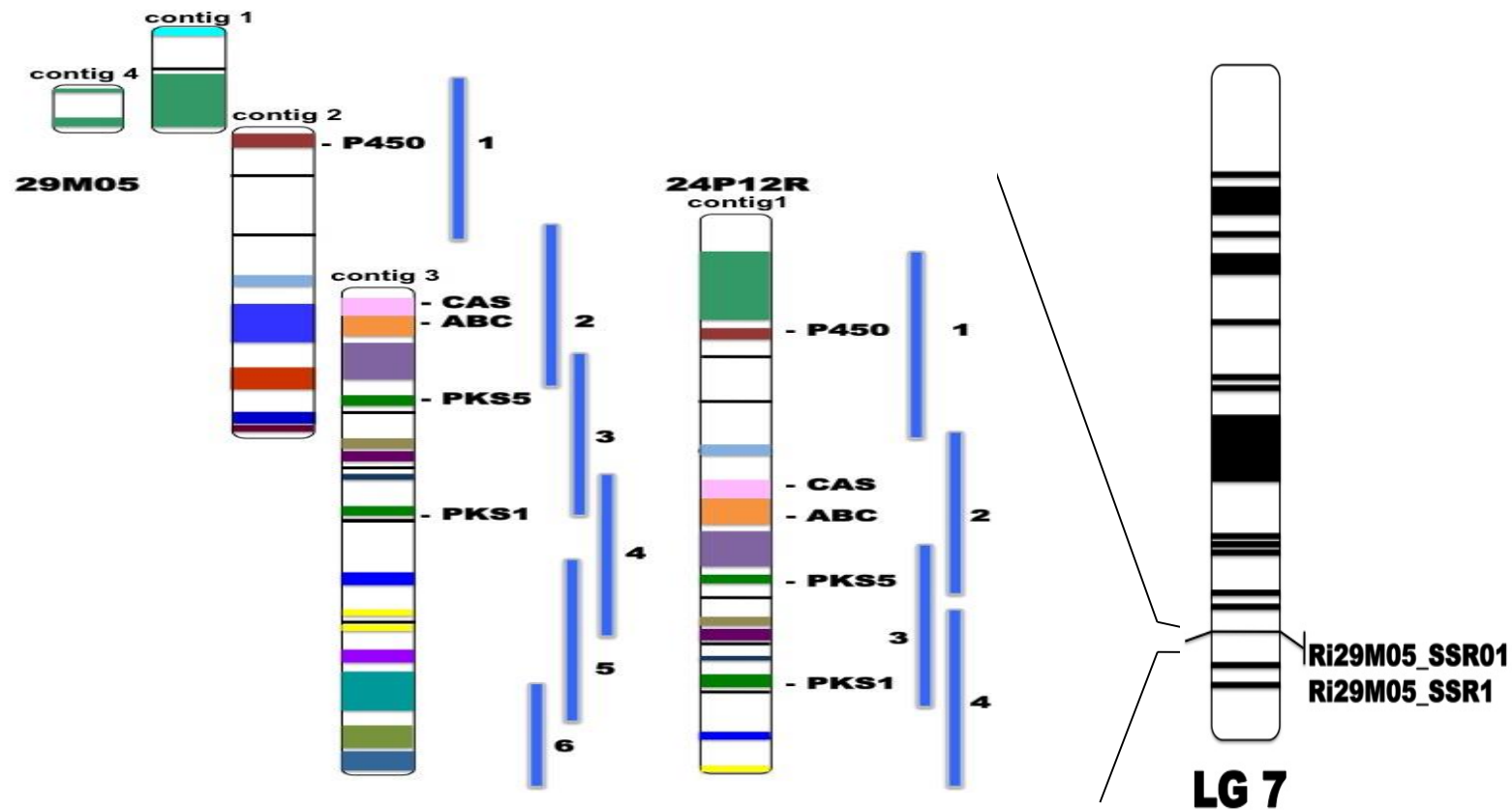


V Construction of Physical Map





The MTP and mini physical maps for 117 kb of BAC24P12 and 98 kb of BAC29M05.



Construction of the mini physical map of the two BACs derived from red raspberry cv. Glen Moy genome



IV. Conclusion

- **Genetic information of PKS genes in red raspberry**
 - **Fosmid library**
 - **Mini Physical Maps**
- The additional information of PKS genes of red raspberry cv. Glen Moy are extensively powerful tool for further studies and applications with the same family and other related fruits as well as other several genes**
- Advanced techniques / Molecular or genetic approach use shorter time / provide higher qualities for plants**





VI. Acknowledgments :

Dr. Paterson A., Dr Ellis E. M.
Special for Dr. Lutz E. M.
NSRU, Thailand
ATPER

