

Rapid Production of Cyclic Citrullinated Peptide Monoclonal Antibody in *Nicotiana benthamiana* for the Early Detection and Diagnosis of Rheumatoid Arthritis

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Table S1. Amino acid sequence of the synthetic CCP and CRP peptide

Peptide	Amino acid sequence	Note
CRP	HQ <u>CH</u> QEST <u>R</u> GRSRGR <u>C</u> GRSGS	Non-citrullinated peptide (CRP) is arginine (R) was changed to citrulline (X) in CCP
CCP	HQ <u>CH</u> QEST X GRSRGR <u>C</u> GRSGS	X stands for citruline amino acid, forming a chemical bond between 2 underline C in the peptide

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DNASIS
Simple Homology Region [mAbCCP LC (BIXI-KIBISI)]

No.	Target	File	Mode	Key	Target	Overlap	Match	Percentage
1	CCP	LC1-T7	N	1	52	751	751	100.00%
1	10	20	30	40	50	60	70	
5'	TTTGGTCTCAAGGTTCTAGAA	GGTATCCACAGCTCAGTTTCTTGAATCTTGTTGCTCTGGTTTCCAG						
*****	*****	*****	*****	*****	*****	*****	*****	*****
5'	TTTGGTCTCAAGGTTCTAGAA	GGTATCCACAGCTCAGTTTCTTGAATCTTGTTGCTCTGGTTTCCAG						
52	61	71	81	91	101	111	121	
71	80	90	100	110	120	130	140	
GTATCAAATGTGACATCAAGATGACCCAGTCTCCATCTTCCATGTATGCATCTCTAGGAGAGAGAGTACAC								
*****	*****	*****	*****	*****	*****	*****	*****	*****
GTATCAAATGTGACATCAAGATGACCCAGTCTCCATCTTCCATGTATGCATCTCTAGGAGAGAGAGTACAC								
122	131	141	151	161	171	181	191	
141	150	160	170	180	190	200	210	
TATCACTTGCAAGGCGAGTCAGGACATTAATAGCTATTTAAGCTGGTTCCAGCAGAAACCAGGGAAATCT								
*****	*****	*****	*****	*****	*****	*****	*****	*****
TATCACTTGCAAGGCGAGTCAGGACATTAATAGCTATTTAAGCTGGTTCCAGCAGAAACCAGGGAAATCT								
192	201	211	221	231	241	251	261	
211	220	230	240	250	260	270	280	
CCTAAGACCCTGATCTATCGTGCAAACAGATGGTAGATGGGGTCCCATCAAGGTTCAAGTGGCAGTGGAT								
*****	*****	*****	*****	*****	*****	*****	*****	*****
CCTAAGACCCTGATCTATCGTGCAAACAGATGGTAGATGGGGTCCCATCAAGGTTCAAGTGGCAGTGGAT								
262	271	281	291	301	311	321	331	
281	290	300	310	320	330	340	350	
CTGGGCAAGATTATCTCTCACCATCAGCAGCCTGGAGTATGAAGATATGGGAATTTATTATGTCTACA								
*****	*****	*****	*****	*****	*****	*****	*****	*****
CTGGGCAAGATTATCTCTCACCATCAGCAGCCTGGAGTATGAAGATATGGGAATTTATTATGTCTACA								
332	341	351	361	371	381	391	401	
351	360	370	380	390	400	410	420	
GTATGATGAGTTTCCGTGGACGTTCCGTGGAGGCACCAAGCTGGAAATCAAACGGGGTGTATGCTGCACCA								
*****	*****	*****	*****	*****	*****	*****	*****	*****
GTATGATGAGTTTCCGTGGACGTTCCGTGGAGGCACCAAGCTGGAAATCAAACGGGGTGTATGCTGCACCA								
402	411	421	431	441	451	461	471	
421	430	440	450	460	470	480	490	
ACTGTATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCCTCAGTCGTGTGCTTCTTGA								
*****	*****	*****	*****	*****	*****	*****	*****	*****
ACTGTATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCCTCAGTCGTGTGCTTCTTGA								
472	481	491	501	511	521	531	541	
491	500	510	520	530	540	550	560	
ACAACTTCTACCCCAAAGACATCAATGTCAAGTGAAGATTGATGGCAGTGAACGACAAAATGGCGTCCT								
*****	*****	*****	*****	*****	*****	*****	*****	*****
ACAACTTCTACCCCAAAGACATCAATGTCAAGTGAAGATTGATGGCAGTGAACGACAAAATGGCGTCCT								
542	551	561	571	581	591	601	611	
561	570	580	590	600	610	620	630	
GAACAGTTGGACTGATCAGGACAGCAAAGACAGCACCTACAGCATGAGCAGCACCCCTCAGTTGACCAAG								
*****	*****	*****	*****	*****	*****	*****	*****	*****
GAACAGTTGGACTGATCAGGACAGCAAAGACAGCACCTACAGCATGAGCAGCACCCCTCAGTTGACCAAG								
612	621	631	641	651	661	671	681	
631	640	650	660	670	680	690	700	
GACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATGTGTA								
*****	*****	*****	*****	*****	*****	*****	*****	*****
GACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATGTGTA								
682	691	701	711	721	731	741	751	
701	710	720	730	740	750			
AGAGCTTCAACAGGAATGAGTGTGTGATAGGTACCGCTTTGAGACCGAGCTC 3'								
*****	*****	*****	*****	*****	*****	*****	*****	*****
AGAGCTTCAACAGGAATGAGTGTGTGATAGGTACCGCTTTGAGACCGAGCTC 3'								
752	761	771	781	791	801			

Cont.

Supplementary Data

Simple Homology Region [mAbCCP LC (BIXI-KIBISI)]							
No.	Target File	Mode	Key	Target	Overlap	Match	Percentage
1	CCP LC1-SP6	C	1	74	751	751	100.00%
1	10	20	30	40	50	60	70
5'	GAGCTCGGTCTCAAAGCGGTACCTATCAACACTCATTCCTGTTGAAGCTCTTGACAATGGGTGAAGTTGA						

5'	GAGCTCGGTCTCAAAGCGGTACCTATCAACACTCATTCCTGTTGAAGCTCTTGACAATGGGTGAAGTTGA						
74	83	93	103	113	123	133	143
71	80	90	100	110	120	130	140
TGTCCTTGAGTGGCCTCACAGGTATAGCTGTTATGTGCTTCATACTCGTCCTTGGTCAACGTGAGGGTG							

TGTCCTTGAGTGGCCTCACAGGTATAGCTGTTATGTGCTTCATACTCGTCCTTGGTCAACGTGAGGGTG							
144	153	163	173	183	193	203	213
141	150	160	170	180	190	200	210
CTGCTCATGCTGTAGGTGCTGCTCTTGTCTGTCCTGATCAGTCCAAGTGTTCAGGACGCCAATTTGTCTGTT							

CTGCTCATGCTGTAGGTGCTGCTCTTGTCTGTCCTGATCAGTCCAAGTGTTCAGGACGCCAATTTGTCTGTT							
214	223	233	243	253	263	273	283
211	220	230	240	250	260	270	280
CACTGCCATCAATCTTCCACTTGACATTTGATGCTCTTGGGGTAGAAGTTGTTCAAGAAGCACACGACTGA							

CACTGCCATCAATCTTCCACTTGACATTTGATGCTCTTGGGGTAGAAGTTGTTCAAGAAGCACACGACTGA							
284	293	303	313	323	333	343	353
281	290	300	310	320	330	340	350
GGCACCTCCAGATGTTAACTGCTCACTGGATGGTGGGAAGATGGATACAGTTGGTGCAGCATCAGCCCGT							

GGCACCTCCAGATGTTAACTGCTCACTGGATGGTGGGAAGATGGATACAGTTGGTGCAGCATCAGCCCGT							
354	363	373	383	393	403	413	423
351	360	370	380	390	400	410	420
TTGATTTCCAGCTTGGTGCCTCCACCGAACGTCCACGAAACTCATCACTGTAGACAATAATAAATTC							

TTGATTTCCAGCTTGGTGCCTCCACCGAACGTCCACGAAACTCATCACTGTAGACAATAATAAATTC							
424	433	443	453	463	473	483	493
421	430	440	450	460	470	480	490
CCATATCTTCATACTCCAGGCTGCTGATGGTGAGAGAATAATCTTGCCAGATCCACTGCCACTGAACCT							

CCATATCTTCATACTCCAGGCTGCTGATGGTGAGAGAATAATCTTGCCAGATCCACTGCCACTGAACCT							
494	503	513	523	533	543	553	563
491	500	510	520	530	540	550	560
TGATGGGACCCCATCTACCAATCTGTTTGACGATAGATCAGGGTCTTAGGAGATTTCCTGGTTTCTGC							

TGATGGGACCCCATCTACCAATCTGTTTGACGATAGATCAGGGTCTTAGGAGATTTCCTGGTTTCTGC							
564	573	583	593	603	613	623	633
561	570	580	590	600	610	620	630
TGGAACCAGCTTAAATAGCTATTAATGTCTGACTCGCCTTGCAAGTGATAGTGACTCTCTCTCCTAGAG							

TGGAACCAGCTTAAATAGCTATTAATGTCTGACTCGCCTTGCAAGTGATAGTGACTCTCTCTCCTAGAG							
634	643	653	663	673	683	693	703
631	640	650	660	670	680	690	700
ATGCATACATGGAAGATGGAGACTGGGTTCATCTTGATGTACATTTGATACCTGGAAACCAGAGCAACAA							

ATGCATACATGGAAGATGGAGACTGGGTTCATCTTGATGTACATTTGATACCTGGAAACCAGAGCAACAA							
704	713	723	733	743	753	763	773
701	710	720	730	740	750		
GATTCCAAGAACTGAGCTGTGGATACCATTTCTAGAACCTTGAGACCAAAA 3'							

GATTCCAAGAACTGAGCTGTGGATACCATTTCTAGAACCTTGAGACCAAAA 3'							
774	783	793	803	813	823		

Figure S1. DNA sequence agliment of CCP-LC using SP6 and T7 primer.

Supplementary Data

No.	Target	File	Mode	Key	Target	Overlap	Match	Percentage
1	CCP	HC11-T7	N	1	52	1043	891	61.70%
1	10	20	30	40	50	60	70	
5'	TTTTGGTCTCAAGGTTCTAGAATGGAATGGAGTGGGGTCTTTATCTTCTCTTGTCAGTAAGTGCAGGTG							
5'	TTTTGGTCTCAAGGTTCTAGAATGGAATGGAGTGGGGTCTTTATCTTCTCTTGTCAGTAAGTGCAGGTG							
52	61	71	81	91	101	111	121	
71	80	90	100	110	120	130	140	
	TCCACTCCCAGGTCCAGTTGCAGCAGTCTGGAGCTGAGCTGGTAAGGCCTGGGACTTCAGTGAAGATATC							
	TCCACTCCCAGGTCCAGTTGCAGCAGTCTGGAGCTGAGCTGGTAAGGCCTGGGACTTCAGTGAAGATATC							
122	131	141	151	161	171	181	191	
141	150	160	170	180	190	200	210	
	CTGCAAGGCTTCTGGATACGGCTTCACTAAGTCTGGCTCGGTTGGATAAAGCAGAGGCCTGGACATGGA							
	CTGCAAGGCTTCTGGATACGGCTTCACTAAGTCTGGCTCGGTTGGATAAAGCAGAGGCCTGGACATGGA							
192	201	211	221	231	241	251	261	
211	220	230	240	250	260	270	280	
	CTTGAGTGGATTGGAGATATTTACCCTGGAAGTGGTAATCTTACTACAATGAGAAGTTCAAGGGCAAAG							
	CTTGAGTGGATTGGAGATATTTACCCTGGAAGTGGTAATCTTACTACAATGAGAAGTTCAAGGGCAAAG							
262	271	281	291	301	311	321	331	
281	290	300	310	320	330	340	350	
	CCCACTGACTGCAGACAAATCCTCGAGCAGCTCTATATGCAGCTCAGTAGCCTGACATCTGAAGACTC							
	CCCACTGACTGCAGACAAATCCTCGAGCAGCTCTATATGCAGCTCAGTAGCCTGACATCTGAAGACTC							
332	341	351	361	371	381	391	401	
351	360	370	380	390	400	410	420	
	TACTGTCTATTTCTGTGCAAGAGAGGGGGGAATTACTACGGCTCCGTACCCGTTTGTTTACTGGGGCCAA							
	TACTGTCTATTTCTGTGCAAGAGAGGGGGGAATTACTACGGCTCCGTACCCGTTTGTTTACTGGGGCCAA							
402	411	421	431	441	451	461	471	
421	430	440	450	460	470	480	490	
	GGGACTCTGGTCACTGTCTCTGCAGCAAAACGACACCCCATCTGTCTATCCACTGGCCCTGGATCTG							
	GGGACTCTGGTCACTGTCTCTGCAGCAAAACGACACCCCATCTGTCTATCCACTGGCCCTGGATCTG							
472	481	491	501	511	521	531	541	
491	500	510	520	530	540	550	560	
	CTGCCAACTAACTCCATGGTGACCTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGT							
	CTGCCAACTAACTCCATGGTGACCTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGT							
542	551	561	571	581	591	601	611	
561	570	580	590	600	610	620	630	
	GACCTGGAACCTGGATCCCTGTCCAGCGGTGTGCACACCTCCAGCTGTCTGACGTCTGACCTCTAC							
	GACCTGGAACCTGGATCCCTGTCCAGCGGTGTGCACACCTCCAGCTGTCTGACGTCTGACCTCTAC							
612	621	631	641	651	661	671	681	
631	640	650	660	670	680	690	700	
	ACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCAGCGAGACCTCACCTGCAACGTTGCC							
	ACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCAGCGAAACGTCACCTGCAACGTTGCC							
682	691	701	711	721	731	741	751	
701	710	720	730	740	750	760	770	
	ACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCAGGGATTGGTTGTAAGCCTTGATATG							
	ACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCAGGGATTGGTTGTAAGCCTTGATATG							
752	761	771	781	791	801	811	821	
771	780	790	800	810	820	830	840	
	TACAGTCCCAGAAGTATCATCTGTCTTCTTCTTCCCCCAAAGCCCAAGGATGTGCTCACCATTACTCTG							
	TACAGTCCCAGAAGTATCATCTGTCTTCTTCTTCCCCCAAAGCCCAAGGATGTGCTCACCATTACTCTG							
822	831	841	851	861	871	881	891	
841	850	860	870	880	890	900	910	
	ACTCCTAAGTACAGTGTGTTGGTAGACATCAGCAAGGATGATCCCGAGTCCAGTTCAGCTGGTTTG							
	ACTCCTAAGTACAGTGTGTTGGTAGACATCAGCAAGGATGATCCCGAGTCCAGTTCAGCTGGTTTG							
892	901	911	921	931	941	951	961	

Cont.

Supplementary Data

Simple Homology Region [mabCCP HC(BIXI-KIBISI)]

No.	Target File	Mode	Key	Target	Overlap	Match	Percentage	
1	CCP HC11-SP6	C	1	218	920	697	48.26%	
1	10	20	* *	30	40	50	60	70
5'	GAGCTCGGTCTCAAAGCGGTACCTATCATTTACCAGGAGAGTGGGAGAGGCTCTTCTCAGTATGGTGTT							
*****	*****	*****	*****	*****	*****	*****	*****	*****
5'	GAGCTCGGTCTCAAAGCGGTACCTATCATTTACCAGGAGAGTGGGAGAGGCTCTTCTCAGTATGGTGTT							
218	227	237	247	257	267	277	287	
71	80	90	100	110	120	130	140	
GTGCAGGCCCTCATGTAACACAGAGCAGGTAAAGTATTTCCTGCCTCCCAGTTGCTCTTCTGCACATTG								
*****	*****	*****	*****	*****	*****	*****	*****	*****
GTGCAGGCCCTCATGTAACACAGAGCAGGTAAAGTATTTCCTGCCTCCCAGTTGCTCTTCTGCACATTG								
288	297	307	317	327	337	347	357	
141	150	160	170	180	190	200	210	
AGCTTGTCTGTAGACGAAGTAAGAGCCATCTGTGTCCATGATGGGCTGAGTGTCTTGTAGTTCTCCGCTG								
*****	*****	*****	*****	*****	*****	*****	*****	*****
AGCTTGTCTGTAGACGAAGTAAGAGCCATCTGTGTCCATGATGGGCTGAGTGTCTTGTAGTTCTCCGCTG								
358	367	377	387	397	407	417	427	
211	220	230	240	250	260	270	280	
GCTGCCCATTTCCA CTGCCACTCCACAGTAATGTCTTCAGGGAAGAAGTCTGTTATCATGCAGGTCAGACT								
*****	*****	*****	*****	*****	*****	*****	*****	*****
GCTGCCCATTTCCA CTGCCACTCCACAGTAATGTCTTCAGGGAAGAAGTCTGTTATCATGCAGGTCAGACT								
428	437	447	457	467	477	487	497	
281	290	300	310	320	330	340	350	
GACTTTATCCTTTGGCCATCTGCTCCTTTGGGAGGTGGAATGGTGTACACCTGTGGAGCCTTCGGTCTGCCT								
*****	*****	*****	*****	*****	*****	*****	*****	*****
GACTTTATCCTTTGGCCATCTGCTCCTTTGGGAGGTGGAATGGTGTACACCTGTGGAGCCTTCGGTCTGCCT								
498	507	517	527	537	547	557	567	
351	360	370	380	390	400	410	420	
TTGGTTT TGGAGATGGTTTTCTCGATGGGGCAGGGAAAGCTGCACTGTTGACCTGCAITTTGAAC TCCT								
*****	*****	*****	*****	*****	*****	*****	*****	*****
TTGGTTT TGGAGATGGTTTTCTCGATGGGGCAGGGAAAGCTGCACTGTTGACCTGCAITTTGAAC TCCT								
568	577	587	597	607	617	627	637	
421	430	440	450	460	470	480	490	
TGCCATTTAGCCAGTCTCTGGTGCATGATGGGAAGTTCACTGACTGAGCGGAAAGTGCTGTTGAAC TGCTC								
*****	*****	*****	*****	*****	*****	*****	*****	*****
TGCCATTTAGCCAGTCTCTGGTGCATGATGGGAAGTTCACTGACTGAGCGGAAAGTGCTGTTGAAC TGCTC								
638	647	657	667	677	687	697	707	
491	500	510	520	530	540	550	560	
CTCCCCGGGGTTGCGTCTGAGCTGTGTGCACCTCCACATCATCTACAAACCAGCTGAAC TTGACCTCGGGA								
*****	*****	*****	*****	*****	*****	*****	*****	*****
CTCCCCGGGGTTGCGTCTGAGCTGTGTGCACCTCCACATCATCTACAAACCAGCTGAAC TTGACCTCGGGA								
708	717	727	737	747	757	767	777	
561	570	580	590	600	610	620	630	
TCATCCTTTGCTGATGTCTACCACAACACAGTGACCTTAGGAGTCAGAGTAATGGTGAGCACATCCTTGG								
*****	*****	*****	*****	*****	*****	*****	*****	*****
TCATCCTTTGCTGATGTCTACCACAACACAGTGACCTTAGGAGTCAGAGTAATGGTGAGCACATCCTTGG								
778	787	797	807	817	827	837	847	
631	640	650	660	670	680	690	700	
GCTTTGGGGGGAAGATGAAGACAGATGATACTTCTGGGACTGTACATATGCAAGGCTTACAACCACAATC								
*****	*****	*****	*****	*****	*****	*****	*****	*****
GCTTTGGGGGGAAGATGAAGACAGATGATACTTCTGGGACTGTACATATGCAAGGCTTACAACCACAAT								
848	857	867	877	887	897	907	917	

CCT = GGA
CCC = GGG

Figure S2. DNA sequence agliment of CCP-HC using SP6 and T7 primer.

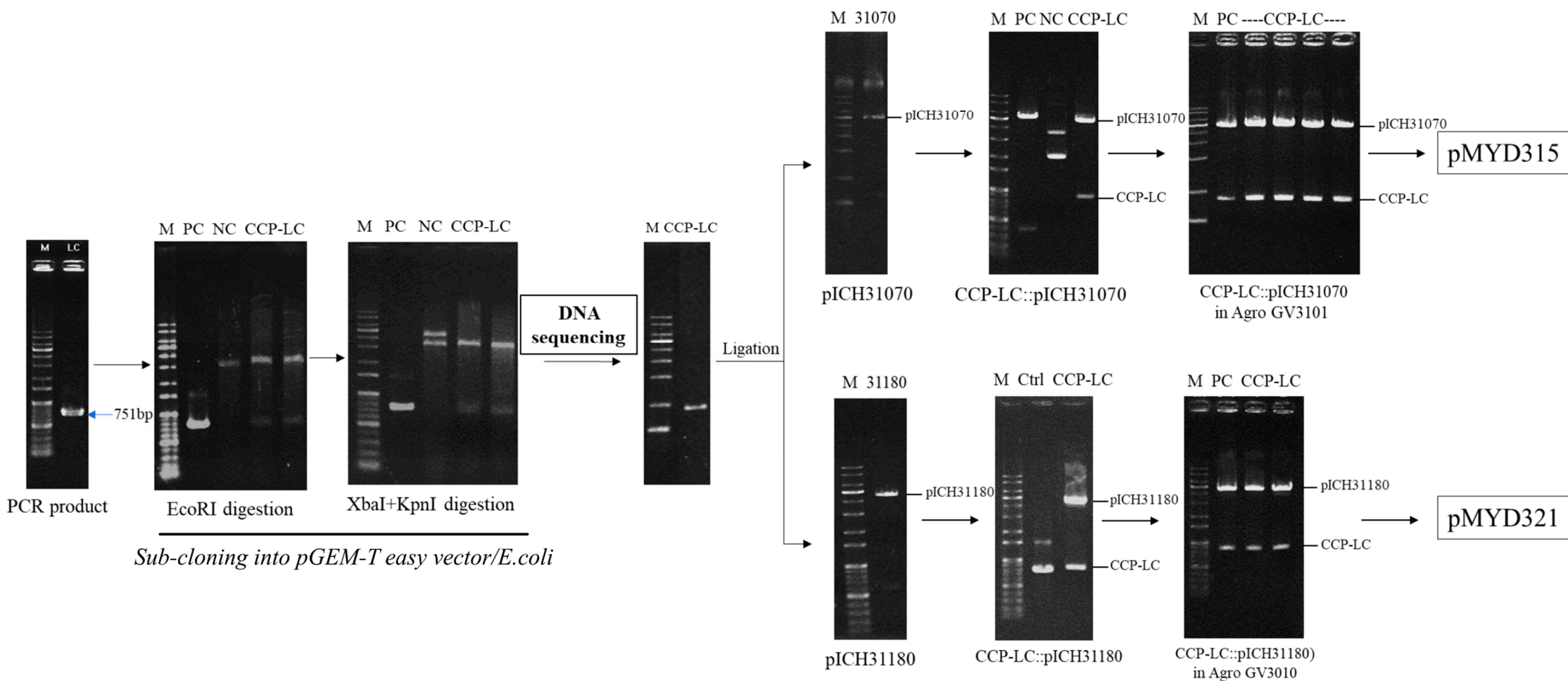


Figure S3. Cloning and construction of expression vectors for CCP-LC. The CCP-LC containing a BsaI site at the 5' end and the 3' end were subcloned into the pGEM-T easy vector and then confirmed by enzyme digestion before DNA sequencing. The BsaI-digested fragment of CCP-LC was eluted and cleaned up from agarose gel using the fragment DNA purification kit (iNtRON Biotechnology) then cloning into the plant expression vector pICH31070 and pICH31180, which resulted in pMYD315 and pMYD321, respectively. The expression vectors were transformed into *A. tumefaciens* GV3101 and then their plasmid DNA was isolated and confirmed by BsaI enzyme digestion. M is DNA ladder

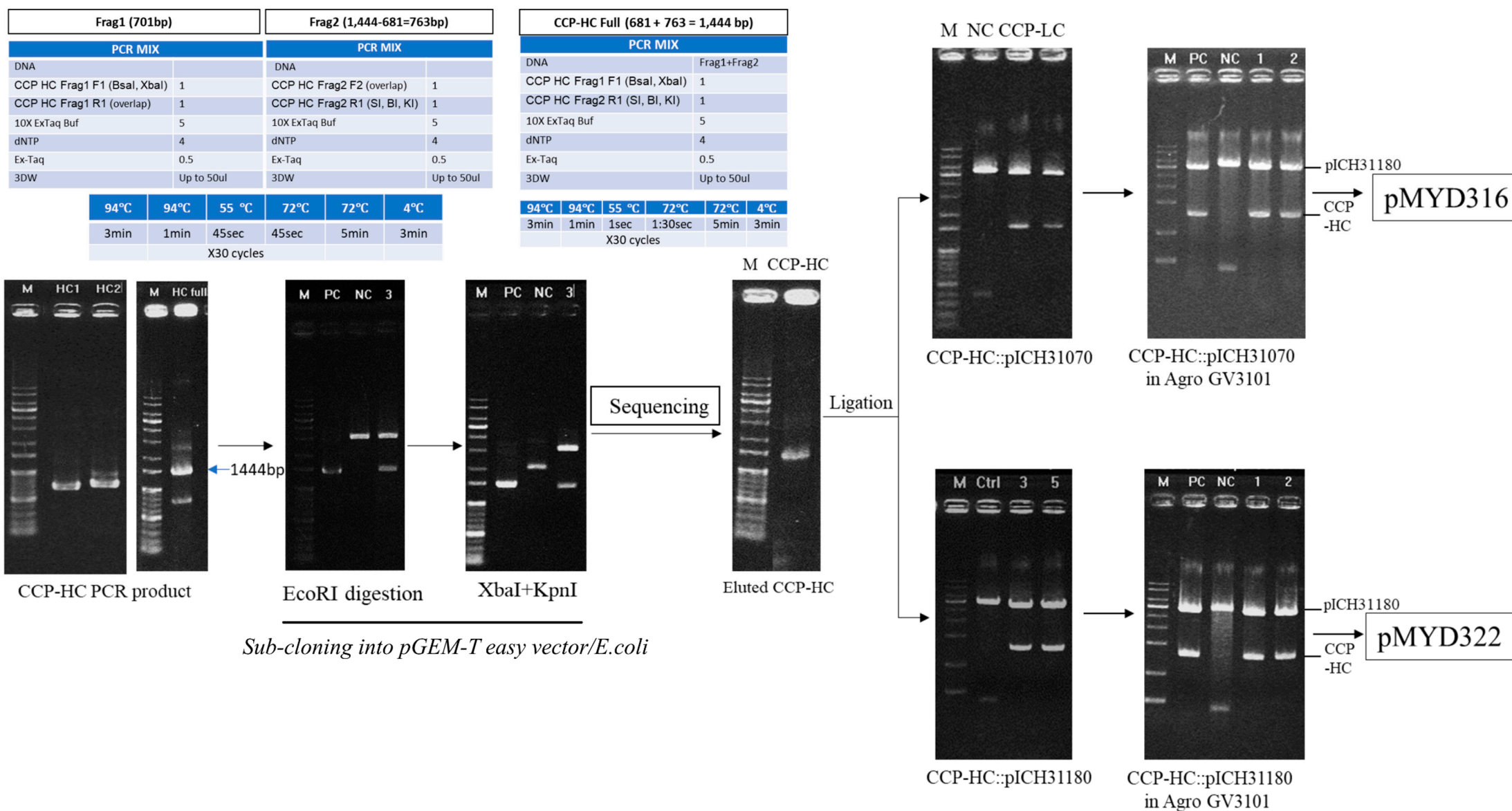


Figure S4. Cloning and construction of expression vectors for CCP-HC. The CCP-HC containing a BsaI site at the 5' end and the 3' end were subcloned into the pGEM-Teasy vector and then confirmed by enzyme digestion before DNA sequencing. The BsaI-digested fragment of CCP-HC was eluted and cleaned up from agarose gel and then cloning into the plant expression vector pICH31070 and pICH31180, which resulted in pMYD316 and pMYD322, respectively. The expression vectors were transformed into *A. tumefaciens* GV3101 and then their plasmid DNA was isolated and confirmed by BsaI enzyme digestion. M is DNA ladder.