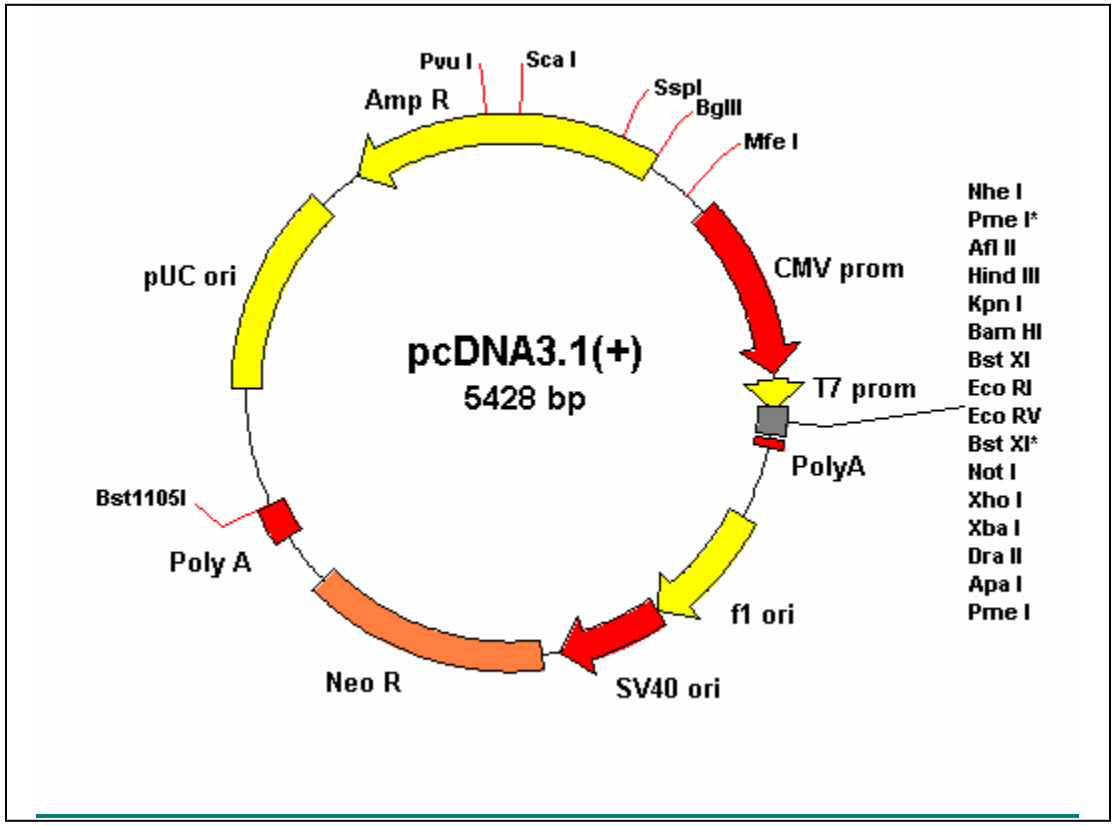
pcDNA3.1(+) [Invitrogen]



pcDNA 3.1 DNA Sequence

CMV forward primer: 5’ CGCAAATGGGCGGTAGGCGTG 3’

T7 Universal primer: 5’ TAATACGACTCACTATAGG 3’

MCS: shaded

BGH_rev primer: 5’ TAGAAGGCACAGTCGAGG 3’ (complement shown in blue – 5’CCTCGACTGTGCCTTCTA 3’)

Rheoswitch promoter forward primer: 5’ TTCGCGATGTACGGGCCA 3’

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   1 GACGGATCGG GAGATCTCCC GATCCCCTAT GGTGCACTCT CAGTACAATC TGCTCTGATG CCGCATAGTT AAGCCAGTAT CTGCTCCCTG CTTGTGTGTT
 101 GGAGGTCGCT GAGTAGTGCG CGAGCAAAAT TTAAGCTACA ACAAGGCAAG GCTTGACCGA CAATTGCATG AAGAATCTGC TTAGGGTTAG GCGTTTTGCG
 201 CTGCTTCGCG ATGTACGGGC CAGATATACG CGTTGACATT GATTATTGAC TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA
 301 TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG CCCAACGACC CCCGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT
 401 AACGCCAATA GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT AAACTGCCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC
 501 CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCAGTA CATGACCTTA TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA
 601 TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA
 701 TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG
 801 GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA CTGCTTACTG GCTTATCGAA ATTAATACGA CTCACTATAG GGAGACCCAA GCTGGCTAGC
 901 GTTTAAACTT AAGCTTGGTA CCGAGCTCGG ATCCACTAGT CCAGTGTGGT GGAATTCTGC AGATATCCAG CACAGTGGCG GCCGCTCGAG TCTAGAGGGC
1001 CCGTTTAAAC CCGCTGATCA GCCTCGACTG TGCCTTCTAG TTGCCAGCCA TCTGTTGTTT GCCCCTCCCC CGTGCCTTCC TTGACCCTGG AAGGTGCCAC
1101 TCCCACTGTC CTTTCCTAAT AAAATGAGGA AATTGCATCG CATTGTCTGA GTAGGTGTCA TTCTATTCTG GGGGGTGGGG TGGGGCAGGA CAGCAAGGGG
1201 GAGGATTGGG AAGACAATAG CAGGCATGCT GGGGATGCGG TGGGCTCTAT GGCTTCTGAG GCGGAAAGAA CCAGCTGGGG CTCTAGGGGG TATCCCCACG
1301 CGCCCTGTAG CGGCGCATTA AGCGCGGCGG GTGTGGTGGT TACGCGCAGC GTGACCGCTA CACTTGCCAG CGCCCTAGCG CCCGCTCCTT TCGCTTTCTT
1401 CCCTTCCTTT CTCGCCACGT TCGCCGGCTT TCCCCGTCAA GCTCTAAATC GGGGGCTCCC TTTAGGGTTC CGATTTAGTG CTTTACGGCA CCTCGACCCC
1501 AAAAAACTTG ATTAGGGTGA TGGTTCACGT AGTGGGCCAT CGCCCTGATA GACGGTTTTT CGCCCTTTGA CGTTGGAGTC CACGTTCTTT AATAGTGGAC
1601 TCTTGTTCCA AACTGGAACA ACACTCAACC CTATCTCGGT CTATTCTTTT GATTTATAAG GGATTTTGCC GATTTCGGCC TATTGGTTAA AAAATGAGCT
1701 GATTTAACAA AAATTTAACG CGAATTAATT CTGTGGAATG TGTGTCAGTT AGGGTGTGGA AAGTCCCCAG GCTCCCCAGC AGGCAGAAGT ATGCAAAGCA
1801 TGCATCTCAA TTAGTCAGCA ACCAGGTGTG GAAAGTCCCC AGGCTCCCCA GCAGGCAGAA GTATGCAAAG CATGCATCTC AATTAGTCAG CAACCATAGT
1901 CCCGCCCCTA ACTCCGCCCA TCCCGCCCCT AACTCCGCCC AGTTCCGCCC ATTCTCCGCC CCATGGCTGA CTAATTTTTT TTATTTATGC AGAGGCCGAG
2001 GCCGCCTCTG CCTCTGAGCT ATTCCAGAAG TAGTGAGGAG GCTTTTTTGG AGGCCTAGGC TTTTGCAAAA AGCTCCCGGG AGCTTGTATA TCCATTTTCG
2101 GATCTGATCA AGAGACAGGA TGAGGATCGT TTCGCATGAT TGAACAAGAT GGATTGCACG CAGGTTCTCC GGCCGCTTGG GTGGAGAGGC TATTCGGCTA
2201 TGACTGGGCA CAACAGACAA TCGGCTGCTC TGATGCCGCC GTGTTCCGGC TGTCAGCGCA GGGGCGCCCG GTTCTTTTTG TCAAGACCGA CCTGTCCGGT
2301 GCCCTGAATG AACTGCAGGA CGAGGCAGCG CGGCTATCGT GGCTGGCCAC GACGGGCGTT CCTTGCGCAG CTGTGCTCGA CGTTGTCACT GAAGCGGGAA
2401 GGGACTGGCT GCTATTGGGC GAAGTGCCGG GGCAGGATCT CCTGTCATCT CACCTTGCTC CTGCCGAGAA AGTATCCATC ATGGCTGATG CAATGCGGCG
2501 GCTGCATACG CTTGATCCGG CTACCTGCCC ATTCGACCAC CAAGCGAAAC ATCGCATCGA GCGAGCACGT ACTCGGATGG AAGCCGGTCT TGTCGATCAG
2601 GATGATCTGG ACGAAGAGCA TCAGGGGCTC GCGCCAGCCG AACTGTTCGC CAGGCTCAAG GCGCGCATGC CCGACGGCGA GGATCTCGTC GTGACCCATG
2701 GCGATGCCTG CTTGCCGAAT ATCATGGTGG AAAATGGCCG CTTTTCTGGA TTCATCGACT GTGGCCGGCT GGGTGTGGCG GACCGCTATC AGGACATAGC
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2801 GTTGGCTACC CGTGATATTG CTGAAGAGCT TGGCGGCGAA TGGGCTGACC GCTTCCTCGT GCTTTACGGT ATCGCCGCTC CCGATTCGCA GCGCATCGCC
2901 TTCTATCGCC TTCTTGACGA GTTCTTCTGA GCGGGACTCT GGGGTTCGAA ATGACCGACC AAGCGACGCC CAACCTGCCA TCACGAGATT TCGATTCCAC
3001 CGCCGCCTTC TATGAAAGGT TGGGCTTCGG AATCGTTTTC CGGGACGCCG GCTGGATGAT CCTCCAGCGC GGGGATCTCA TGCTGGAGTT CTTCGCCCAC
3101 CCCAACTTGT TTATTGCAGC TTATAATGGT TACAAATAAA GCAATAGCAT CACAAATTTC ACAAATAAAG CATTTTTTTC ACTGCATTCT AGTTGTGGTT
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3701 GAGCATCACA AAAATCGACG CTCAAGTCAG AGGTGGCGAA ACCCGACAGG ACTATAAAGA TACCAGGCGT TTCCCCCTGG AAGCTCCCTC GTGCGCTCTC
3801 CTGTTCCGAC CCTGCCGCTT ACCGGATACC TGTCCGCCTT TCTCCCTTCG GGAAGCGTGG CGCTTTCTCA TAGCTCACGC TGTAGGTATC TCAGTTCGGT
3901 GTAGGTCGTT CGCTCCAAGC TGGGCTGTGT GCACGAACCC CCCGTTCAGC CCGACCGCTG CGCCTTATCC GGTAACTATC GTCTTGAGTC CAACCCGGTA
4001 AGACACGACT TATCGCCACT GGCAGCAGCC ACTGGTAACA GGATTAGCAG AGCGAGGTAT GTAGGCGGTG CTACAGAGTT CTTGAAGTGG TGGCCTAACT
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5401 ATTTCCCCGA AAAGTGCCAC CTGACGTC

pcDNA3.1 Restriction Map

RESTRICTION MAP: ALL ENZYMES

Enzyme	Cuts	[--------- Base 5' to Cleave Sites ----------]
AatII	5	375 428 511 697 5427
AccI	2	3235 3242
AclI	2	4731 5104
AcyI	9	372 425 508 694 2264 2966 3045 5042 5424
AflII	1	908
AflIII	2	228 3615
AgeI	0	
AhaIII	5	904 1006 4371 4390 5082
AluI	27	135 816 891 913 925 1274 1441 1698 2018 2072 2082 2370 2828 3119 3252 3258 3280 3375 3439 3557 3783 3873 3919 4176 4694 4794 4857
AlwNI	2	80 4031
ApaBI	2	1800 1872
ApaI	1	1001
ApaLI	3	32 3929 5172
ApoI	4	127 952 1711 3154
AscI	0	

AsuI	11	217	333	526	997	998	1534	2780	4547
		4626	4643	4865					
AsuII	1	2946							
AvaI	2	985	2075						
AvaII	3	2780	4643	4865					
AvrII	1	2054							
BalI	1	2346							
BamHI	1	929							
BclI	2	1015	2105						
BetI	4	2295	3821	3968	4796				
BglI	4	340	462	533	4625				
BglII	1	12							
BsaAI	3	590	1528	2568					
BsaBI	1	2123							
BseMII	4	53	3904	4310	4476				
BsePI	1	2661							
BsiYI	12	299	1309	1635	1962	2245	2789	3070	3463
		3637	3655	3821	4100				
Bsp1407I	0								
BspHI	2	4332	5340						
BspLU11I	1	3615							
BspMII	0								

BstEII	0									
BstXI	2	948	974							
Cac8I	33	122	336	529	893	897	1045	1222	1226	
		1368	1382	1425	1781	1800	1853	1872	2158	
		2344	2563	2629	2635	2663	2667	2708	2712	
		2766	3049	3408	3437	3546	3632	3669	4226	4617
CauII	8	2076	2077	2268	2428	3041	3995	4688	5039	
Cfr10I	5	1423	2583	2764	3047	4585				
CfrI	7	979	2170	2344	2735	2762	3454	4893		
ClaI	0									
CviJI	101	73	135	151	219	292	334	343	527	
		816	824	851	891	895	913	925	981	
		999	1021	1047	1244	1252	1274	1280	1427	
		1441	1455	1536	1678	1698	1771	1843	1966	
		1995	2001	2018	2041	2053	2059	2072	2082	
		2172	2189	2197	2224	2249	2333	2342	2346	
		2370	2408	2484	2501	2520	2583	2627	2637	
		2654	2737	2764	2768	2805	2828	2844	3024	
		3051	3119	3252	3258	3280	3332	3353	3375	
		3439	3456	3538	3557	3630	3641	3659	3685	
		3783	3873	3919	3924	3949	4028	4093	4104	
		4147	4176	4536	4548	4589	4615	4619	4628	
		4694	4784	4794	4857	4895				
CviRI	24	34	166	959	1135	1793	1802	1865	1874	
		1989	2065	2156	2315	2490	2504	3116	3184	
		3442	3931	4224	4559	4649	4842	4930	5174	
DdeI	11	39	109	180	1147	1256	2014	2927	3890	
		4296	4462	5002						
DpnI	28	6	14	22	931	1017	2102	2107	2126	
		2437	2515	2596	2605	2683	3059	3075	4183	
		4255	4266	4274	4352	4364	4469	4810	4828	

		4874	5132	5149	5185				
DraII	1	997							
DraIII	1	1531							
DrdI	3	1575	2291	3723					
DsaI	3	610	1961	2696					
Eam1105I	1	4505							
Eco47III	0								
EcoNI	0								
EcoRI	1	952							
EcoRII	11	338	531	1085	1766	1821	1838	2649	3353
		3641	3762	3775					
EcoRV	1	964							
EspI	0								
Fnu4HI	39	61	201	979	982	1311	1325	1347	2002
		2173	2225	2236	2326	2331	2368	2409	2496
		2499	2502	2738	2834	2875	2889	3003	3117
		3440	3521	3539	3542	3660	3815	3958	4023
		4026	4229	4557	4896	4923	5018	5247	
FnuDII	19	120	208	230	310	1300	1324	1344	1720
		2330	2631	2663	3069	3462	3464	3662	4240
		4570	5063	5395					
FseI	0								
HaeI	5	2053	2346	3630	3641	4093			
HaeII	5	1373	1381	2267	3493	3863			
HaeIII	22	219	334	527	981	999	1536	1678	1995

		2001	2053	2172	2346	2737	2764	3456	3630
		3641	3659	4093	4548	4628	4895		
HgiAI	8	36	818	927	2377	2567	3933	5091	5176
HgiCI	8	715	917	1093	1487	2263	2298	3359	4453
HgiJII	7	818	927	1001	1246	1282	1457	2629	
HhaI	30	120	200	1302	1315	1324	1346	1372	1380
		2258	2266	2330	2367	2633	2663	2665	2893
		3069	3399	3464	3492	3525	3795	3862	3962
		4136	4242	4635	4728	5065	5397		
HincII	2	234	3243						
HindIII	1	911							
HinfI	16	173	660	869	988	1576	1598	2749	2883
		2935	2993	3030	3450	3515	3590	3986	4500
HpaI	0								
HpaII	22	1424	2076	2169	2246	2268	2296	2427	2517
		2584	2765	3040	3048	3333	3822	3969	3995
		4185	4586	4620	4687	4797	5039		
KpnI	1	921							
MaeI	15	250	829	896	936	992	1037	1283	1375
		2055	3189	3249	3253	4110	4360	4695	
MaeII	16	372	384	425	508	589	694	1417	1527
		1570	1582	2380	2567	4315	4731	5104	5424
MaeIII	16	311	398	747	1338	1350	2384	2690	3128
		3971	4034	4150	4430	4761	4819	4972	5160
MboI	28	4	12	20	929	1015	2100	2105	2124
		2435	2513	2594	2603	2681	3057	3073	4181
		4253	4264	4272	4350	4362	4467	4808	4826
		4872	5130	5147	5183				

McrI	6	982	2173	3531	3955	4875	5024		
MfeI	1	161							
MluI	1	228							
MseI	23	69	131	257	862	903	909	1005	1318
		1589	1687	1704	1715	1725	3386	3445	4318
		4370	4375	4389	4442	4677	4716	5081	
MslI	5	615	2701	4757	4916	5275			
MstI	2	2366	4727						
MwoI	35	197	340	462	494	533	626	650	1234
		1258	1319	1321	1363	1390	1420	1799	1871
		1922	2001	2007	2255	2339	2362	2501	2507
		2624	2660	2707	2974	3359	3403	3487	3554
		3668	4237	4625					
NaeI	3	1425	2766	3049					
NarI	1	2264							
NcoI	3	610	1961	2696					
NdeI	1	484							
NheI	1	895							
NlaIII	22	170	554	614	1228	1802	1874	1965	2138
		2483	2669	2700	2726	3082	3230	3273	3619
		4336	4827	4837	4915	4951	5344		
NlaIV	20	717	919	931	999	1095	1456	1468	1489
		1772	1844	2265	2300	3361	3647	3686	4455
		4549	4590	4801	5391				
NotI	1	979							
NruI	1	208							

NspBII	7	1013	1274	2370	3439	3957	4202	5140	
NspI	5	1228	1802	1874	2669	3619			
PacI	0								
PflMI	0								
PmaCI	0								
PmeI	2	904	1006						
PpuMI	0								
PshAI	0								
PstI	2	961	2317						
PvuI	1	4875							
PvuII	3	1274	2370	3439					
RsaI	11	44	214	469	494	549	582	633	790
		919	2570	4985					
RsrII	1	2780							
SacI	2	818	927						
SacII	0								
SalI	1	3241							
SanDI	0								
SauI	0								
ScaI	1	4985							
ScrFI	19	340	533	1087	1768	1823	1840	2076	2077
		2268	2428	2651	3041	3355	3643	3764	3777

		3995	4688	5039					
SduI	15	36	818	927	1001	1246	1282	1457	2210
		2303	2377	2567	2629	3933	5091	5176	
SecI	12	610	1085	1766	1838	1961	1996	2054	2075
		2427	2696	3354	3775				
SexAI	1	1821							
SfeI	7	875	957	1305	2313	3880	4071	4746	
SfiI	0								
SgfI	0								
SgrAI	0								
SmaI	1	2077							
SmlI	7	908	985	2655	3721	3983	4257	5125	
SnaBI	1	590							
SpeI	2	249	935						
SphI	4	1228	1802	1874	2669				
SplI	0								
SrfI	0								
Sse8387I	0								
Sse8647I	0								
SspI	1	5309							
StuI	1	2053							
StyI	4	610	1961	2054	2696				

SwaI	0									
TaqI	14	856	986	1024	1493	2377	2533	2557	2593	
		2755	2946	2991	3242	3715	5156			
TatI	6	42	467	547	580	631	4983			
TfiI	7	173	2749	2883	2993	3030	3450	3590		
TseI	18	200	1346	2224	2325	2367	2408	2501	2888	
		3116	3439	3520	3538	3957	4022	4025	4228	
		4556	4922							
Tsp45I	5	1350	2384	2690	4761	4972				
Tsp4CI	17	326	448	519	793	974	1029	1107	1554	
		2644	2760	2868	3240	3579	3650	4120	4430	4945
TspEI	19	127	161	268	859	952	1130	1711	1722	
		1726	1808	1880	1972	3154	3295	3312	3387	
		4372	4678	4933						
TspRI	15	845	948	979	1110	2393	3186	3408	3517	
		4023	4036	4304	4453	4558	4905	4932		
Tth111I	1	2382								
VspI	6	257	862	1725	3386	3445	4677			
XbaI	1	991								
XcmI	0									
XhoI	1	985								
XhoII	12	12	929	2100	2435	2681	3073	4253	4264	
		4350	4362	5130	5147					
XmaIII	2	979	2170							
XmnI	2	1726	5104							

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