

# Systematic comparison of plant promoters in *Nicotiana* spp. expression systems

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**Table S1.** Level 1 vectors used in this study.

Type	ID	Structure	Sequence
Virus promoter	pNK955	Level1   p35s_0.4kb - 5'UTR TMV omega - EGFP - tOCS	<a href="https://benchling.com/s/seq-Xv4yj8acyzr5Ms9vWE92?m=slm-Z04z3oSgMyZVnFCU5oP3">https://benchling.com/s/seq-Xv4yj8acyzr5Ms9vWE92?m=slm-Z04z3oSgMyZVnFCU5oP3</a>
	pNK1481	Level1   p35s_0.4kb - 5'UTR AtRBCS2B - EGFP - tOCS	<a href="https://benchling.com/s/seq-NWaRFM9UiqVg3Kk62CD3?m=slm-yd34JT03rKFJl2FQ2FrR">https://benchling.com/s/seq-NWaRFM9UiqVg3Kk62CD3?m=slm-yd34JT03rKFJl2FQ2FrR</a>
	pX088	Level1   pFMV - EGFP - tOCS	<a href="https://benchling.com/s/seq-IBV1TXfJMIvmcuLdRbTg?m=slm-PJ0oohwynpUw1tVppfWr">https://benchling.com/s/seq-IBV1TXfJMIvmcuLdRbTg?m=slm-PJ0oohwynpUw1tVppfWr</a>
	pX089	Level1   pCmYLCV - EGFP - tOCS	<a href="https://benchling.com/s/seq-BzVQwacCASJ3QiXyT5LS?m=slm-mGNqUdrifxQz9BzGYI1n">https://benchling.com/s/seq-BzVQwacCASJ3QiXyT5LS?m=slm-mGNqUdrifxQz9BzGYI1n</a>
Synthetic promoter	pNK951	Level1   pMinSyn104 - 5'UTR AtRBCS2B - EGFP - tOCS	<a href="https://benchling.com/s/seq-0kYMTsYtcpEFoA5rVmsy?m=slm-3b27GbcFKiY6r614Pwxz">https://benchling.com/s/seq-0kYMTsYtcpEFoA5rVmsy?m=slm-3b27GbcFKiY6r614Pwxz</a>
	pNK1513	Level1   pMinSyn105 - 5'UTR AtRBCS2B - EGFP - tOCS	<a href="https://benchling.com/s/seq-jgWZjw7OI5PjWVqSbXAh?m=slm-dR4dUflhyHbZQS2LoZ4f">https://benchling.com/s/seq-jgWZjw7OI5PjWVqSbXAh?m=slm-dR4dUflhyHbZQS2LoZ4f</a>
	pNK1141	Level1   pMinSyn108 - 5'UTR AtRBCS2B - EGFP - tOCS	<a href="https://benchling.com/s/seq-OS9XK2vW90gpwnk5SYAb?m=slm-viewEeMhX16F6R1HambEF">https://benchling.com/s/seq-OS9XK2vW90gpwnk5SYAb?m=slm-viewEeMhX16F6R1HambEF</a>
	pNK1137	Level1   pMinSyn110 - 5'UTR AtRBCS2B - EGFP - tOCS	<a href="https://benchling.com/s/seq-VpQlZc8dgtwbPd3iUmvX?m=slm-OLWrMFaWIEgMlHGlbtLx">https://benchling.com/s/seq-VpQlZc8dgtwbPd3iUmvX?m=slm-OLWrMFaWIEgMlHGlbtLx</a>
	pNK952	Level1   pMinSyn159x - 5'UTR AtRBCS2B - EGFP - tOCS	<a href="https://benchling.com/s/seq-s5B6tzYHfvqwuXx2UoVu?m=slm-qic0dr0QGeRLKIVGsKAi">https://benchling.com/s/seq-s5B6tzYHfvqwuXx2UoVu?m=slm-qic0dr0QGeRLKIVGsKAi</a>
	pNK1112	Level1   pMinSyn159x - 5'UTR AtRBCS2B - EGFP - tAct2	<a href="https://benchling.com/s/seq-oYCEmyaF71RfBpTabEFg?m=slm-riK7MJUwcGiEKw96thBA">https://benchling.com/s/seq-oYCEmyaF71RfBpTabEFg?m=slm-riK7MJUwcGiEKw96thBA</a>
	pNK1412	Level1   pMinSyn159x - 5'UTR AtRBCS2B - EGFP - tRBCS3C	<a href="https://benchling.com/s/seq-ztYZj76YosOM2A2iNW1U?m=slm-DtHx6UiaPicCpug1FOyH">https://benchling.com/s/seq-ztYZj76YosOM2A2iNW1U?m=slm-DtHx6UiaPicCpug1FOyH</a>
	pNK1236	Level1   pMinSyn159x - 5'UTR AtRBCS2B - EGFP - tATPase	<a href="https://benchling.com/s/seq-aPufVoUSKizTaFhT2E3E?m=slm-NrKKtuxswElZN1Ax1N82">https://benchling.com/s/seq-aPufVoUSKizTaFhT2E3E?m=slm-NrKKtuxswElZN1Ax1N82</a>
	pNK1111	Level1   pMinSyn159x - 5'UTR AtRBCS2B - EGFP - tHSP18.2	<a href="https://benchling.com/s/seq-wjSuFYeCnattBzyfJgW4?m=slm-RI5kiSudgkfkJeObr49">https://benchling.com/s/seq-wjSuFYeCnattBzyfJgW4?m=slm-RI5kiSudgkfkJeObr49</a>
	pNK1140	Level1   pMinSyn1556x - 5'UTR AtRBCS2B - EGFP - tOCS	<a href="https://benchling.com/s/seq-hLOY4zQtQts9lCyJgKBs?m=slm-CGqSZUiNYxwabad00fWO">https://benchling.com/s/seq-hLOY4zQtQts9lCyJgKBs?m=slm-CGqSZUiNYxwabad00fWO</a>
	pNK1138	Level1-1   pMinSyn1569x - 5'UTR AtRBCS2B - EGFP - tOCS	<a href="https://benchling.com/s/seq-19omt2Y0LD6NW9aeAK1G?m=slm-uflaYDAe812s51fEk8YE">https://benchling.com/s/seq-19omt2Y0LD6NW9aeAK1G?m=slm-uflaYDAe812s51fEk8YE</a>
	pNK1515	Level1   pMinSyn1637x - 5'UTR AtRBCS2B - EGFP - tOCS	<a href="https://benchling.com/s/seq-SyrFSUGSJwj2uGYBEQ7A?m=slm-0kpvhriPePECa20BKkm5">https://benchling.com/s/seq-SyrFSUGSJwj2uGYBEQ7A?m=slm-0kpvhriPePECa20BKkm5</a>
	pNK1036	Level1   pMinSyn1824x - 5'UTR AtRBCS2B - EGFP - tOCS	<a href="https://benchling.com/s/seq-gyWD4RBezBWQxFdQl6rm?m=slm-t9BfguUZgXrirE5XYSQs">https://benchling.com/s/seq-gyWD4RBezBWQxFdQl6rm?m=slm-t9BfguUZgXrirE5XYSQs</a>
	pNK1037	Level1   pMinSyn1904x - 5'UTR AtRBCS2B - EGFP - tOCS	<a href="https://benchling.com/s/seq-TU9mjtZ0iPxozRGdvp0G?m=slm-tPn3qpoBsNjcnzy6rUG">https://benchling.com/s/seq-TU9mjtZ0iPxozRGdvp0G?m=slm-tPn3qpoBsNjcnzy6rUG</a>

Plant promoter	pNK953	Level1   <b>pAtTCTP</b> - EGFP - tOCS	<a href="https://benchling.com/s/seq-8fQel0byzUI350uKtDYr?m=slm-EVEL4TI37GQodCF8wJq6">https://benchling.com/s/seq-8fQel0byzUI350uKtDYr?m=slm-EVEL4TI37GQodCF8wJq6</a>
	pNK1145	Level1   <b>pAtTCTP</b> - EGFP - <b>tAct2</b>	<a href="https://benchling.com/s/seq-rV4DWhZy2F8GIzrsT9NS?m=slm-BfB3mJNUvII4Od4GDlb">https://benchling.com/s/seq-rV4DWhZy2F8GIzrsT9NS?m=slm-BfB3mJNUvII4Od4GDlb</a>
	pNK1152	Level1   <b>pAtTCTP</b> - EGFP - <b>tRBCS3C</b>	<a href="https://benchling.com/s/seq-8pJOK3HtycSzLCRpLpAz?m=slm-EjZVbezI09ucYHnsBqNz">https://benchling.com/s/seq-8pJOK3HtycSzLCRpLpAz?m=slm-EjZVbezI09ucYHnsBqNz</a>
	pNK1143	Level1-1   <b>pAtTCTP</b> - EGFP - <b>tATPase</b>	<a href="https://benchling.com/s/seq-YYeDtylG69ZQrAqnUlyD?m=slm-waMZwW0kseP60KbNrp80">https://benchling.com/s/seq-YYeDtylG69ZQrAqnUlyD?m=slm-waMZwW0kseP60KbNrp80</a>
	pNK1114	Level1   <b>pAtTCTP</b> - EGFP - <b>tHSP18.2</b>	<a href="https://benchling.com/s/seq-9kcntvkxWSNYzQyvoUuM?m=slm-Hrv8rcIAXuKELmISuTbE">https://benchling.com/s/seq-9kcntvkxWSNYzQyvoUuM?m=slm-Hrv8rcIAXuKELmISuTbE</a>
	pNK954	Level1   <b>pdeI5_MtHP</b> - EGFP - tOCS	<a href="https://benchling.com/s/seq-QvJzk8QaFH1e108jAYbl?m=slm-fXzAyVAzwBKLxr87DQNf">https://benchling.com/s/seq-QvJzk8QaFH1e108jAYbl?m=slm-fXzAyVAzwBKLxr87DQNf</a>
	pNK1110	Level1   <b>pdeI5_MtHP</b> - EGFP - <b>tAct2</b>	<a href="https://benchling.com/s/seq-ivePFin9Q5nAZf4C0VnD?m=slm-l44K4zjb90hxGAS1PMTv">https://benchling.com/s/seq-ivePFin9Q5nAZf4C0VnD?m=slm-l44K4zjb90hxGAS1PMTv</a>
	pNK1109	Level1   <b>pdeI5_MtHP</b> - EGFP - <b>tRBCS3C</b>	<a href="https://benchling.com/s/seq-Y0p5GIBsbAcEV4hn84MI?m=slm-EEdXCcENRhGd7bJjCeQT">https://benchling.com/s/seq-Y0p5GIBsbAcEV4hn84MI?m=slm-EEdXCcENRhGd7bJjCeQT</a>
	pNK1113	Level1   <b>pdeI5_MtHP</b> - EGFP - <b>tATPase</b>	<a href="https://benchling.com/s/seq-l1VojRlInf0GEhwhl1f3m?m=slm-gVPTKyqzFagJyE41nb5z">https://benchling.com/s/seq-l1VojRlInf0GEhwhl1f3m?m=slm-gVPTKyqzFagJyE41nb5z</a>
	pNK1144	Level1   <b>pdeI5_MtHP</b> - EGFP - <b>tHSP18.2</b>	<a href="https://benchling.com/s/seq-StzkvqM01NeKpD4eTwmn?m=slm-yFiElb0eKdoydeRkdEDL">https://benchling.com/s/seq-StzkvqM01NeKpD4eTwmn?m=slm-yFiElb0eKdoydeRkdEDL</a>
	pNK931	Level1   <b>pAtUBQ10</b> - EGFP - tOCS	<a href="https://benchling.com/s/seq-TzWi1PDZB5OVr2ws61GE?m=slm-mqXjDgtkE7aeSCsH399o">https://benchling.com/s/seq-TzWi1PDZB5OVr2ws61GE?m=slm-mqXjDgtkE7aeSCsH399o</a>
	pNK1106	Level1   <b>pAtUBQ10</b> - EGFP - <b>tAct2</b>	<a href="https://benchling.com/s/seq-ApnWp2eG3BQdHu664IMh?m=slm-weypOv2dQBHSmaZvEk8l">https://benchling.com/s/seq-ApnWp2eG3BQdHu664IMh?m=slm-weypOv2dQBHSmaZvEk8l</a>
	pNK1108	Level1   <b>pAtUBQ10</b> - EGFP - <b>tRBCS3C</b>	<a href="https://benchling.com/s/seq-hVLNKYvVIK1qf11tVblC?m=slm-DDzyAFgWHYyiC2Fmyh5z">https://benchling.com/s/seq-hVLNKYvVIK1qf11tVblC?m=slm-DDzyAFgWHYyiC2Fmyh5z</a>
	pNK1107	Level1   <b>pAtUBQ10</b> - EGFP - <b>tATPase</b>	<a href="https://benchling.com/s/seq-cvAPHcypUt5fYh2j2Wdu?m=slm-MTzgxDRKhI2tGC1YmooF">https://benchling.com/s/seq-cvAPHcypUt5fYh2j2Wdu?m=slm-MTzgxDRKhI2tGC1YmooF</a>
	pNK1142	Level1   <b>pAtUBQ10</b> - EGFP - <b>tHSP18.2</b>	<a href="https://benchling.com/s/seq-twwPDlyKDsdzux0R0Dvc?m=slm-UykClrMtTr0IIBWOL904">https://benchling.com/s/seq-twwPDlyKDsdzux0R0Dvc?m=slm-UykClrMtTr0IIBWOL904</a>
	pNK930	Level1   <b>pAtRPS5a</b> - EGFP - tOCS	<a href="https://benchling.com/s/seq-ykQtGbOXM8pmSlZjgnNa?m=slm-C7lg4SOTyizdhjTqbfYu">https://benchling.com/s/seq-ykQtGbOXM8pmSlZjgnNa?m=slm-C7lg4SOTyizdhjTqbfYu</a>
	pNK932	Level1   <b>pAtPD7</b> - EGFP - tOCS	<a href="https://benchling.com/s/seq-PXTVGBjl2QJHHahIFIDR?m=slm-wAJ0Fs00laLRH0eL5hUj">https://benchling.com/s/seq-PXTVGBjl2QJHHahIFIDR?m=slm-wAJ0Fs00laLRH0eL5hUj</a>
	pX083	Level1   <b>pAtAct2</b> - 5'UTR TMV omega - EGFP - tOCS	<a href="https://benchling.com/s/seq-0ZfjzZWajg0osBlilCcA?m=slm-5BILr6n3iYcUyQEzrefo">https://benchling.com/s/seq-0ZfjzZWajg0osBlilCcA?m=slm-5BILr6n3iYcUyQEzrefo</a>

**Table S2.** Level 0 plasmids with promoters and terminators used in this study.

ID	Name of the construct	Sequence	DNA part type	Source of plasmid
pICH51277 MoClo Plant Parts Kit Addgene (Kit #1000000047)	Level 0 - promoter   <b>p35s_0.4kb</b> - 5'UTR TMV omega	<a href="https://www.addgene.org/50268/">https://www.addgene.org/50268/</a>	PROM + 5UTR	[7]
pICH41388 MoClo Plant Parts Kit Addgene (Kit #1000000047)	Level 0 - promoter   <b>p35s_0.4kb</b>	<a href="https://www.addgene.org/50253/">https://www.addgene.org/50253/</a>	PROM	[7]
pNK883	Level 0 - promoter   <b>pMinSyn104</b>	<a href="https://benchling.com/s/seq-bwu4KtFbPI7C3f7wrm5m?m=slm-LX8478zw5MY3eK6LxsCS">https://benchling.com/s/seq-bwu4KtFbPI7C3f7wrm5m?m=slm-LX8478zw5MY3eK6LxsCS</a>	PROM	[5]
pNK880	Level 0 - promoter   <b>pMinSyn105</b>	<a href="https://benchling.com/s/seq-mtPe4uNuJkUREyk1E9TB?m=slm-4P6pmRnEleWIYE24locP">https://benchling.com/s/seq-mtPe4uNuJkUREyk1E9TB?m=slm-4P6pmRnEleWIYE24locP</a>	PROM	
pNK879	Level 0 - promoter   <b>pMinSyn108</b>	<a href="https://benchling.com/s/seq-qahowDDKd9M3JvNHfeDg?m=slm-CGsjRUx8x3bmlA2Y40xn">https://benchling.com/s/seq-qahowDDKd9M3JvNHfeDg?m=slm-CGsjRUx8x3bmlA2Y40xn</a>	PROM	
pNK887	Level 0 - promoter   <b>pMinSyn110</b>	<a href="https://benchling.com/s/seq-Cn85nhOm0I2DMeEDzHBO?m=slm-cp1QzkzwES1QmA7dk7c6">https://benchling.com/s/seq-Cn85nhOm0I2DMeEDzHBO?m=slm-cp1QzkzwES1QmA7dk7c6</a>	PROM	
pNK882	Level 0 - promoter   <b>pMinSyn159x</b>	<a href="https://benchling.com/s/seq-lAviApCq4kkds4HrfgNf?m=slm-ASMYEq0pM5bqFNVDpJql">https://benchling.com/s/seq-lAviApCq4kkds4HrfgNf?m=slm-ASMYEq0pM5bqFNVDpJql</a>	PROM	

pNK881	Level 0 - promoter   <b>pMinSyn1556x</b>	<a href="https://benchling.com/s/seq-lvkyH4l3dhhdUx569J8A?m=slm-bztFB6F96JxkqnhuDyP6">https://benchling.com/s/seq-lvkyH4l3dhhdUx569J8A?m=slm-bztFB6F96JxkqnhuDyP6</a>	PROM	
pNK884	Level 0 - promoter   <b>pMinSyn1569x</b>	<a href="https://benchling.com/s/seq-fmxiLzQJdsRj4vd3332T?m=slm-mSIQsnDH5xkF3BDAwtKA">https://benchling.com/s/seq-fmxiLzQJdsRj4vd3332T?m=slm-mSIQsnDH5xkF3BDAwtKA</a>	PROM	
pNK6030	Level 0 - promoter   <b>pMinSyn1637x</b>	<a href="https://benchling.com/s/seq-UhSCuiyG3wbeYAofc97i?m=slm-M1s4yJgnDkcb08MPIMTj">https://benchling.com/s/seq-UhSCuiyG3wbeYAofc97i?m=slm-M1s4yJgnDkcb08MPIMTj</a>	PROM	
pNK885	Level 0 - promoter   <b>pMinSyn1824x</b>	<a href="https://benchling.com/s/seq-JeaHhnuxRXrv2CifdrMi?m=slm-gNO7kcdpJJfknvoNcgvH">https://benchling.com/s/seq-JeaHhnuxRXrv2CifdrMi?m=slm-gNO7kcdpJJfknvoNcgvH</a>	PROM	
pNK878	Level 0 - promoter   <b>pMinSyn1904x</b>	<a href="https://benchling.com/s/seq-lw4GyN7iasauX3DyXzuS?m=slm-WcW504iKeOw6zwznAFHg">https://benchling.com/s/seq-lw4GyN7iasauX3DyXzuS?m=slm-WcW504iKeOw6zwznAFHg</a>	PROM	
pNK875	Level 0 - promoter + 5U   <b>pAtTCTP</b>	<a href="https://benchling.com/s/seq-clKcYEcm2UbC2DJcZuiz?m=slm-dV56BTwMS6j3n0TySGfb">https://benchling.com/s/seq-clKcYEcm2UbC2DJcZuiz?m=slm-dV56BTwMS6j3n0TySGfb</a>	PROM + 5UTR	[14]
pNK928	Level 0 - promoter + 5U   <b>pdel5_MtHP</b>	<a href="https://benchling.com/s/seq-fMZgG4tPifg3OGQTTPVX?m=slm-Y5CtJNoPrUwOWApIRSTM">https://benchling.com/s/seq-fMZgG4tPifg3OGQTTPVX?m=slm-Y5CtJNoPrUwOWApIRSTM</a>	PROM + 5UTR	[15]
pJOG684 MoClo Plant Parts II and Infrastructure Kit Addgene (Kit #1000000135)	Level 0 - promoter + 5U   <b>pAtUBQ10</b>	<a href="https://www.addgene.org/105355/">https://www.addgene.org/105355/</a>	PROM + 5UTR	[16]

pJOG603 MoClo Plant Parts II and Infrastructure Kit Addgene (Kit #1000000135)	Level 0 - promoter + 5U   <b>pAtRPS5a</b>	<a href="https://www.addgene.org/105356/">https://www.addgene.org/105356/</a>	PROM + 5UTR	[16]
pNK869	Level 0 - promoter + 5U   <b>pAtPD7</b>	<a href="https://benchling.com/s/seq-CNlr9MZN9n6irNXQMYKb?m=slm-Rs0KA3oOcLiXcUold34X">https://benchling.com/s/seq-CNlr9MZN9n6irNXQMYKb?m=slm-Rs0KA3oOcLiXcUold34X</a>	PROM + 5UTR	[17]
pICH87644 MoClo Plants Part Kit Addgene (Kit #1000000047)	Level 0 - promoter + 5U   <b>pAtAct2 + 5'UTR TMV omega</b>	<a href="https://www.addgene.org/50274/">https://www.addgene.org/50274/</a>	PROM + 5UTR	[7]
pNK6032	Level 0 - promoter + 5U   <b>pFMV</b>	<a href="https://benchling.com/s/seq-iXRBZ2D4pW0tMjyhjBJT?m=slm-B4U18SGhRBGriHoJIABC">https://benchling.com/s/seq-iXRBZ2D4pW0tMjyhjBJT?m=slm-B4U18SGhRBGriHoJIABC</a>	PROM + 5UTR	[12]
pNK6031	Level 0 - promoter + 5U   <b>pCmYLCV</b>	<a href="https://benchling.com/s/seq-4oY2mNHqVNuj4XYIAkUY?m=slm-QUqDDSQAFmhUsuZLBvgI">https://benchling.com/s/seq-4oY2mNHqVNuj4XYIAkUY?m=slm-QUqDDSQAFmhUsuZLBvgI</a>	PROM + 5UTR	[13]
pICH44179 MoClo Plants Part Kit Addgene (Kit #1000000047)	Level 0 - 5U   <b>AtRBCS2B</b>	<a href="https://www.addgene.org/50290/">https://www.addgene.org/50290/</a>	5UTR	[7]
pICH41432 MoClo Plants Part Kit Addgene (Kit #1000000047)	Level 0 - 3U + terminator   <b>tOCS</b>	<a href="https://www.addgene.org/50343/">https://www.addgene.org/50343/</a>	3UTR+TE RM	[7]

pICH44300 MoClo Plants Part Kit Addgene (Kit #1000000047)	Level 0 - 3U + terminator   <b>tAtAct2</b>	<a href="https://www.addgene.org/50340/">https://www.addgene.org/50340/</a>	3UTR+TE RM	[7]
pICH71411 MoClo Plants Part Kit Addgene (Kit #1000000047)	Level 0 - 3U + terminator   <b>tRBCS3C</b>	<a href="https://www.addgene.org/50345/">https://www.addgene.org/50345/</a>	3UTR+TE RM	[7]
pICH71431 MoClo Plants Part Kit Addgene (Kit #1000000047)	Level 0 - 3U + terminator   <b>tATPase</b>	<a href="https://www.addgene.org/50344/">https://www.addgene.org/50344/</a>	3UTR+TE RM	[7]
pNK1198	Level 0 - 3U + terminator   <b>tHSP18.2</b>	<a href="https://benchling.com/s/seq-aw4gwtvqbls9HeZ40NTA?m=slm-bYfZI7MnwrijyEz63vfML">https://benchling.com/s/seq-aw4gwtvqbls9HeZ40NTA?m=slm-bYfZI7MnwrijyEz63vfML</a>	3UTR+TE RM	[18]

**Table S3.** Primers used in this study.

Primer name	Sequence
pr748	atgcGGTCTCaacatGGAGataggcaaccgtggacttcttcactagctcatcgagatagcaatgccactagctaatttcttac gttgatcttatttgtttactttggggcatgacatggttaaaccatcaaagagaaagtgttcacttagtcaatatacgaattc attcgaatttatccctgtaaactcctag
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pr750	atgcGGTCTCaacatctcaataaactaaagctaccaattaccacaatatagtcttTcatatccacttagatataaaagataaaag taaacaaatattaaatttcatatgcacgcataggaaactcatgatcttatcttttaaatagacatctagttttcttaggtataaatagac atgttcctagaacttctcact
pr751	attaccacaatatagtcttTcatatccacttagatataaaagataaaagtaaacaaatattaaatttcatatgcacgcataggaaact catgatcttatcttttaaatagacatctagttttcttaggtataaatagacattttgtcctagaacttctcactactaaaacctagcctc aaATGTTGTtGAGACCgcat