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TECHNICAL RESEARCH REPORT

Additional information on Chromovert® Technology is available at this link:
<https://link.springer.com/article/10.1007/s10529-021-03101-5>



Chromover Technology:

Shekdar, K., Langer, J., Venkatachalan, S. *et al.* Cell engineering method using fluorogenic oligonucleotide signaling probes and flow cytometry. *Biotechnol Lett* **43**, 949–958 (2021). <https://doi.org/10.1007/s10529-021-03101-5>

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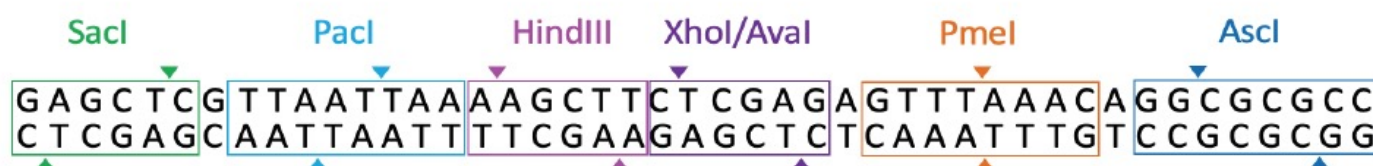
All plasmids are supplied as 10 µg aliquots provided in 10 mM Tris-HCl, 1mM EDTA, pH 8.0 at a concentration of 0.5 µg/µL. The concentration reported in the table below is the exact measure recorded by the Nanodrop (+/- 10% is the standard deviation of the Nanodrop quantification method).

Note: Partial sequencing was used to confirm the identify of all plasmid preparations prior to shipping. All plasmids were prepared from single colonies obtained from glycerol stocks established using fully sequence-confirmed plasmids.

Plasmid Name	CAT #	Conc. (ng/mL)	OD 260/280	OD260/230
pChromo-NeoO	40-2201NO-01	539.9	1.94	2.18
pChromo-NeoA	40-2201NA-01	526.0	1.95	2.19
pChromo-NeoB	40-2201NB-01	522.0	1.95	2.17
pChromo-NeoC	40-2201NC-01	517.7	1.96	2.19
pChromo-PuroO	40-2201PO-01	510.9	1.97	2.17
pChromo-PuroA	40-2201PA-01	523.1	1.96	2.16
pChromo-PuroB	40-2201PB-01	531.7	1.94	2.08
pChromo-PuroC	40-2201PC-01	510.1	1.92	2.17
pChromo-HygroO	40-2201HO-01	546.0	1.95	2.20
pChromo-HygroA	40-2201HA-01	534.6	1.94	2.17
pChromo-HygroB	40-2201HB-01	523.5	1.96	2.19
pChromo-HygroC	40-2201HC-01	516.6	1.96	2.19
pChromo-ZeoO	40-2201ZO-01	507.6	1.96	2.20
pChromo-ZeoA	40-2201ZA-01	528.7	1.95	2.13
pChromo-ZeoB	40-2201ZB-01	527.4	1.96	2.18
pChromo-ZeoC	40-2201ZC-01	530.9	1.94	2.16
pChromo-BlastO	40-2201BO-01	483.0	1.95	2.16
pChromo-BlastA	40-2201BA-01	533.1	1.94	2.16
pChromo-BlastB	40-2201BB-01	546.1	1.95	2.21
pChromo-BlastC	40-2201BC-01	529.0	1.96	2.17

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The Multiple Cloning Site (MCS) is the same across all plasmids:



Additional plasmid features and elements are provided in the table below. Full sequences for all plasmids are available at the Secondcell website and further below.

Plasmid Name	Gene Link CAT#	Chromo-Tag™	Mammalian Antibiotic-Resistance Gene	Total Size (bases)	CMV Promoter	Multiple Cloning Site (MCS)	Spacer SEQ (Sequence between MCS and Chromo-Tag)	Chromo-Tag	Bacterial Ampicillin Resistance Gene (Reverse Complement)	Mammalian Antibiotic-Resistance Gene
pChromo-NeoO	40-2201NO-01	None	Neomycin	5193	1-602	651-695	None	N/A	1149-2010	3095-3887
pChromo-NeoA	40-2201NA-01	A	Neomycin	5338	1-602	651-695	696-747	748-846	1294-2155	3240-4032
pChromo-NeoB	40-2201NB-01	B	Neomycin	5348	1-602	651-695	696-759	760-856	1304-2165	3250-4042
pChromo-NeoC	40-2201NC-01	C	Neomycin	5324	1-602	651-695	696-733	734-832	1280-2141	3226-4018
pChromo-PuroO	40-2201PO-01	None	Puromycin	4980	1-602	651-695	None	N/A	1149-2010	3068-3668
pChromo-PuroA	40-2201PA-01	A	Puromycin	5123	1-602	651-695	696-747	748-846	1294-2155	3213-3813
pChromo-PuroB	40-2201PB-01	B	Puromycin	5133	1-602	651-695	696-759	760-856	1304-2165	3223-3823
pChromo-PuroC	40-2201PC-01	C	Puromycin	5109	1-602	651-695	696-733	734-832	1280-2141	3199-3779
pChromo-HygroO	40-2201HO-01	None	Hygromycin	5400	1-602	651-695	None	N/A	1149-2010	3068-4094
pChromo-HygroA	40-2201HA-01	A	Hygromycin	5545	1-602	651-695	696-747	748-846	1294-2155	3213-4239
pChromo-HygroB	40-2201HB-01	B	Hygromycin	5555	1-602	651-695	696-759	760-856	1304-2165	3223-4249
pChromo-HygroC	40-2201HC-01	C	Hygromycin	5531	1-602	651-695	696-733	734-832	1280-2141	3199-4225
pChromo-ZeoO	40-2201ZO-01	None	Zeomycin	4749	1-602	651-695	None	N/A	1149-2010	3068-3443
pChromo-ZeoA	40-2201ZA-01	A	Zeomycin	4894	1-602	651-695	696-747	748-846	1294-2155	3213-3588
pChromo-ZeoB	40-2201ZB-01	B	Zeomycin	4904	1-602	651-695	696-759	760-856	1304-2165	3223-3598
pChromo-ZeoC	40-2201ZC-01	C	Zeomycin	4880	1-602	651-695	696-733	734-832	1280-2141	3199-3574
pChromo-BlastO	40-2201BO-01	None	Blasticidin	4803	1-602	651-695	None	N/A	1149-2010	3068-3467
pChromo-BlastA	40-2201BA-01	A	Blasticidin	4948	1-602	651-695	696-747	748-846	1294-2155	3213-3612
pChromo-BlastB	40-2201BB-01	B	Blasticidin	4958	1-602	651-695	696-759	760-856	1304-2165	3223-3622
pChromo-BlastC	40-2201BC-01	C	Blasticidin	4934	1-602	651-695	696-733	734-832	1280-2141	3199-3598

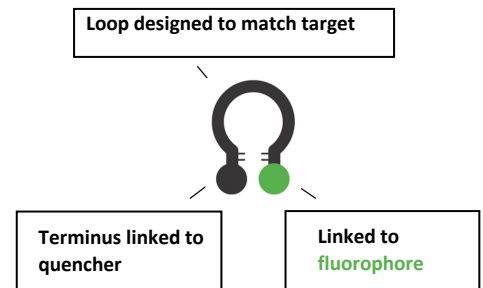
Additional information on Chromo-Tags™ and protocols to operate Chromovert® Technology are available at: <https://link.springer.com/article/10.1007/s10529-021-03101-5>

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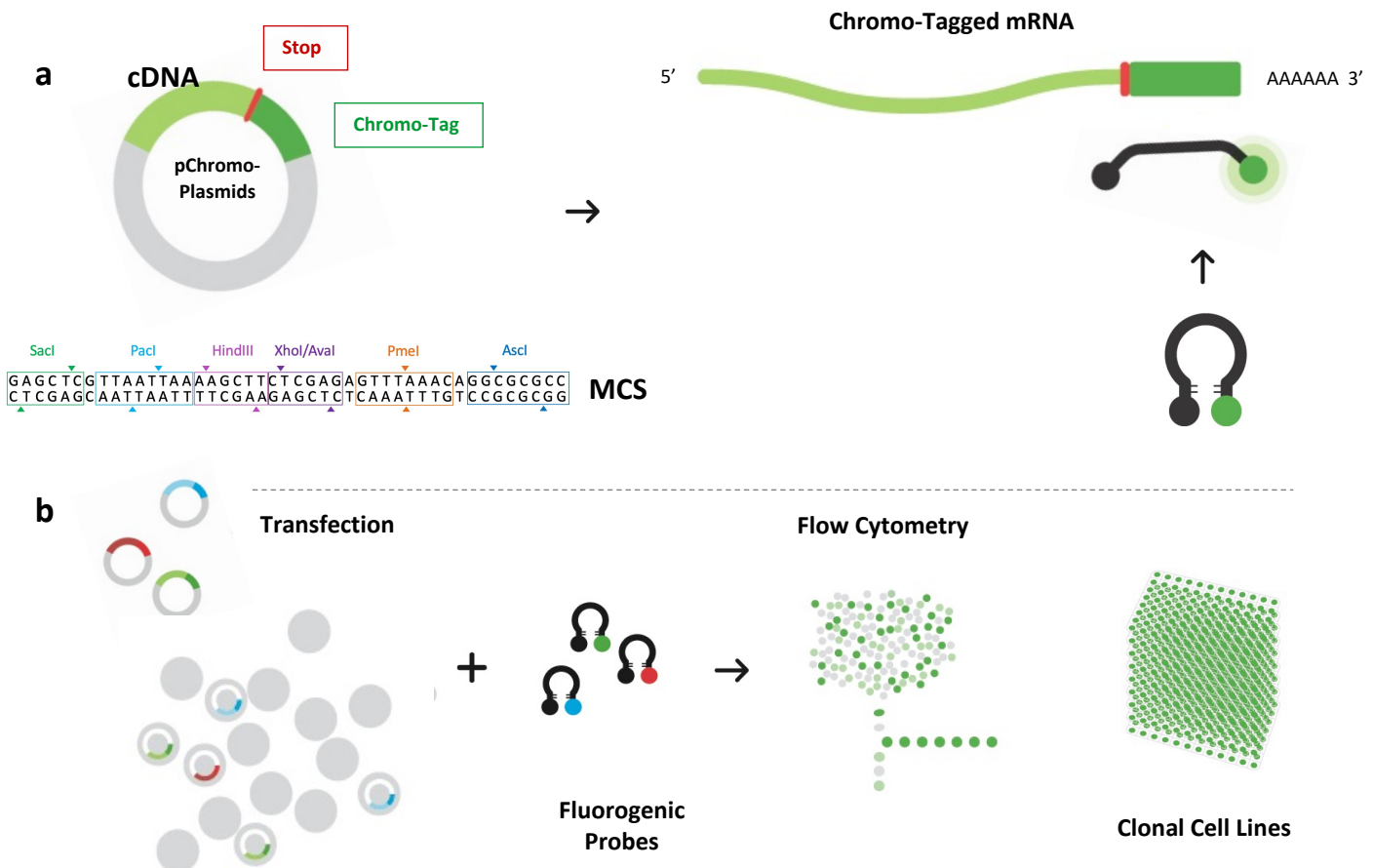
Chromovert® Technology is a new research tool for rapid creation of stable cell lines. The technology utilizes fluorogenic oligonucleotide signaling probes and flow cytometry to detect and isolate individual living cells expressing one or more genes.

For more information or to obtain pChromo-Plasmids™ or signaling probes from our preferred eCommerce partner at Gene Link, Inc. please go to <http://www.secondcellbio.com/>.

Fluorogenic Oligonucleotide Probes



Process



a) cDNAs are subcloned for expression of mRNAs comprising 3' untranslated plasmid-encoded Chromo-Tag™ sequences for detection using fluorogenic oligonucleotide signaling probes. Protein expression products remain untagged. **b)** To create cell lines, one or more Chromo-Tagged cDNAs are transfected into cells, the transfected cells are exposed to differentially-labeled signaling probes and individual positive cells are isolated using flow cytometry. Downstream testing is used to select final cell lines.



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Full Sequence: pChromo-NeoO

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<https://link.springer.com/article/10.1007/s10529-021-03101-5>



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Full Sequence: pChromo-NeoA

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Additional information and protocols to operate Chromovert® Technology are available at:

<https://link.springer.com/article/10.1007/s10529-021-03101-5>



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Full Sequence: pChromo-NeoB

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Full Sequence: pChromo-NeoC

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CC

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Full Sequence: pChromo-PuroO

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Full Sequence: pChromo-PuroA

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Full Sequence: pChromo-PuroB

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<https://link.springer.com/article/10.1007/s10529-021-03101-5>

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Full Sequence: pChromo-PuroC

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<https://link.springer.com/article/10.1007/s10529-021-03101-5>



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Full Sequence: pChromo-HygroO

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Full Sequence: pChromo-HygroA

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Full Sequence: pChromo-HygroB

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Additional information and protocols to operate Chromovert® Technology are available at:
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TGATTCTGTGGATAACCGTATTACCGCC

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Full Sequence: pChromo-BlastO

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ATGCATTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGTTACATAAATTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCG
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TGCTGGCCTTTGCTCAGATGTTCTTCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGC
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Full Sequence: pChromo-BlastB

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<https://link.springer.com/article/10.1007/s10529-021-03101-5>

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Full Sequence: pChromo-BlastC

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