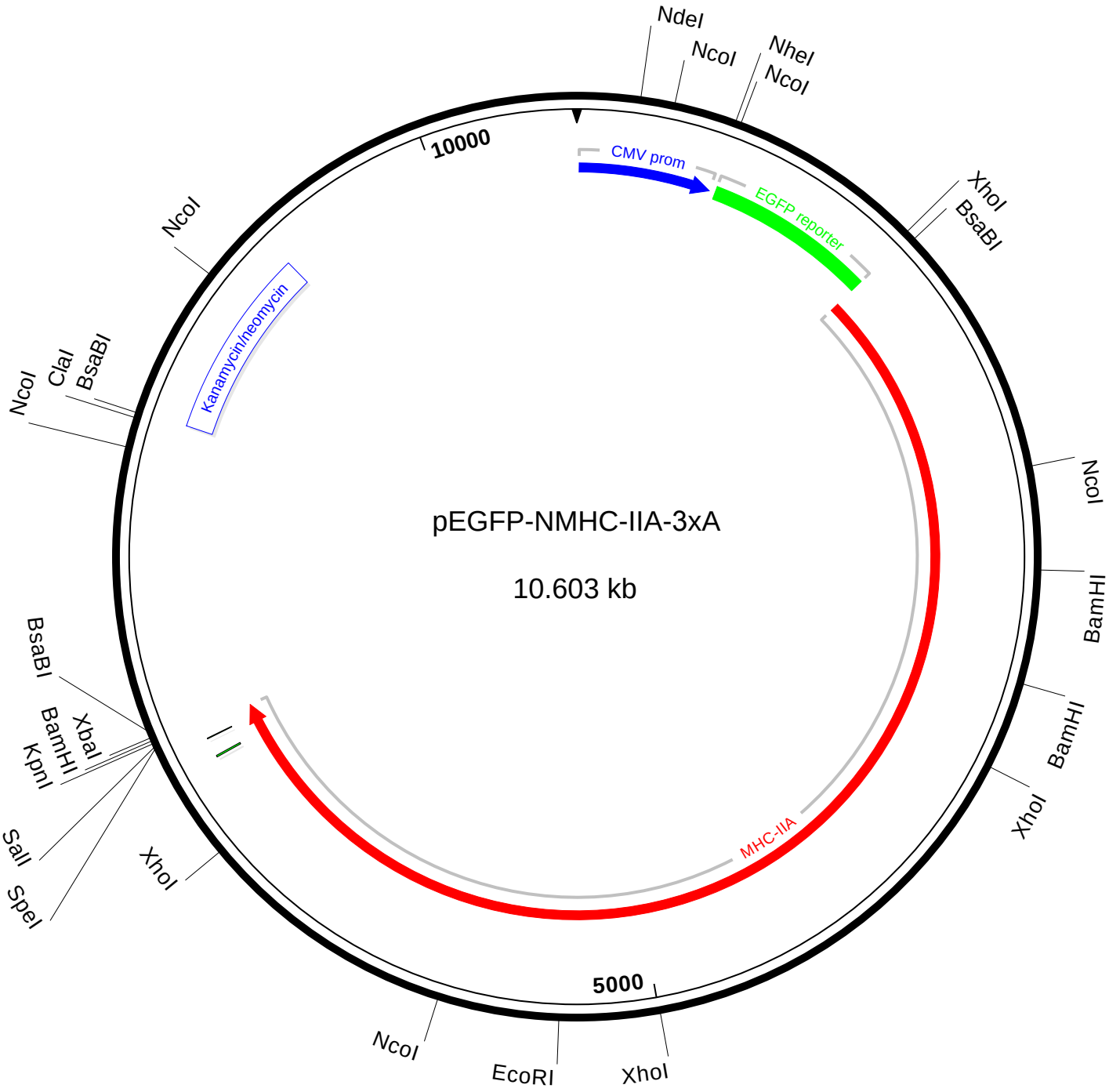
NdeI
NcoI
NheI
NcoI
10000
CMV prom
EGFP reporter
XhoI
BsaBI
NcoI
ClaI
BsaBI
Kanamycin/neomycin
NcoI
BamHI
BamHI
XhoI
MHC-IIA
BsaBI
XbaI
BamHI
KpnI
SalI
SpeI
XhoI
5000
NcoI
EcoRI
XhoI
pEGFP-NMHC-IIA-3xA
10.603 kb

pEGFP-NMHC-IIA-2xA

This plasmid, pEGFP-NMHC-IIA-3xA, carries the human MHC IIA gene with a mutation of the S1916 PKC target site to Alanine and a mutation at putative CKII target site S1943A.

Construct is based upon the plasmid pEGFPMIIAC3, from the Bresnick lab, which expresses full length GFP-MHC IIA
from a CMV promoter.

Briefly, a synthetic piece of DNA carrying the mutations was swapped into the wild type backbone.

Note that at the PKC target site S1916, we ALSO MUTATED adjacent residue S1915 TO ALA. This was done to be sure no PKC phosphorylation could occur at the adjacent S residue (hence "3xA construct").

Also note that in this mutant an artificial SpeI site was introduced just after MHC IIA stop codon but before the
SalI site, to distinguish mutant from parental version of sequence.

Absent Sites	0	NotI, PacI
BamHI	3	2700 (421), 3121 (4142), 7263 (6040)
BsaBI	3	1377 (5913), 7290 (1197), 8487 (3493)
ClaI	1	8468 (10603)
EcoRI	1	5368 (10603)
KpnI	1	7252 (10603)
NcoI	6	361 (251), 612 (1707), 2319 (3498), 5817 (2540), 8357 (703), 9060 (1904)
NdeI	1	235 (10603)
NheI	1	592 (10603)
SalI	1	7242 (10603)
SpeI	1	7238 (10603)
XbaI	1	7275 (10603)
XhoI	4	1340 (2106), 3446 (1551), 4997 (1791), 6788 (5155)