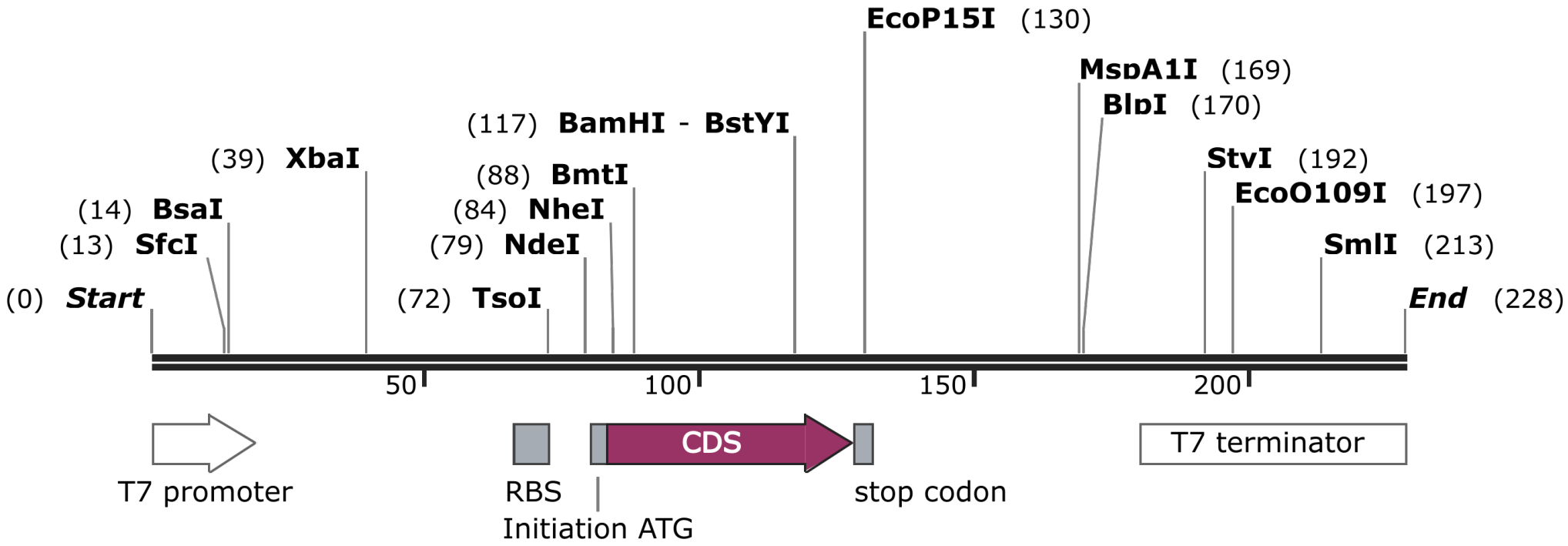
Cloning Club: Multiple proteins from one plasmid

http://blog.addgene.org/plasmids-101-multicistronic-vectors



Bacterial expression construct

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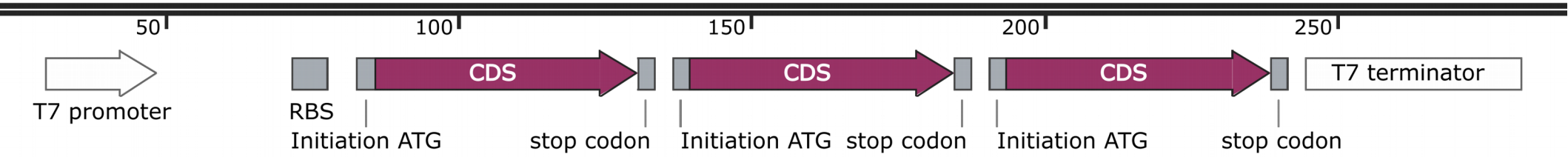
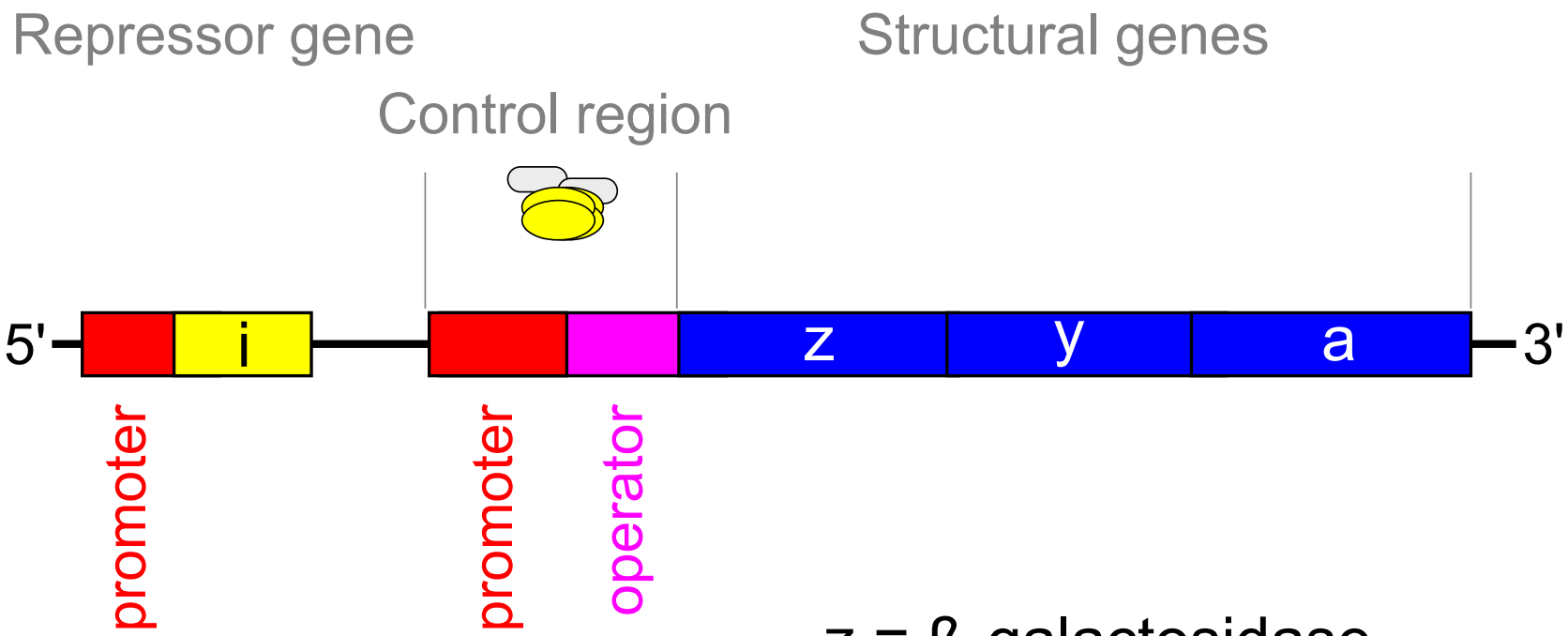
You can clone an *E. coli* expression vector like this (multicistronic expression):



But why can't you clone a mammalian vector like this?

The lac operon (3 genes under one promoter)



z = ß-galactosidase
y = galactoside permease
a = transacetylase
i = lac repressor

Initiation of translation

E. coli

RBS
(= Shine-Dalgarno sequence)

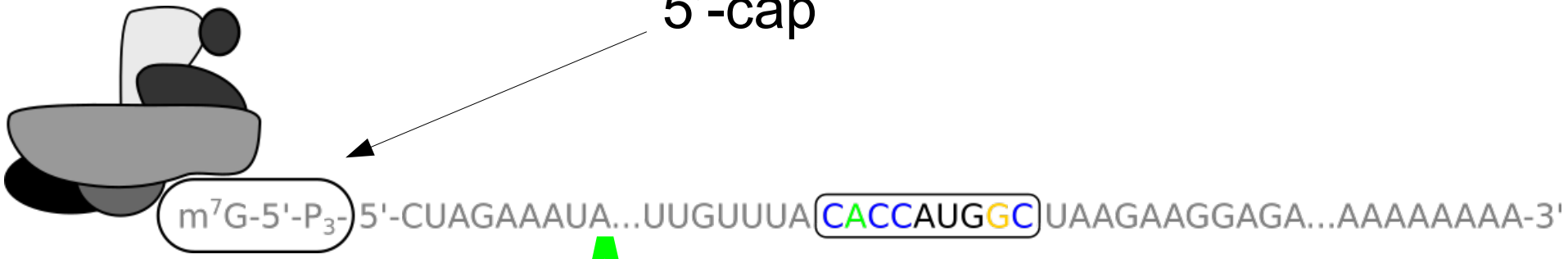
UAAGGAGG

5-9 nt

ATG: 80%
CTG: 15%
TTG&GTG: 5%

Mammalian cells

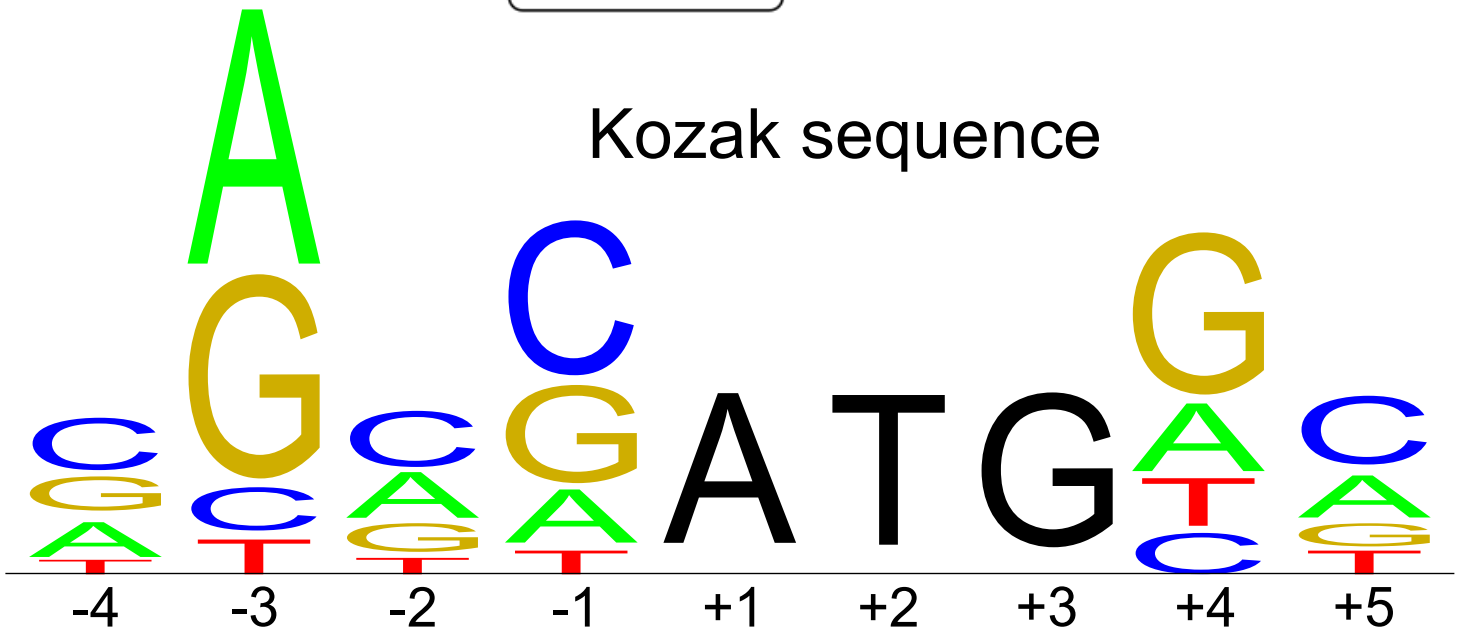
5’-cap



The 5’-cap is (mostly) needed for the initiation of scanning

Cap-independent Translation → IRES

Kozak sequence



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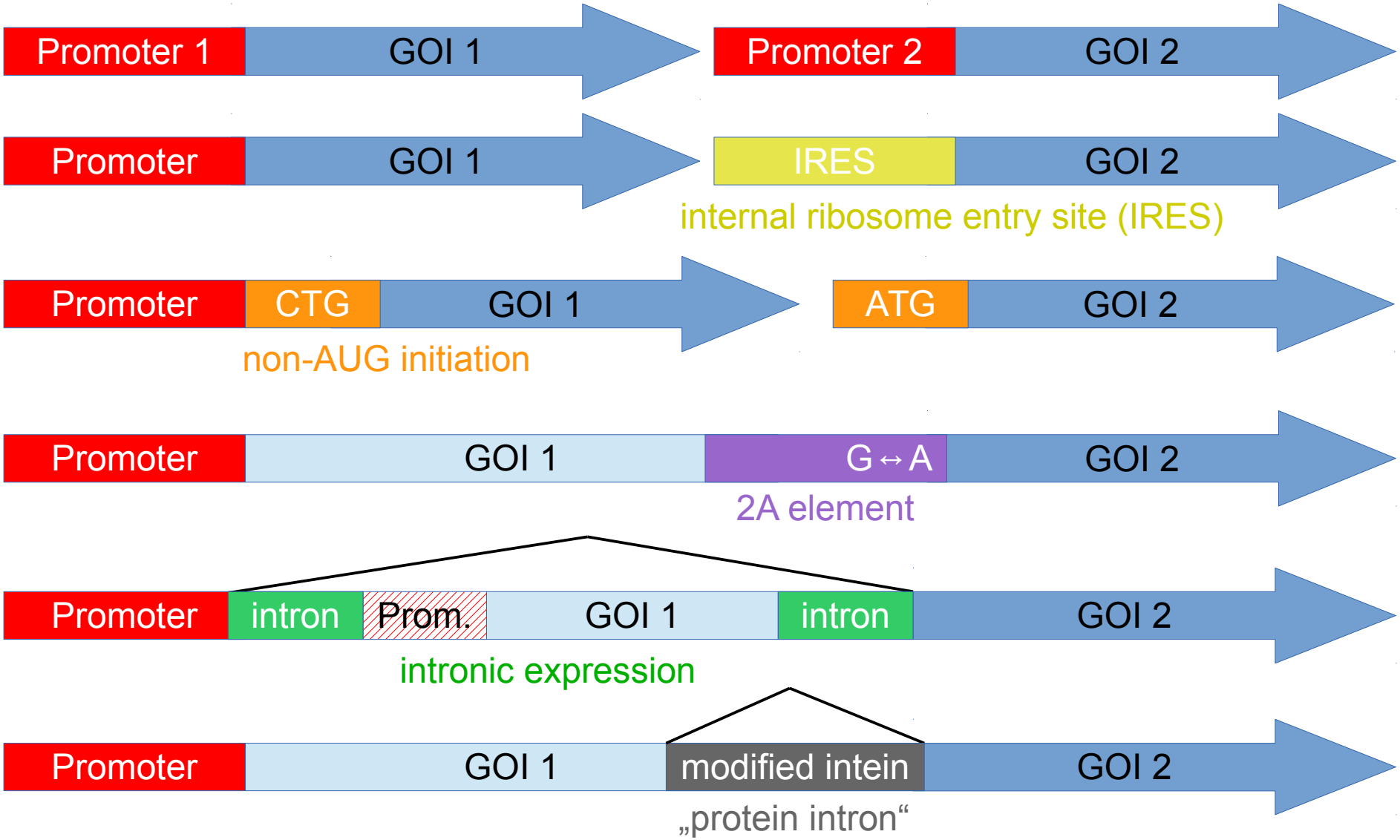
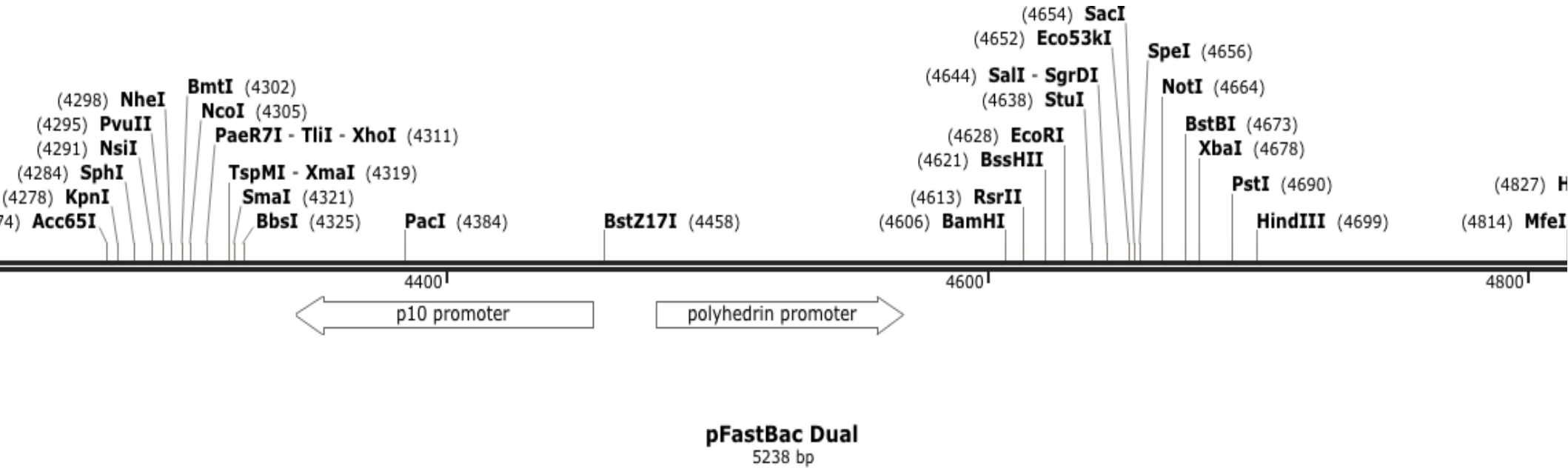


Ratio of expression between GOI1 and GOI2 can be regulated: IRES < cap-dependent translation, CTG << ATG

Baculovirus System

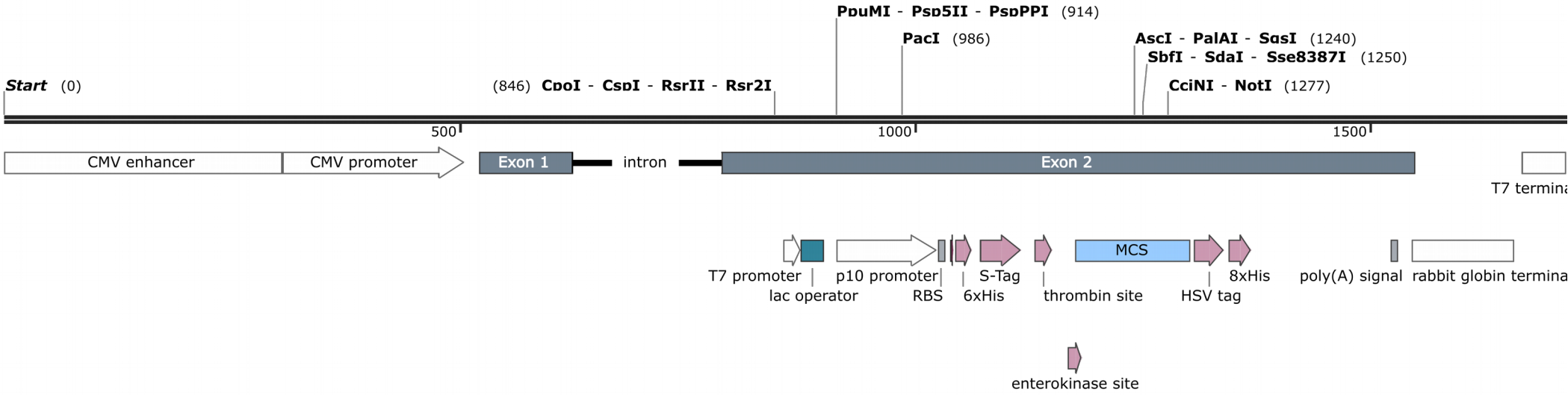


pFastBac Dual
5238 bp

More than one Gene Of Interest (GOI)

- 2 GOIs: pFastBac Dual (LifeTechnologies)
- Many GOIs: MultiBac (Geneva Biotech)

Baculovirus System



The same plasmid for bacterial, baculoviral and mammalian expression

- pTriEx (Novagen→Merck/Millipore)
- pQE-TriSystem (Qiagen)

Next meeting

- Topic: Gateway and Golden Gate Cloning
- Again in room KOK7 (7th floor)