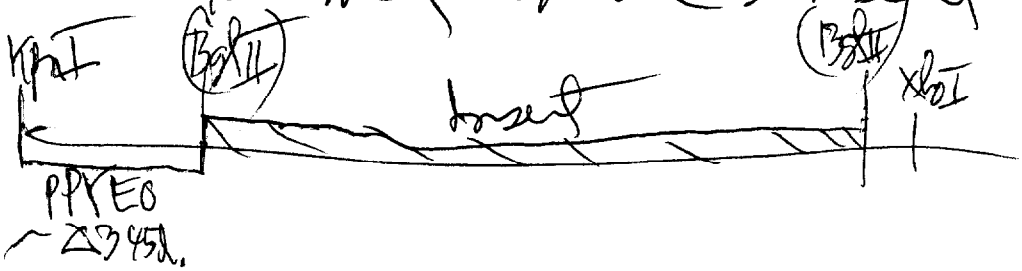
3-21-88

*** Idea - To Sequence ⟹ Make Primer near insert



Use - 40 Primer

→

If make nested set of dones. Which end is fixed

Henikoff Method

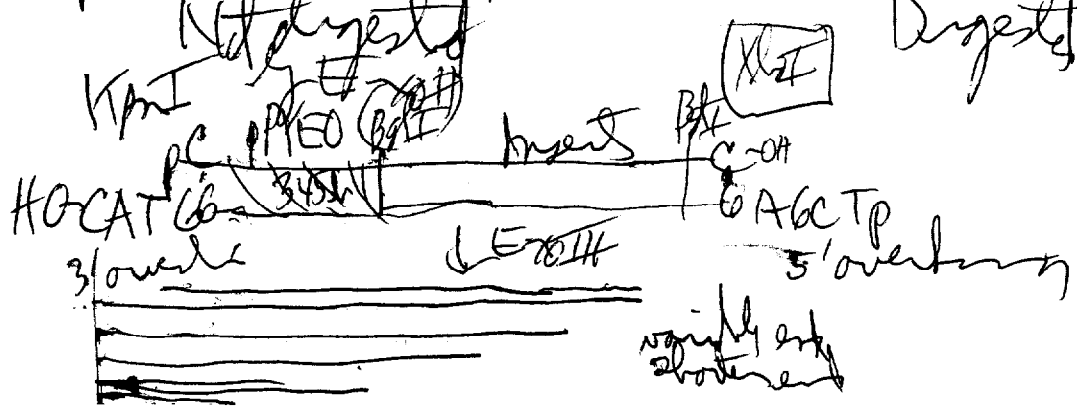
Exo III digests 1) Protruding 5' terms
or 2) Blunt end

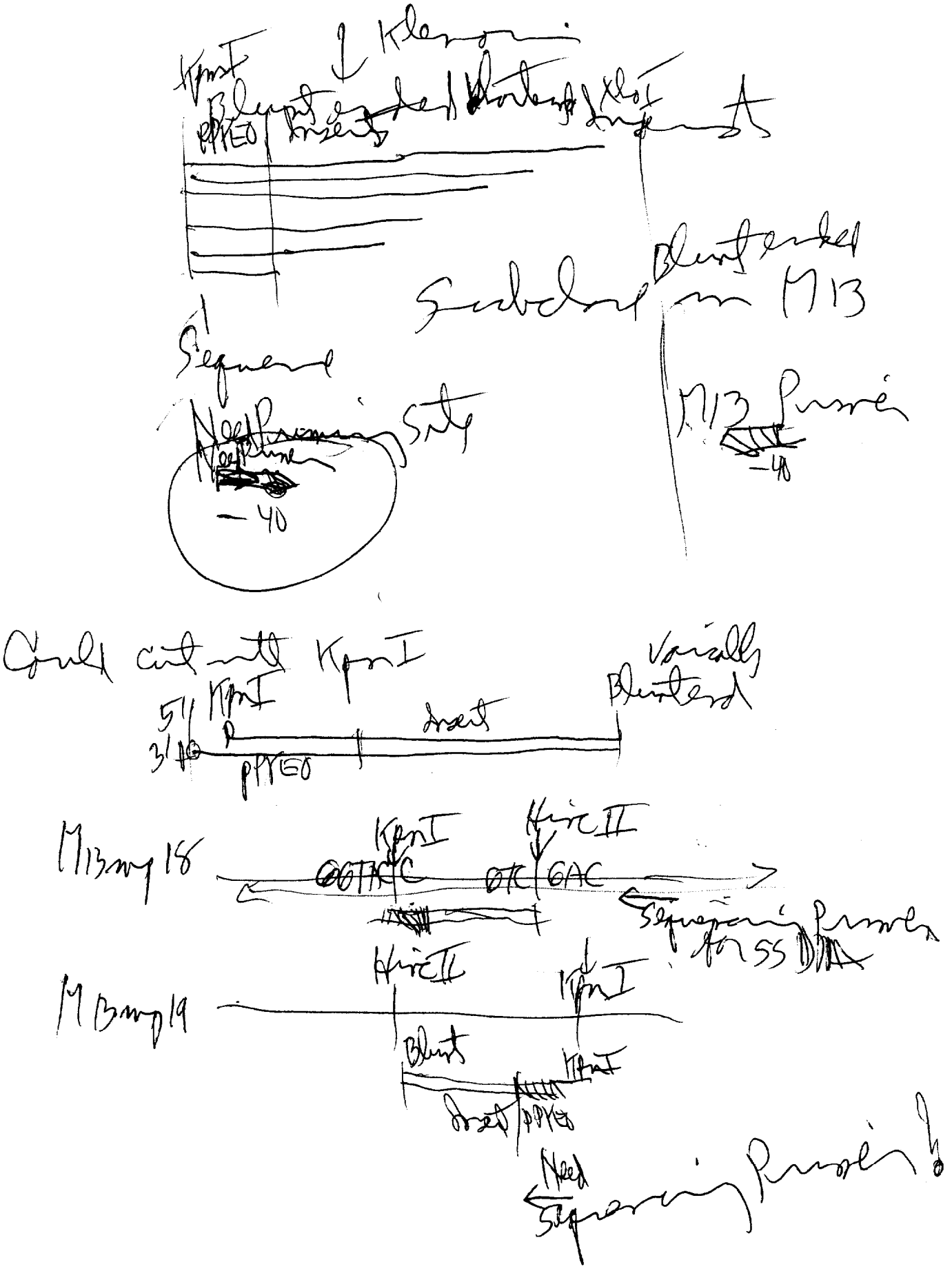
Exo III does not digest a 4 h 3' Protruding end
or an α-phosphorothioate filled end

KpnI = 4 h 3' overhang — Insert — XhoI end = 5' Protruding end

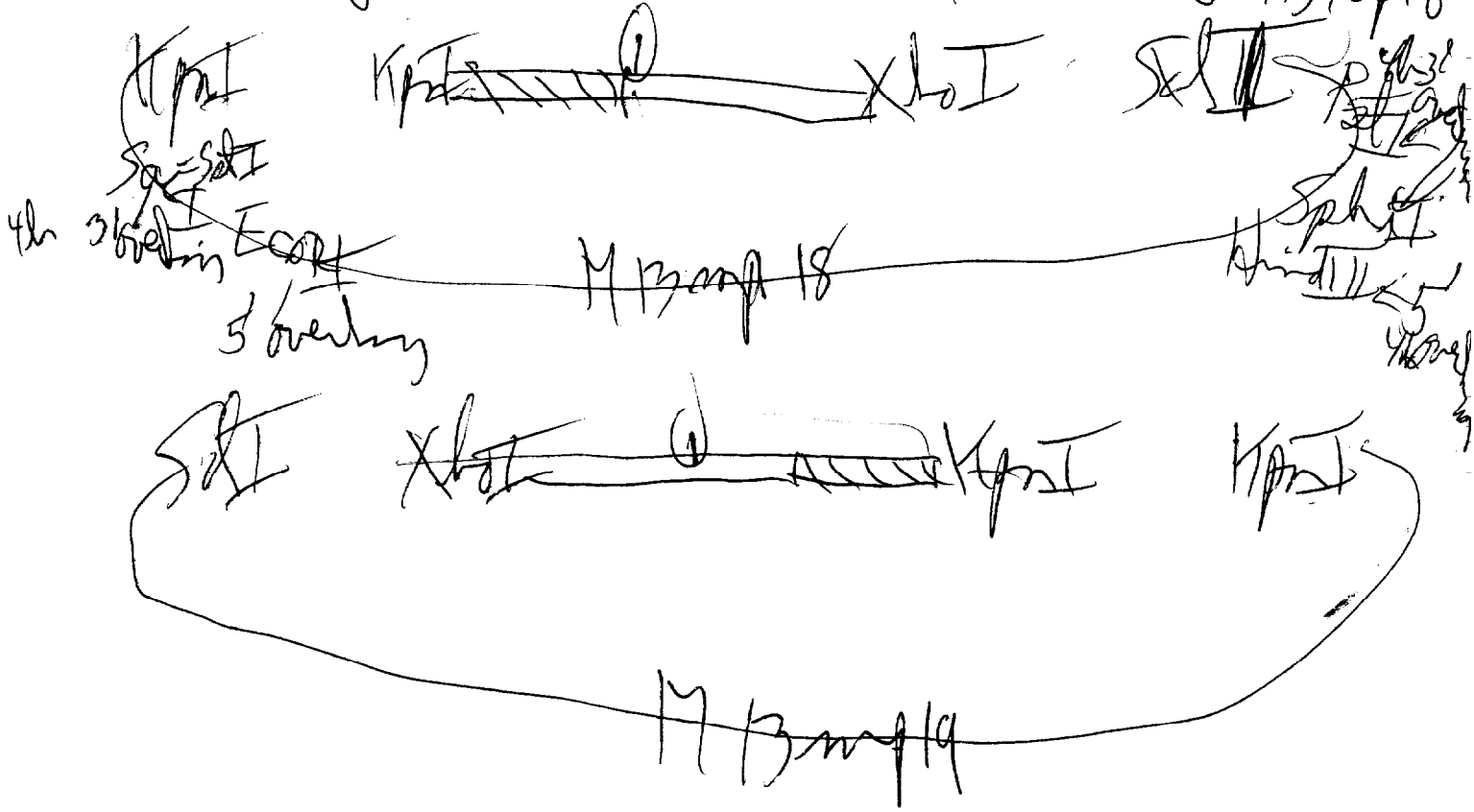
Not digested by Exo III

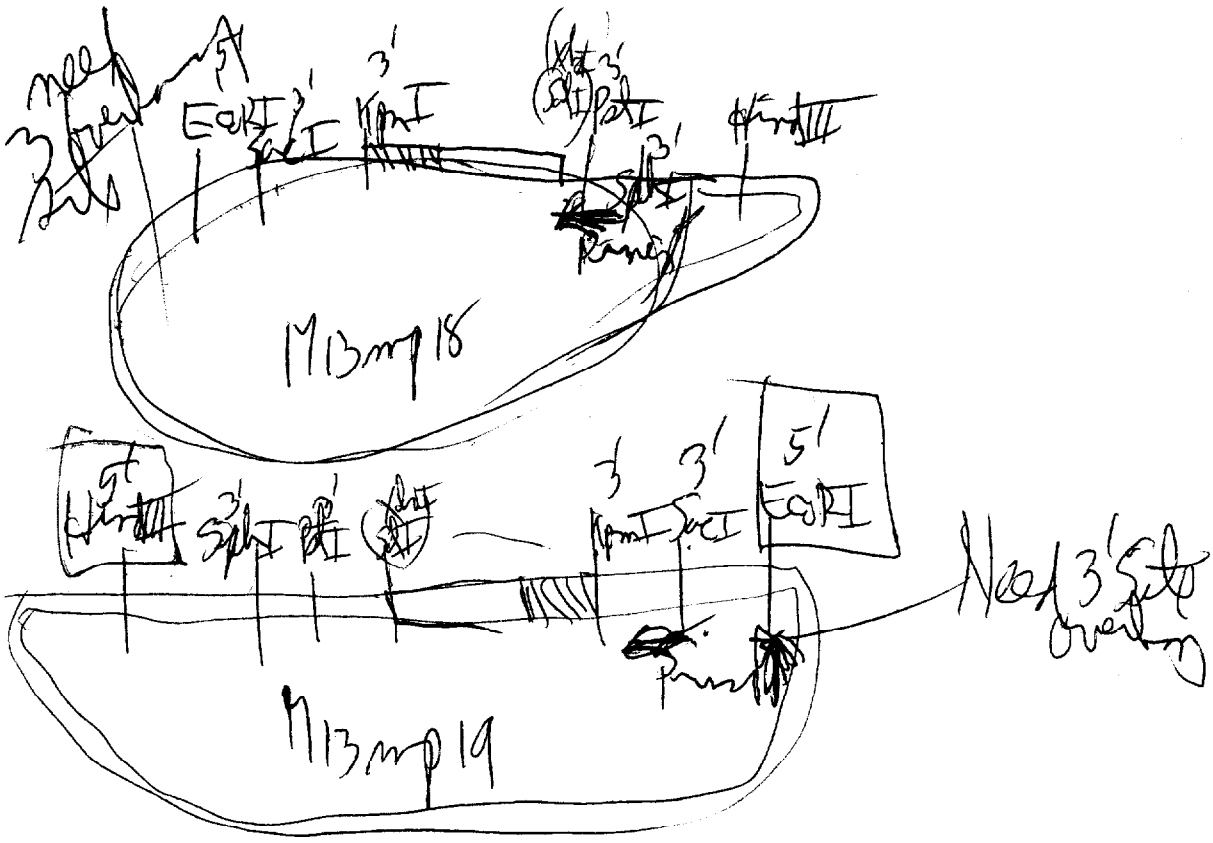
Digested by Exo III

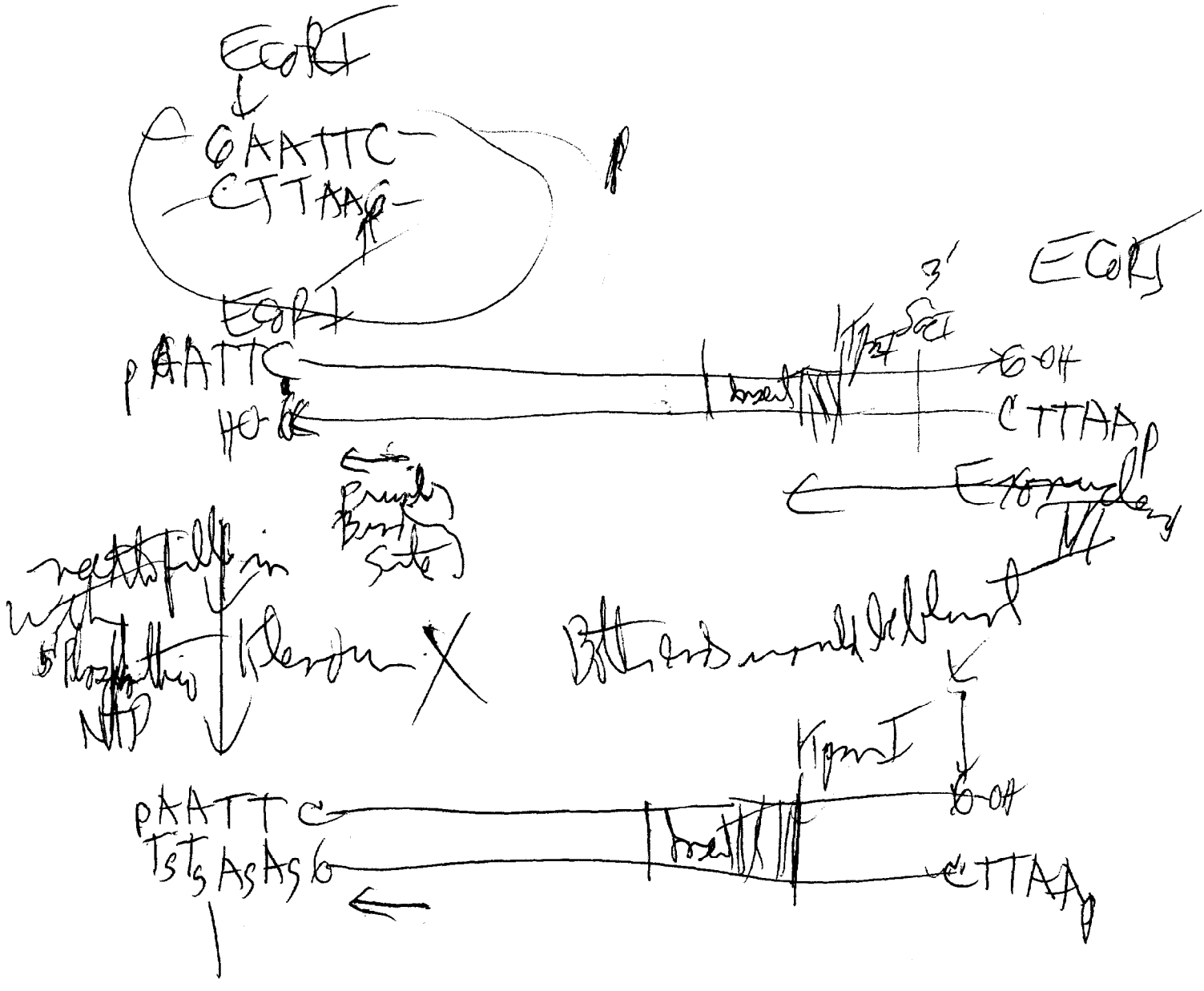


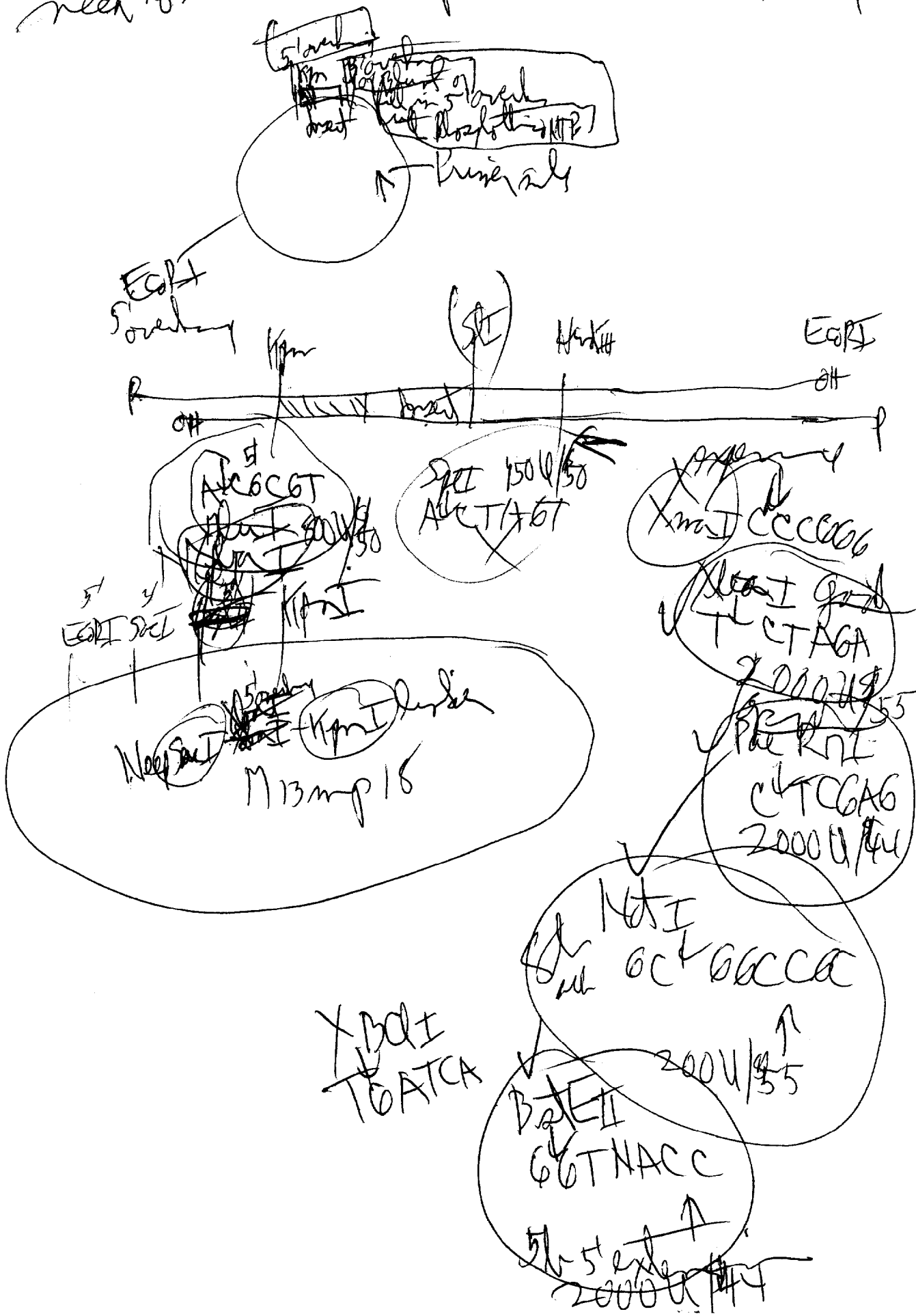

Klenow
KpnI
Blunt
pPTEO
Insert
Blunt
XbaI
Blunt ended
M13
Sequenced
Need Primer Site
-40
M13 Primer
-40
Could cut with KpnI
KpnI
Insert
Variably Blunted
5'
3'
pPTEO
M13mp18
KpnI
HincII
GGTACC
GTC GAC
Sequencing Primer for SS DNA
M13mp19
HincII
KpnI
Blunt
KpnI
Insert
pPTEO
Need Sequencing Primer!

Subclone inserts 1st into M13mp18






ECoRI
GAATTC
CTTAAG
ECoRI
pAATTC
HO-K
3'
ECoRI
Insert
G-OH
CTTAAp
Primary Binding Site
Exonuclease
Klenow
Both ends would be blunt
Kpn I
pAATTC
TsTsAsAsG
Insert
G-OH
CTTAAp

For Henikoff Sequence
need to insert a linker in M13mp18 sq

EX 0114