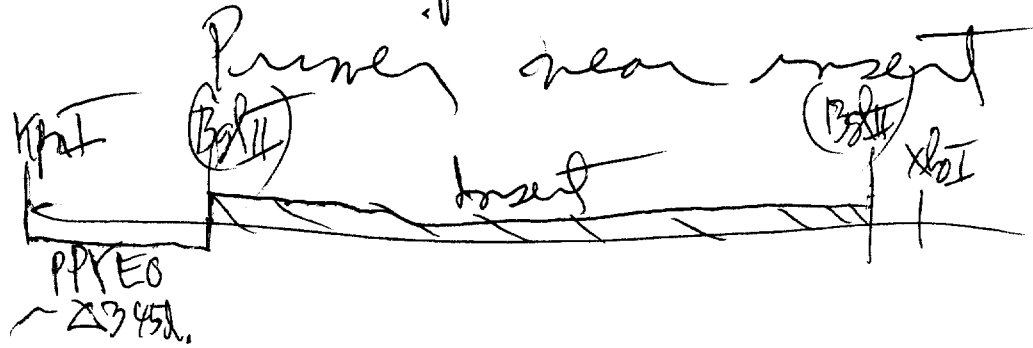


3-21-88

\*\*\* Idea - To Sequence  $\longrightarrow$  Make



Use - 40 Primer

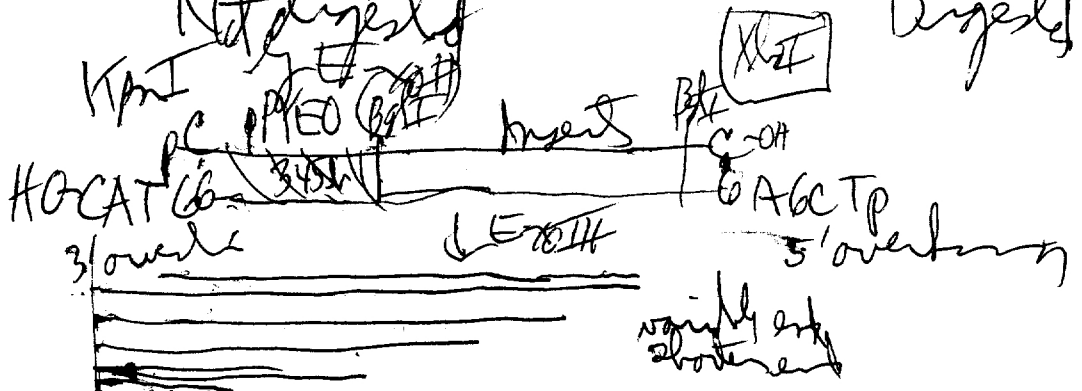
$\longrightarrow$   
If make rest of clones, which end is fixed

Henickoff Melt

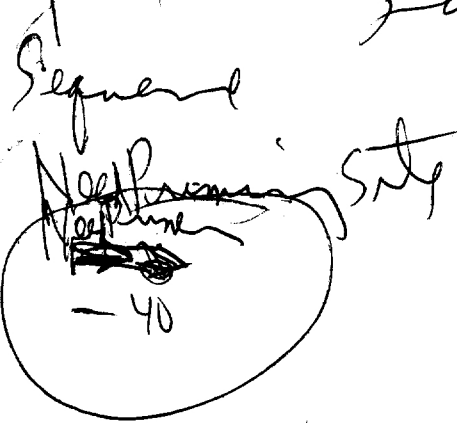
Exo III digests protruding 5' term  
92' Blunt end

Exo III does not digest a 4h 3' Protruding end  
or an  $\alpha$ -phosphorylated filled end

KAT = 4h 3' overhang  $\xrightarrow{\text{insert}}$  XbaI site = 5' Protruding  
Not digested Digested by Exo III



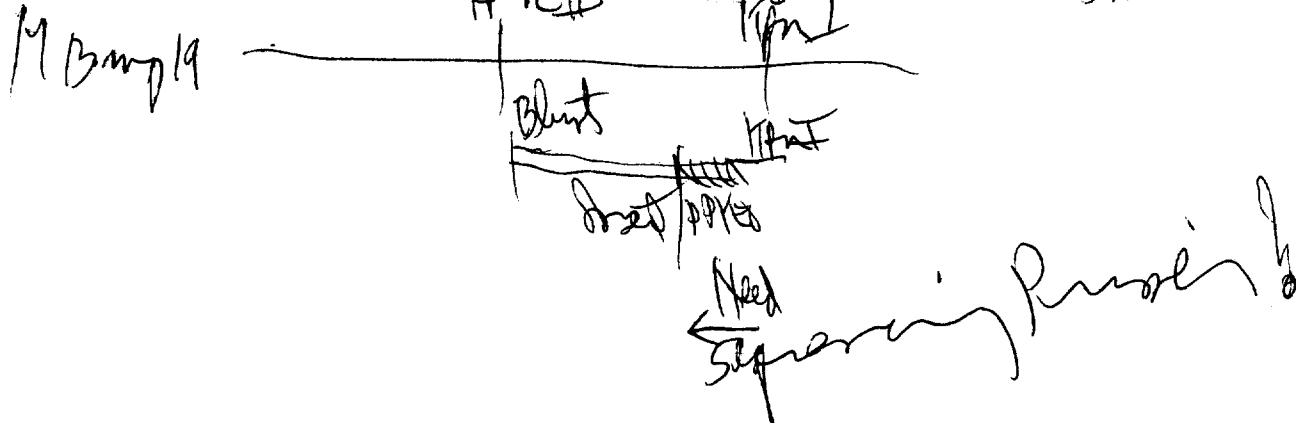
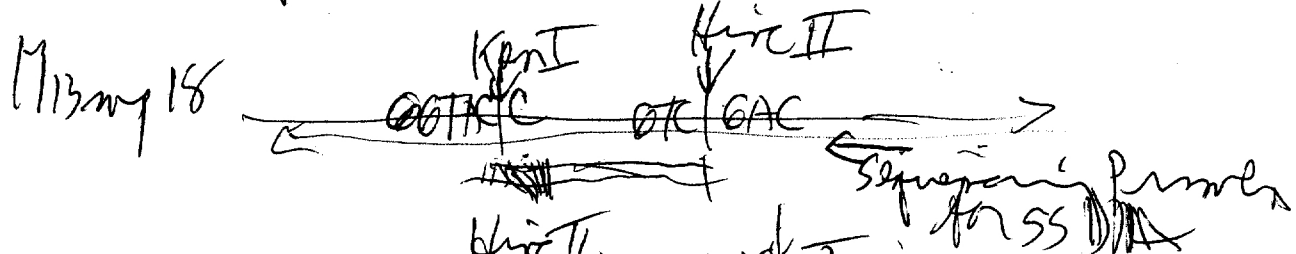
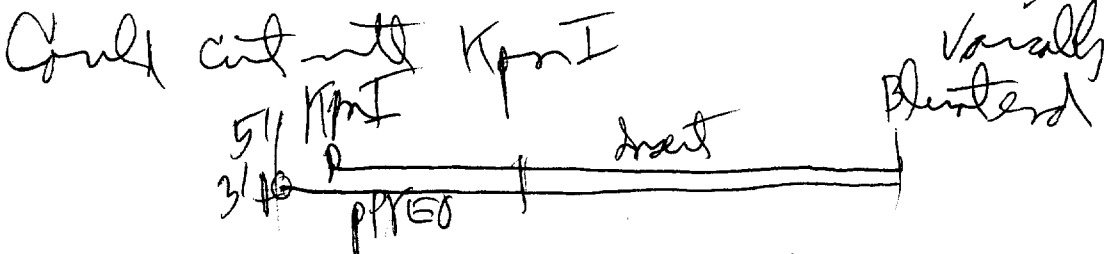
KpnI & Klenow  
 Blunt ended  
 PPEO inserts



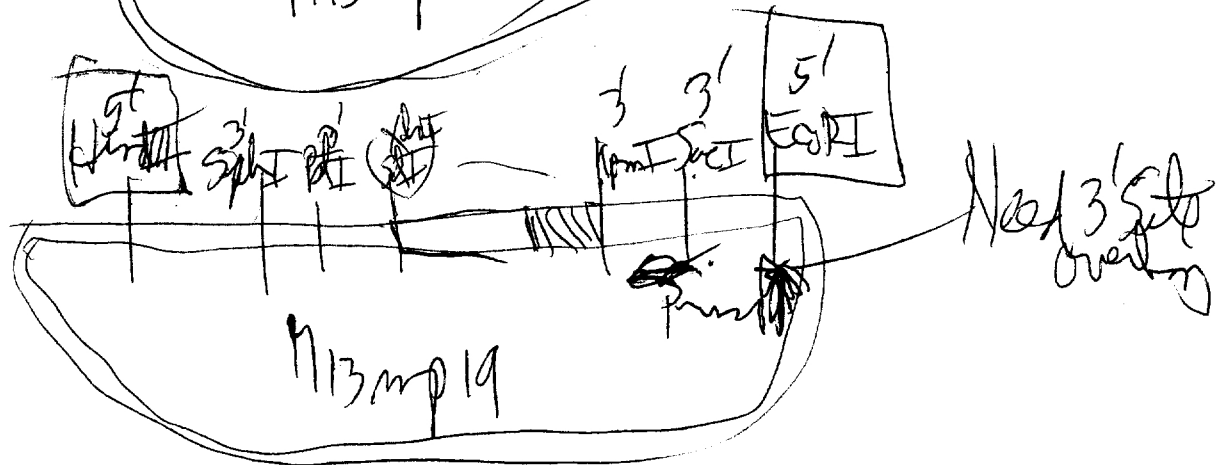
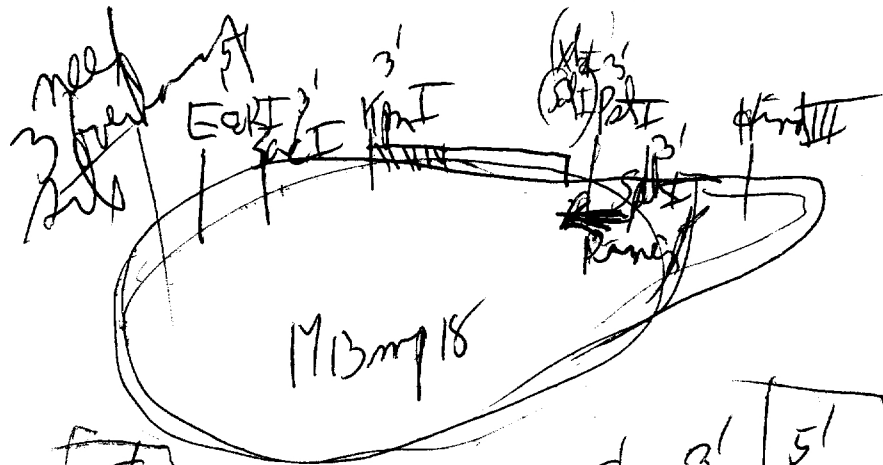
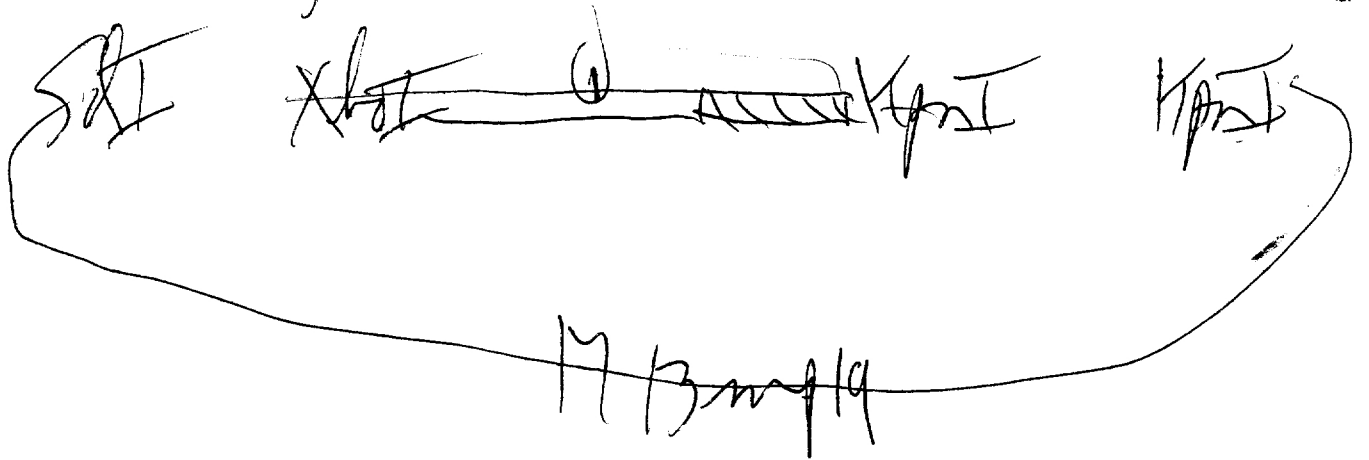
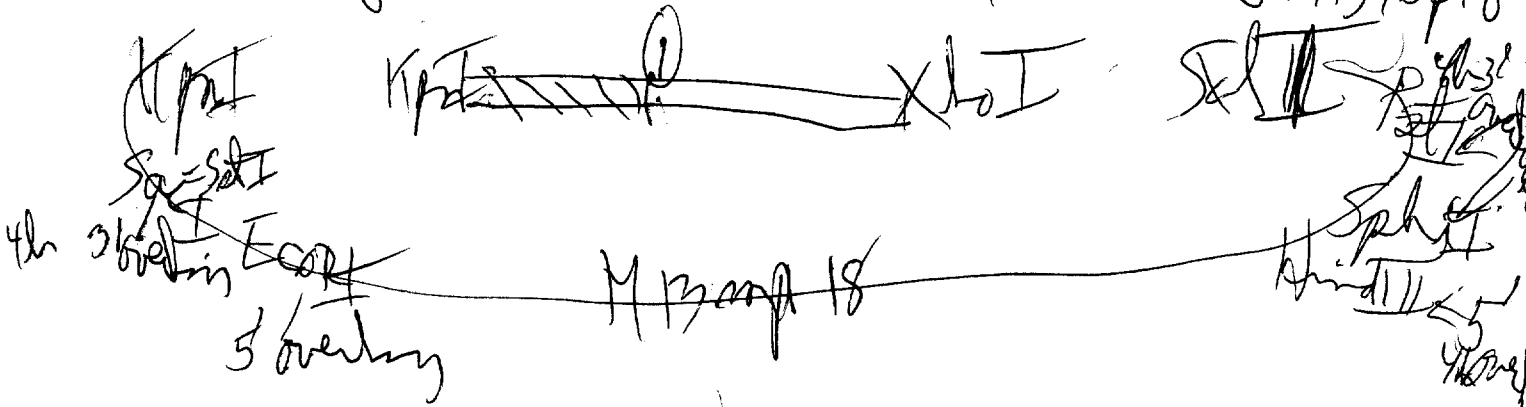
Subcloned blunt ended  
 in M13

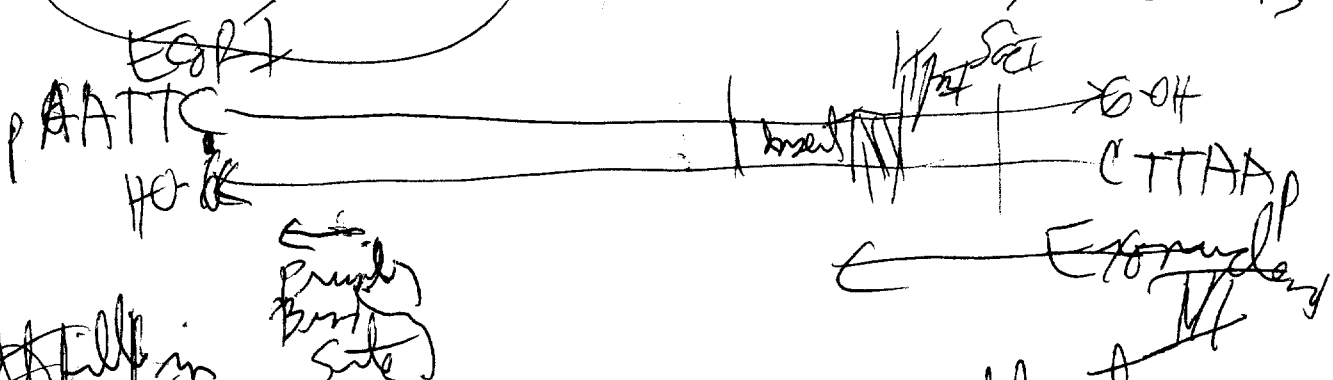
M13 Purified

A small rectangular fragment with a diagonal line through it, representing a purified M13 fragment. Below it, the number '-40' is written.



Subclones inserts 1st into M13mp18





next to fill in  
 with 5' phosphate  
 NTP

$\text{Klenow X}$

$\leftarrow$   $\text{Primer}$   
 $\text{Synth}$   
 $\text{Site}$

$\text{Both ends would be blunt}$

