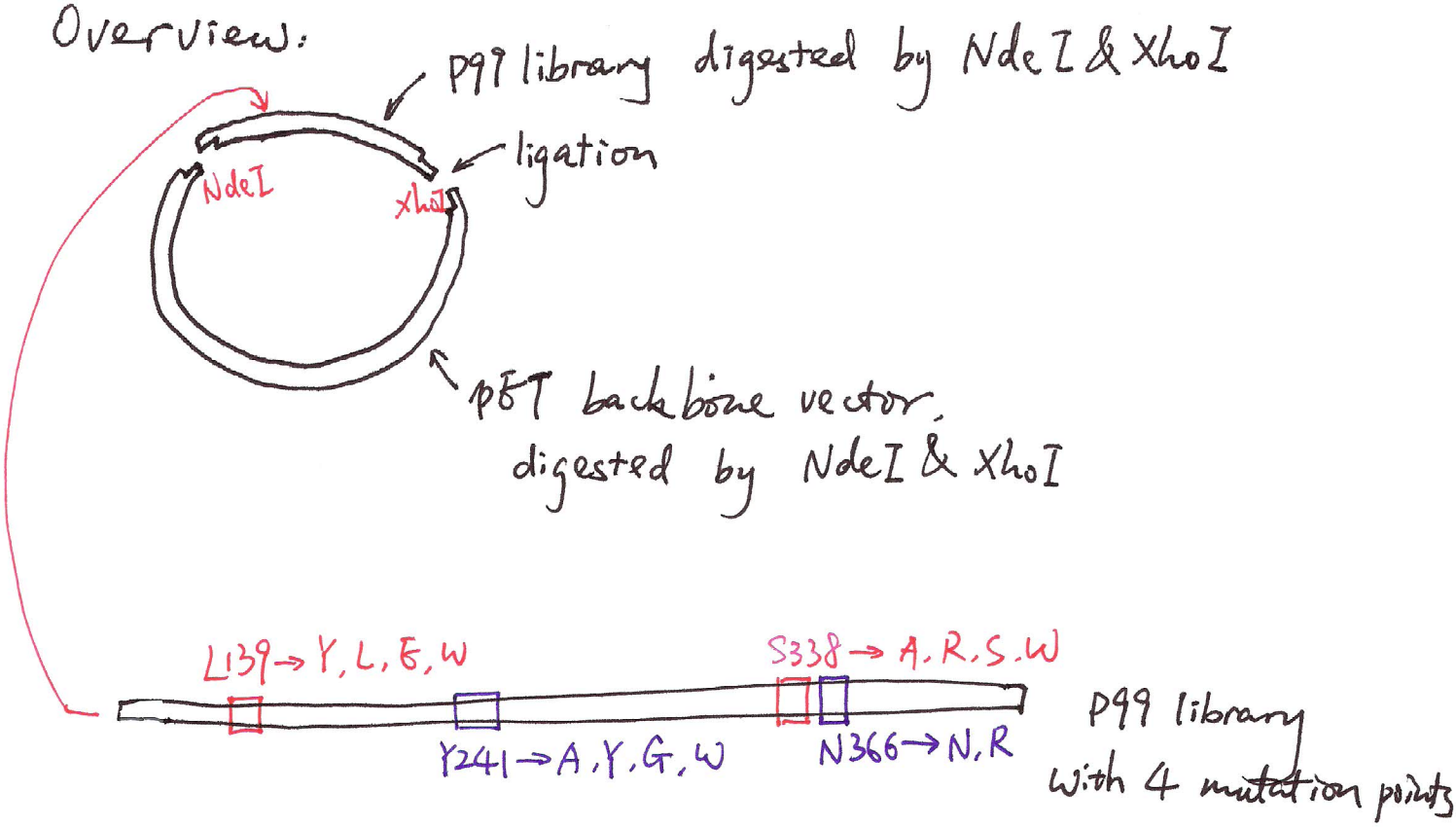
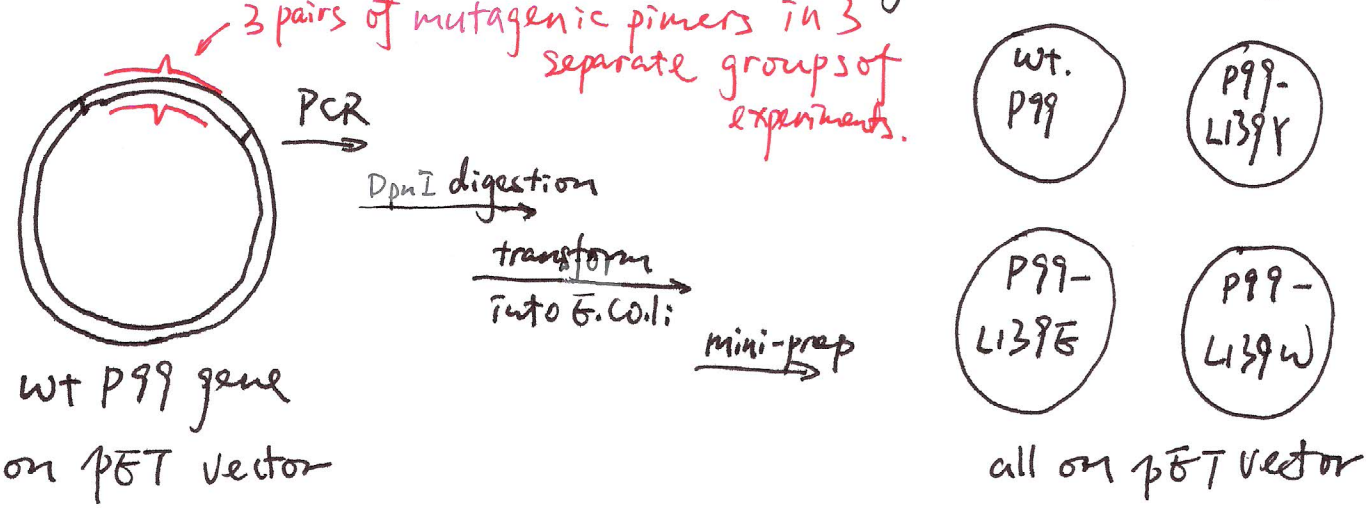
Library construction

① Overview:



The last 2 mutation points are close to each other and are close to the C-terminus of P99.

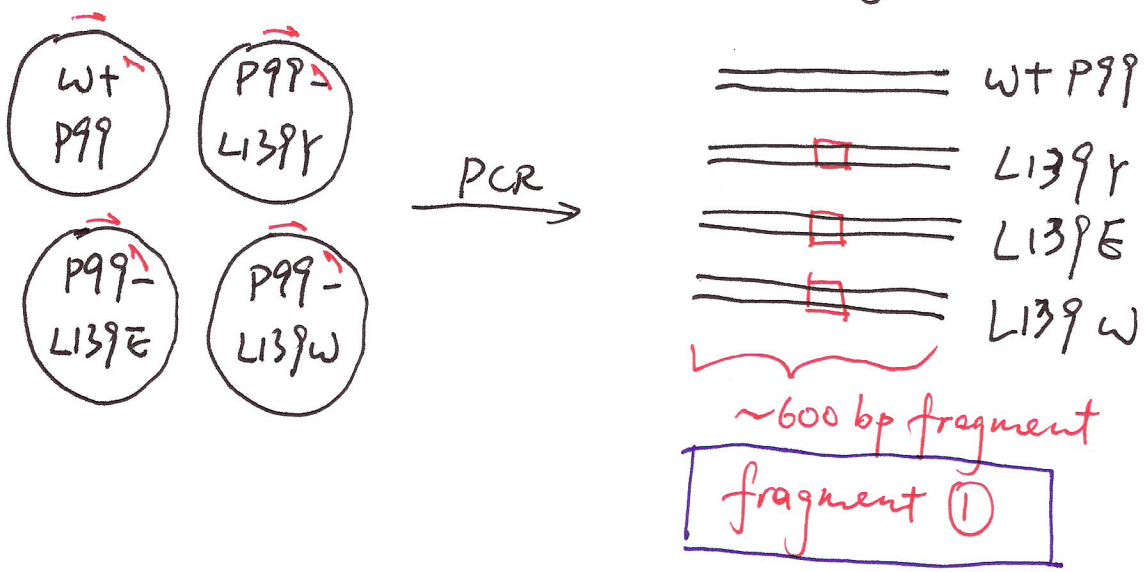
② SDM. The L139 & Y241 mutations are to be created via site-directed mutagenesis (SDM)



In the same way I will make P99-Y241A, P99-Y241G, P99-Y241W on pET vector.

(time estimation : ~ 2 weeks)

③ from vector to linear DNA fragments.



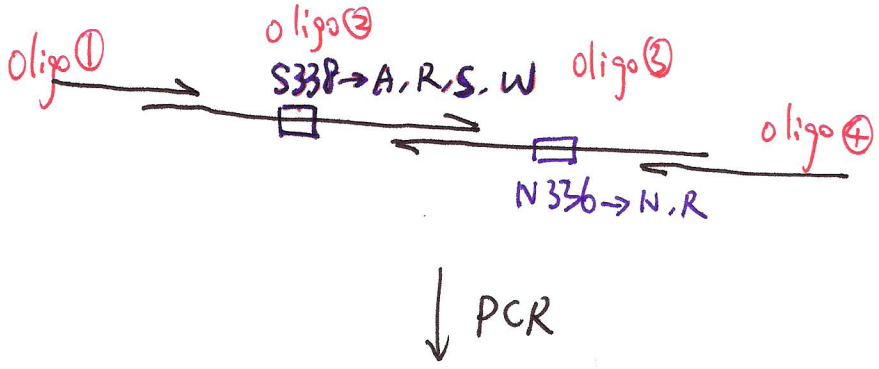
In the same way I will make ~400bp fragments bearing mutations Y241A; Y241G; Y241W.

This is fragment ②

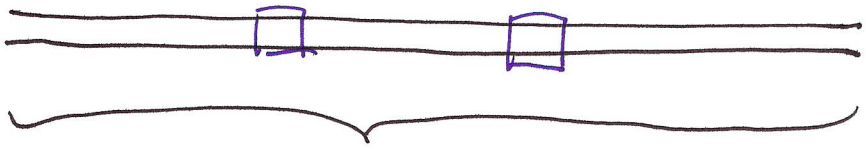
Fragment ② is designed in the way that overlaps with fragment ① for ~20 bp to facilitate fusion PCR. (~ 1 week)

④ gene synthesis:

mutations at S338 & N366 are close to each other, so I'm going to combine them into one fragment. This fragment will be made by PCR-based gene synthesis.



Oligo ② is a mixture of 4 oligos encoding 4 different aa..
Oligo ③ is a mixture of 2.



~200 bp fragment with combinatorial mutations at S338 & N366.

This is **fragment ③**

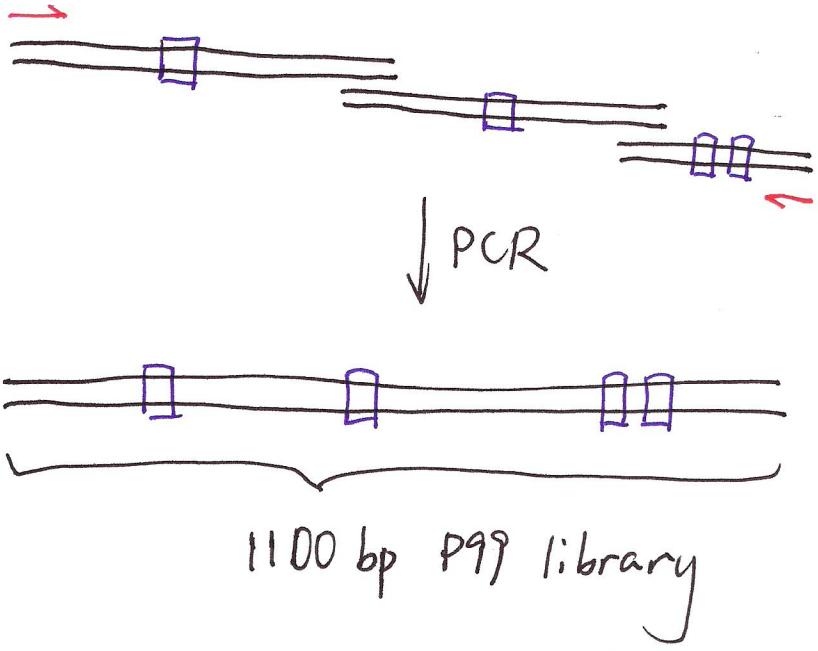
Fragment ③ is designed to overlap fragment ② for ~20bp to facilitate fusion PCR.

(time estimation : ~2 weeks)

⑤ Fusion PCR.

Fragment ①, ②, ③ will be connected by fusion PCR.

Each fragment is itself a mixture of different mutations. In fusion PCR they will make a combinatorial library.



⑥ Digest the library & the back bone vector by NdeI & XhoI, ligate, transform into E. coli. Pick up colonies & sequence for validation.

(time estimation: step ⑤+⑥ 2~3 weeks)

Total time: 6~8 weeks.