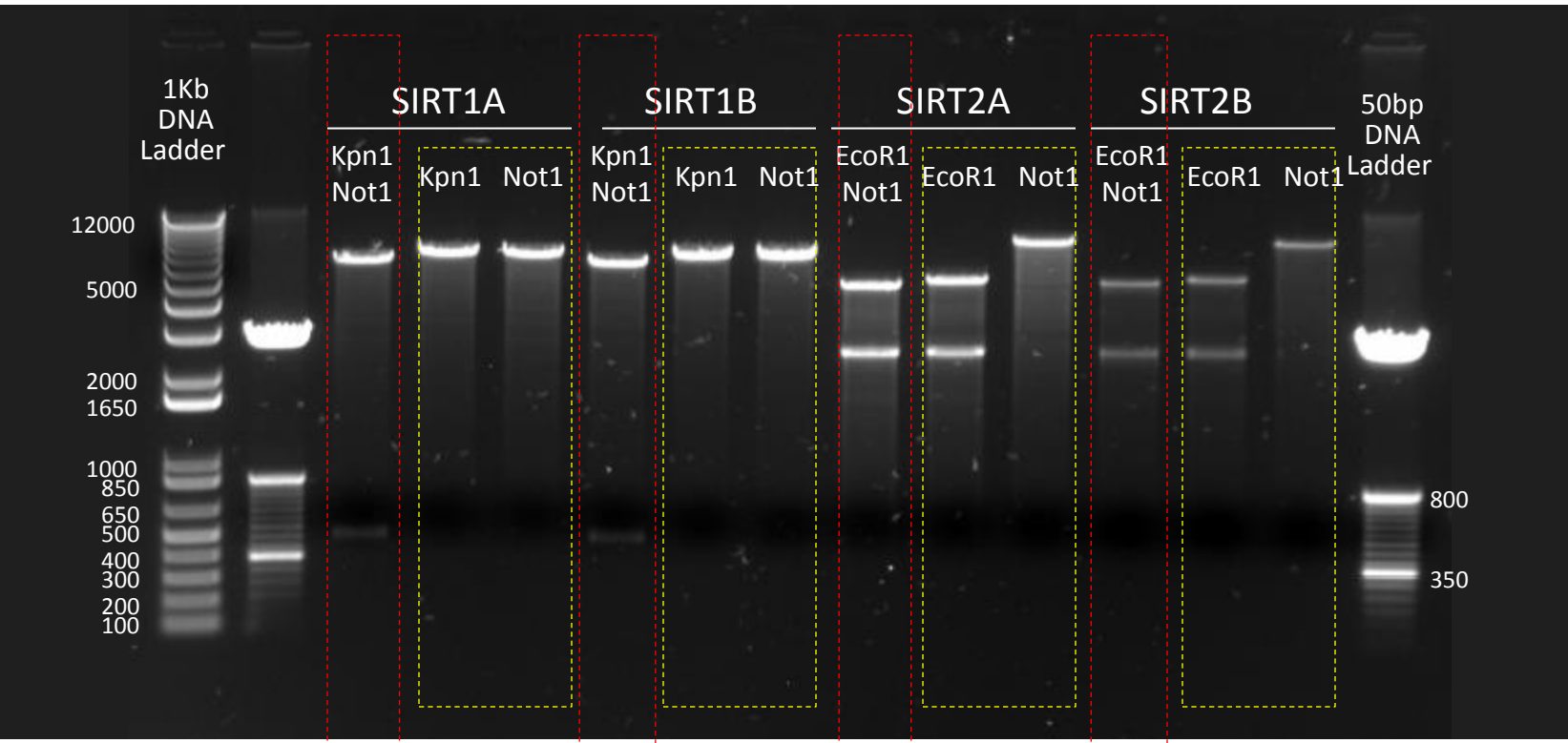


# Miniprep 1<sup>st</sup> run



# Miniprep 2<sup>nd</sup> run



- For each plasmid (SIRT1 and SIRT2) two colonies were selected (A and B) and inoculated.
  - SIRT1A and SIRT1B results are reproducible.
  - SIRT2A and SIRT2B results are reproducible.
- Miniprep for SIRT1 and SIRT2 were performed twice (Run1 and Run2)
  - Run 1 and Run 2 data are reproducible.
- Double digestion results (red highlight) indicated
  - SIRT1A and SIRT1B got 1 strong band (over 5000bp) and 1 light band (~ 500 bp) which are not right size.
  - SIRT2A and SIRT2B got two bands (>5000 bp and >2000bp). We expect to obtain one 1327 bp band which was not observed.
- Single digestion results (yellow highlight) indicated
  - SIRT1A and SIRT1B can be digested with kpn1 and not1.
  - When EcoR1 was applied, two bands were obtained on SIRT2A and SIRT2B digestion product which does not agree with the sequence provided by OriGene.

**Conclusion:** The plasmids of Sirt1 and 2 failed to generate the appropriate proteins. When the restriction digestion was performed, the sequences provided by OriGene don't agree with the results of the restriction enzymatic analysis.

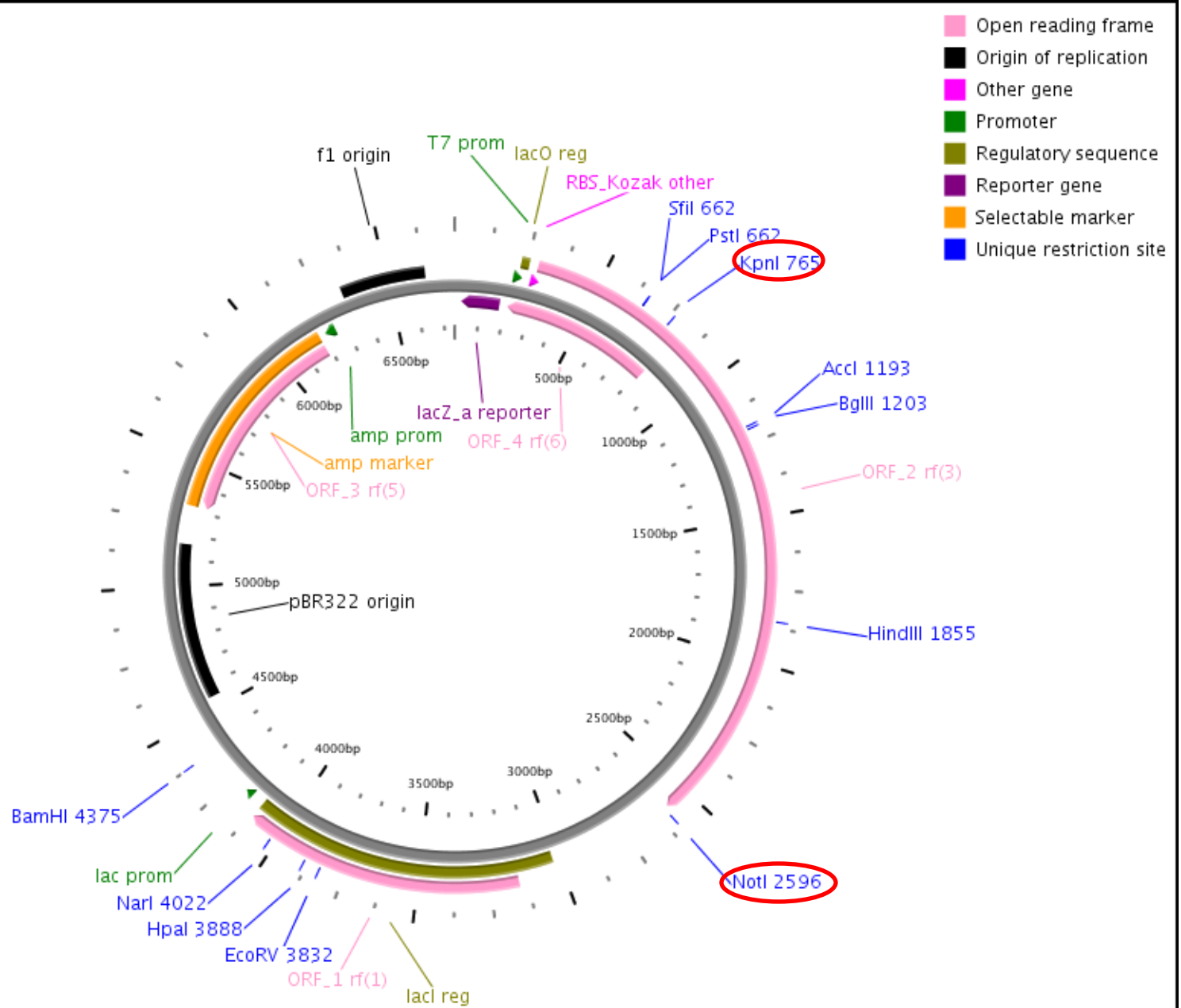
**Next step:**

- (1) Request OriGene analyze SIRT1 and SIRT2 plasmids, and send us both the enzymatic diagnosis and sequencing results.
- (2) If the problem is observed, the new plasmids need to be made without extra charge.

**Solution:**

- Email has been sent to request both the enzymatic diagnostic from OriGene.
- OriGene has set up a free replacement (SO#SR031647) to get another vial of these two custom clones. They will do the same digestion and fully sequence the insert genes for these two clones. This replacement should take 2~3 weeks.

# SIRT1



# SIRT2

