

### RDM Day 2 Interpretation Questions and Answers

1. Interpret your low-melt gel. If you didn't see the three bands you were aiming for, give explanations as to why that happened.

**Answers will vary but hopefully everyone saw something. Explanations for what went wrong could include: partial digestions, forgot to add CIP, added CIP to both vector and insert, forgot to heat samples before loading, didn't keep enzyme on ice, etc.**

2. What three features do pUC19 and pET share making them useful cloning vectors? In addition, what distinct quality does pET have that pUC19 does not? How will this feature come in handy later in the module?

**Both pUC19 and pET have: an origin of replication, restriction enzyme sites (or a polylinker), and a gene for antibiotic resistance (necessary when selecting bacteria with plasmid). In addition, pET has a T7 promoter which will direct transcription of the *gfp* gene in the presence of the T7 RNA Polymerase in *E. coli* BL21.**

3. When group A1 plates their ligations, the following are their results. Explain what might have happened. (Assume they performed all the steps in the experiment correctly and had complete digestions of pET and pUGFP as judged by the low melt gel.)

Ligation #1 – lots of transformants

Ligation #2 – lots of transformants

Ligation #3 – zero transformants

Ligation #4 – lots transformants

**We expected to see lots of transformants from the first ligation and very few or none from the second ligation. Since ligation #2 was a negative control for the CIP, the CIP must have been expired/not working. As a result, the digested pET had its ends intact and most pET plasmids probably recircularized so the cells receiving ligation #2 grew on the plates. Because the majority of the pET recircularized before the GFP insert was added to it, there was a very low chance of the GFP ligating to the vector. Therefore, the transformants from ligation #1 are most likely the same as the transformants from ligations #2, pET without the GFP insert.**