>> Dude: Grrr!

Something is really wrong with this!

>> Sally: What are you playing with?

>> Dude: I downloaded an app called "Part Palette." It lets me plan genetic circuits using Parts from the Registry.

>> Sally: That sounds great.

Can I see?

>> Dude: I can select parts and then wrap them together on the palette.

Or at least I'm suppose to be able to do that!

>> Sally: Hmmm...

I keep getting an error message.

maybe the app doesn't work, or maybe there's something about the parts you're trying to wrap together.

What gene are you trying to build?

>> Dude: I just grabbed these 3 parts to try.

>> Sally: A terminator, an operator and an open reading frame?

That's not a gene...

>> Dude: but it's got an open reading frame...what more do I need?

>> Sally: Walk with me Dude.

For starters you'll need a promoter.

That's a snippet of DNA that the RNA polymerase binds to when it starts transcription.

Promoters come in different strengths so choose carefully.

>> Dude: How do I know if a promoter is strong or weak?

>> Sally: Well the strongest promoters have this DNA sequence.

RNA polymerase can bind these bases well.

The more bases that are different from this consensus sequence, the weaker the promoter is since RNA polymerase will bind less well and transcribe less often...at least...

that's how it usually works.

>> Dude: oh no!

I hear an exception coming...

>> Sally: Well sure Dude...it's very clever really.

There are promoters that are used in different stages of cell growth and those promoters bind a slightly different RNA polymerase so they have a consensus sequence that's a little different from this one.

And then there are still other promoters that have lots of differences from this consensus sequence but they are still strong promoters since they also have DNA sequences nearby that bind activating proteins.

The activators help RNA polymerase find the promoter and that gets transcription really cranking.

Activated promoters can be very strong.

>> Dude: When does it end!?!

>> Sally: Another great question Dude!...there are sequences that come after the open reading frame called terminators.

These tell RNA polymerase to stop transcribing RNA and to leave the DNA.

These terminators are really useful if you have another promoter downstream of this gene.

You'll need a terminator if you don't want the polymerase to read through this gene into the next one downstream.

>> Dude: "Downstream?" Don't you mean "on the right?" >> Sally: Cells don't know their right from their left so we have to use the natural polarity of double stranded DNA when we give directions.

"Downstream" means "more 3-prime" no matter which strand of the DNA you're working on.

>> Dude: So in this app I can wrap any promoter, ORF and terminator together, and I've got a gene!

>> Sally: Well actually you've got a transcription unit, and most of the time you'll want to translate the RNA from that unit into a protein.

To translate it, you'll need to include a ribosome binding site.

The RBS is where ribosomes attach to the RNA message so they can start linking the specified amino acids into a protein chain.

And you can control the strength of the RBS to control how much of the message gets translated.

>> Dude: and I'll bet there's an app for that too...

>> Sally: well if you're interested in working on one, let me tell you about how genes are arranged in eukaryotic cells like plants, yeast and human...

>> Dude: Can we do that another day Sally?

My disk space nearly full.