C4A and C4B GCN qPCR Assay

1. Prepare C4A and C4B Standard Curve
   The C4 genotype of this DNA is 2 copies each of C4A and C4B. This standard DNA is used for both C4A and C4B assays.
   Prepare 10 fold serial dilutions enough to use 4 uL/reaction.
   Standard 1. 2.5x10^5/uL
   Standard 2. 2.5x10^4/uL
   Standard 3. 2.5x10^3/uL
   Standard 4. 2.5x10^2/uL
   Standard 5. 2.5x10^1/uL

2. Prepare a C4A and a C4B PCR mix for 25 uL reaction volume/well.
   12.5 uL Quantitect Reagent/well
   1.25 uL of C4A or C4B primer&probe mix/well
   1.25 uL of ENDO primer&probe mix/well
   6 uL water/well
3. To appropriate wells of a 96-well PCR plate, add 4 uL of each Standard concentration. For the GCN standard curve add 4 uL of C4A or C4B GCN 3, 2, 1 and 0 (DNA provided) to the appropriate wells. Add 100-200 ng of sample DNA + water to 4uL to appropriate wells. For each PCR mix add 4 uL water to one well as negative control. Standards and samples should be run in duplicate.
4. Add 21 uL of the appropriate PCR mix to each well. Seal with optically clear sealing tape and centrifuge briefly to remove air bubbles and make sure all liquid is at the bottom of the well.
5. Run PCR on BioRad CFx 96 Thermal Cycler under the following conditions.
   15 min at 95 C
   40 cycles of
   1 min at 95 C
   1 min at 60 C
6. Analyze data as described in Dr. Yu's paper. (Attached to e-mail.)

C4 Total GCN qPCR Assay

1. Prepare C4 Total Standard Curve
   Prepare 10 fold serial dilutions enough to use 1 uL/reaction.
   Standard 1. 1x10^6/uL
   Standard 2. 1x10^5/uL
   Standard 3. 1x10^4/uL
   Standard 4. 1x10^3/uL
   Standard 5. 1x10^2/uL

2. Prepare a C4 Total PCR mix for 25 uL reaction volume/well.
   12.5 uL Quantitect/well
   1.25 uL of C4A or C4B primer&probe mix/well
   1.25 uL of ENDO primer&probe mix/well
   9 uL water/well
3. To appropriate wells of a 96-well PCR plate, add 1 uL of each Standard concentration. For the GCN standard curve add 1 uL of C4 Total GCN 5, 4, 3 and 2 (DNA provided) to the appropriate wells. Add 1 ul of 50 ng/ul sample DNA to appropriate wells. Add 1 uL water to one well as negative control. Standards and samples should be run in duplicate.
4. Add 24 uL of the appropriate PCR mix to each well. Seal with optically clear sealing tape and centrifuge briefly to remove air bubbles and make sure all liquid is at the bottom of the well.
5. Run PCR on BioRad CFx 96 Thermal Cycler under the following conditions.
   15 min at 95 C
   40 cycles of
   1 min at 95 C
   1 min at 60 C
6. Analyze data as described in Dr. Yu's paper. (Attached to e-mail.)