RT- QuIC example experiment

MATERIALS:

Samples to be tested: Normal (negative control) and TSE infected (positive control) 10% BH (eg. 263K)

Substrate: rPrP-^{sen} Hamster (90-231) (or other appropriate substrate for the samples to be tested)

BH dilutent: N_2 media supplement in **0.1%** SDS/PBS

1. Dilute 100XN_2 media supplement in **0.1%** SDS/PBS as follows: 990ul **0.1%** SDS/PBS + 10ul N2 for BH dilutions

2. Dilute hamster normal and 263K 10% BH in 0.1% SDS/PBS/N2 as follows:

   a. (1:20): 5ul of 10% BH in 95ul 0.1% SDS/PBS/1xN2 [500pg/ul]
   b. (1:10): 5ul of previous dilution (1:20) in 45ul of 0.1% SDS/PBS/1xN2 [50 pg/ul]
   c. (1:10): add 5ul of previous to 45ul of 0.1% SDS/PBS/1xN2 [5 pg/ul]
   d. (1:10): add 10ul of previous to 90ul of 0.1% SDS/PBS/1xN2 [500 fg/ul]
   e. (1:10): add 10ul of previous to 90ul of 0.1% SDS/PBS/1xN2 [50 fg/ul]
   f. (1:10): add 10ul of previous to 90ul of 0.1% SDS/PBS/1xN2 [5 fg/ul]

   Note: With 263K BH we start with a 1:20 dilution to achieve an initial concentration of 500pg/ul based upon quantitative western data. The normal BH controls are equally diluted as parallel negative controls. For other TSE BHs, the dilution series may be significantly different to achieve target Res concentrations. Alternatively, you could do simple 1:10 dilutions and base activity on dilution levels rather than Res concentration.

3. Make 10mM ThT stock in mQH2O (weigh 0.032g ThT/10ml mQH2O, filter and keep on ice)

4. Dilute 10mM ThT stock in mQH2O 1:10

5. Filter recombinant rPrP with a 100kD microtube filter (PALL): 4000 rpm for 5min. Can use filter twice with 500ul of rPrP each time. You may need to add additional spin time on to get all the solution through.

6. Gently transfer filtered recombinant rPrP in new tube and keep on ice (do not vortex the rPrP and try to not pass bubbles through it).

   To determine the volume of rPrP/reaction volume when using a new batch:

   Dilute 1:10 recombinant rPrP in 0.1% SDS/PBS and spec at 280nm.

   [Protein]mg/mL = (280nm reading / PrP Extinction Coefficient (1.44 for hamster 90-231)) * Dilution Factor = **X mg/mL** **note: The extinction coefficient for (23-231) rPrP is ~2.6**

   Determine the volume to add to each 100ul reaction to achieve 0.1 mg/mL rPrP

5. Prepare RT-QuIC cocktails as follows:

Example: Hamster (90-231) rPrPsen, 300mM NaCl, (for 16 wells make a 18X cocktail, 98ul aliquot/well, adding 2ul seed); total vol. 1764ul

	1X	18X
MilliQ water	37.5ul	675ul
5X PBS Buffer:	20ul	360ul
100mM EDTA:	1ul	18ul
1mM ThT:	1ul	18ul
2MNaCl:	8.5ul	153ul
$rPrP^{-sen}$	30ul	540ul

-initially add all the cocktail components together except for the rPrP

-vortex

-Gently add the rPrP volume to the cocktail and mix gently through inversion or by gently pipetting up and down

-gently transfer 98ul of cocktail into each well.

-gently add 2ul of the test sample (positive and negative BH dilutions) into the appropriate wells

Note: it is our standard practice to run 4 wells of each sample on each experimental plate (2ul/well). This allows for some statistical analysis. We do find that positive control BHs need to be diluted down 100 to 1000 fold before activity can be detected. This apparent inhibitory effect is likely due to the Triton X-100 that we commonly use for Brain homogenization.

Also the 2ul volume can be adjusted, but the [SDS] from the BH diluent is an important variable. If you add significantly more than 2ul of the diluent, it will cause a higher incidence of spontaneous reaction (spon) formation. We also know that the [SDS] in this 2ul is critical for detecting low levels of activity. Too much is bad, this much is good.

6. Run RT-QuIC as REPORTED previously with DOUBLE ORBITAL SHACKING: 1min shake/1min rest, **42°C**

7. Shaking program: 1min shaking at 700rpm Double Orbital / 1min resting, except for the final 1min for fluorescence measuring.

Fluorescence measurement settings:

- excitation: 450nm, emission: 480nm
- bottom read, number of flashes: 20
- manual gain: 1000, Integration time: 20us

Seal the plate with plate sealer (Nalge Nunc International 265301)

Incubate the plate in BMG Polarstar plate reader for ~ 60hs at **42°C** (Script mode: Rocky Mountain Shake), and measure ThT fluorescence every ~ 15min with a shaking kinetic cycle