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PiggyBac copy number Kit (catalog #PBC100A-1)

The piggyBac transposon system can efficiently integrate transposons into TTAA sites in genomes of transfected cells. To determine the number of piggyBac transposon integrations, SBI has developed a qPCR-based system to measure the copy number of transposons in genomes relative to a genomic counting primer set. The UCR1 primer mix counts the number of genomes and the PBcopy primer set measures the number of piggyBac transposons present in the transgenic genome. PBcopy primer set compatible only with SBI's piggyBac transposon vectors. The copy number of piggyBac transposons is calculated using the cycle threshold (Ct) values of the UCR1 signals relative to the PBcopy signals. The PBC100A-1 copy number kit comes with primers and cell lysis buffer reagents enough for 20 copy number determinations.

Your cells must be passaged at least once before performing this copy number measurement to ensure that residual, non-integrated piggyBac transposon plasmid will not interfere with the qPCR.

Materials provided in kit

- 25x UCR1 primer mix
- 25x PBcopy primer mix
- 1X cell Lysis buffer

Materials required but not included

- 2X SYBR green mastermix
- qPCR instrument
- 1x PBS

A. Genomic DNA preparation from cells in a 12 well culture plate

1. Remove media and wash cells with 1mL, 1x PBS.
2. Remove all of PBS and add 250uL Lysis buffer to each well. You may freeze cells at -80°C until ready. It is recommended to perform one freeze-thaw cycle with the Lysis buffer to ensure complete cellular lysis, then thaw the plate at room temperature.
3. Pipet cells up and down to detach them, and remove cell clumps.
4. Transfer cell lysates in a 1.5ml eppendorf tube.

5. Heat lysates at 95°C, 2 minutes.
6. Centrifuge at 13,000 rpm, 2 minutes in a standard microcentrifuge to pellet debris.
7. Transfer supernatant to a new tube and place tubes on ice or store at -20°C until ready to proceed.
8.

B. qPCR set-up for copy number quantitation, 384 well plate

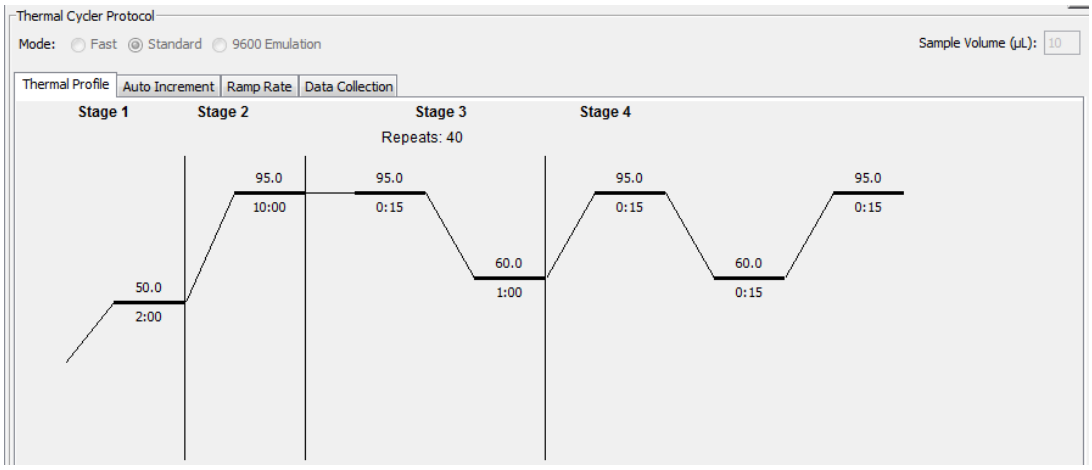
per reaction for PB detection

water	4.75 uL
2x SYBR Green	6.25 uL
PB copy primer mix (5uM)	0.5 uL
lysate (≤500ng DNA)	0.5 uL

per reaction for genomic DNA detection

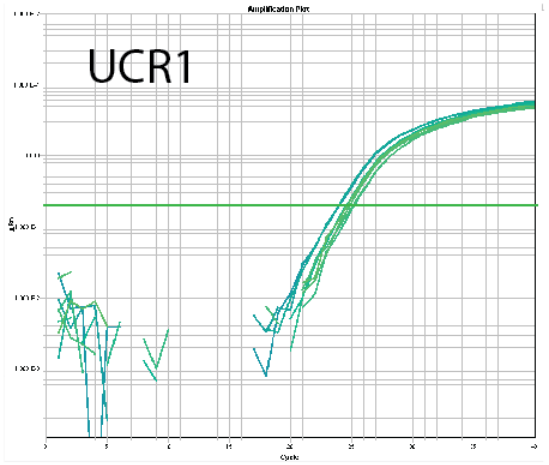
water	4.75 uL
2x SYBR Green	6.25 uL
25x UCR1 primer mix	0.5 uL
lysate (≤500ng DNA)	0.5 uL

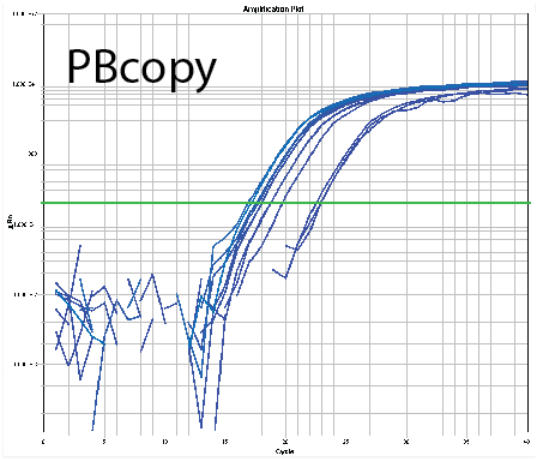
1. Prepare 2 mastermixes of water, 2x SYBR green, and primers (one for UCR1 and one for PBcopy primers) for all samples including duplicates. (e.g. double all reaction ingredients and multiply by the number of samples).
2. Aliquot master mixes in each well in 96-well plate
3. Add 1uL of lysate, and mix by pipetting up and down.
4. Transfer 10uL of lysate/SYBR mix into duplicate wells in a 384-well plate.
5. Place seal cover and briefly spin to collect all the liquid.
6. Standard qPCR program:

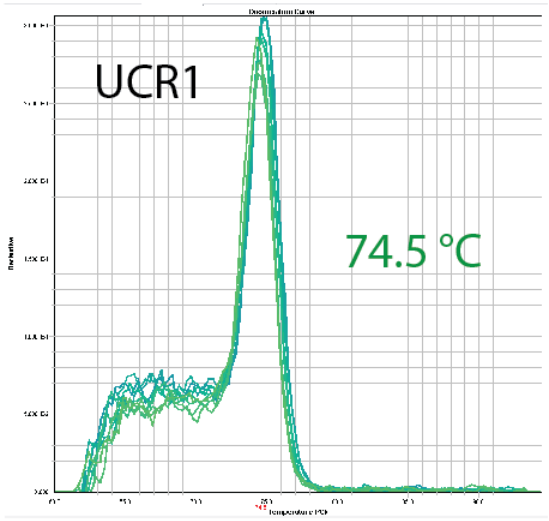


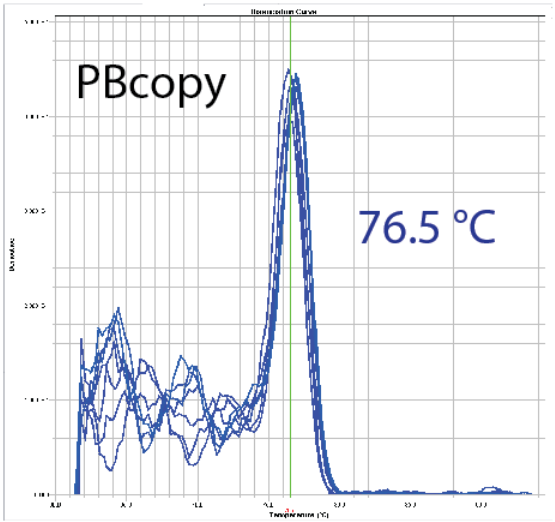
C. Sample Data

Expected amplification curves and dissociation analysis









PB titration examples and copy numbers

Ratios of PB transposon to PB transposase can be done to adjust the copy number of integrations. First, average the Ct values for the UCR1 and PBcopy measurements. The Delta-delta (ΔΔ) Ct calculation = 2^-(avg PBcopy Ct - avg UCR1 Ct)

The copy number is determined by dividing the ΔΔCt by 2, as there are 2 copies of the UCR sequence per genome.

Transposon (ng)	Transposase (ng)	Pbcopy avg Ct	UCR1 avg Ct	ΔΔCt: 2^-(Pbcopy-UCR1)	copy# (ΔΔCt/2)
100	100	23.82	28.43	24.47	12
300	100	19.88	24.74	29.00	15
500	100	19.05	24.69	49.76	25
700	100	18.77	24.97	73.29	37
1000	100	18.15	24.49	80.92	40