



# signal-joint TCR Receptor Excision Circles (sjTREC) quantification

## DNA quantification

### Materials Required:

Quantifiler® Human DNA Quantification Kit (Thermo Fisher Scientific, Waltham, MA) \*  
Real-Time PCR Instrument \*  
Tris-EDTA (TE) buffer, pH 8.0 \*

## Construction of sjTREC-cloned plasmid as a positive control

### Materials Required:

TOPO® TA Cloning Kit including pCR2.1-TOPO vector (Thermo Fisher Scientific)\*  
HiYield Plasmid Mini Kit (RBC Bioscience, Taipei, Taiwan)\*

### sjTREC fragment sequence(131bp)

```
CCATGCTGAC ACCTCTGGTT TTTGTAAAGG TGCCCACTCC TGTGCACGGT GATGCATAGG CACCTGCACC  
CCGTGCCTAA ACCCTGCAGC TGGCACGGGC CCTGTCTGCT CTTCATTAC CGTTCTCAG A
```

### For positive control in each reaction:

- Apply 0.1 pg of plasmid in each reaction  
0.1 pg of plasmid corresponds to 22,727 copies,

calculated as:

$\text{copy} = (\text{ng} \times \text{copy/mole}) / (\text{bp} \times \text{ng/g} \times \text{g/mole of bp})$

### For establishment of the standard curve:

- Dilute the plasmid containing sjTREC sequence serially in 10-fold  
(Recommendation: Dilution sjTREC plasmid ranging from 10<sup>7</sup> to 10<sup>1</sup> copies)

- Generated a standard curve equation calculating the amount of copy from the obtained Ct value -(Eq.)

\* Follow the manufacturer's protocol



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## TaqMan real-time PCR assay

### Materials Required:

TaqMan® Universal PCR Master Mix (Thermo Fisher Scientific) \*  
TaqMan TAMRA probe \*  
Forward and Reverse primer\*

### Primers and Probe :

Target ID	Sequence (5'→3')	Reference
forward primer	TGCTGACACCTCTGGTTTTTGTA	[1]
reverse primer	GTGCCAGCTGCAGGGTTTAG	
probe	(FAM)-CACGGTGATGCATAGGCACCTGC-(TAMRA)	[2]

### SBE Reaction Mixture:

Reaction Component	Vol. (ul)	Final
2X TaqMan Universal Master Mix II	5	1 X
Forward primer (50 µM)	0.16	250 nM
Reverse primer (50 µM)	0.16	250 nM
TaqMan probe (10 µM)	0.25	800 nM
template DNA	( )*	50 ng
distilled water	up to 10	
<b>Total</b>	<b>10</b>	

\*depends on concentration of DNA sample

### Thermal Cycling:

50°C for 2 minutes  
95°C for 10 minutes  
  
95°C for 15 seconds  
60°C for 1 minutes  
for 45 cycles

[1] Gerste-Thompson JL et al. 2010. Clin Chem 56(9):1466-74.  
[2] Zubakov D et al. 2010. Curr Biol 20(22):R970-1.



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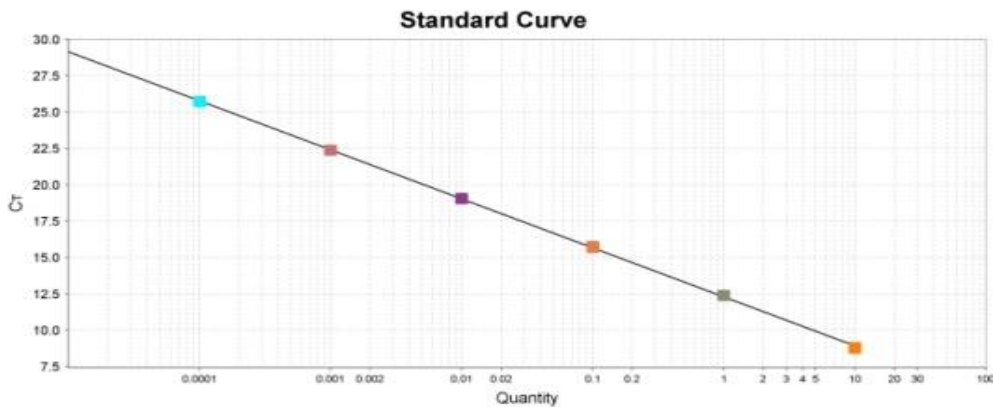
## Data Analysis

### Materials Required:

IBM SPSS Statistics

Microsoft Excel

1. Convert the obtained Ct value to copy number using the equation (Eq.)



2. Calculate  $\text{Log}[\text{sjTREC content per } 1 \mu\text{g of DNA}]$  using the copy number calculated in 1.

3. Calculate the predicted age by applying the sjTREC content into the age estimation model [3]

