Codon bias

ATG AGG TTA AAT AGT CCC AGA CCG
Fast
Slow

Synonymous substitution

Match
<table>
<thead>
<tr>
<th>Codon</th>
<th>Amino Acid</th>
<th>Amount in A</th>
<th>Rank in A</th>
<th>Amount in B</th>
<th>Rank in B</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAU</td>
<td>H</td>
<td>0.3</td>
<td>2</td>
<td>0.8</td>
<td>1</td>
</tr>
<tr>
<td>CAC</td>
<td>H</td>
<td>0.7</td>
<td>1</td>
<td>0.2</td>
<td>2</td>
</tr>
</tbody>
</table>

E(CAU): 5*0.8=4; A(CAU)=3; D(CAU)=1
E(CAC): 5*0.2=1; A(CAC)=2; D(CAC)=-1

**Fast**

**Input Sequence**

CAU CAU CAU CAU CAU

CAU CAU CAC CAC CAU

CAC CAC CAC CAC CAC

**Slow**

**Synonymous Substitution**

CAC CAC CAU CAU CAC

**Match**

CAU CAU CAU CAC CAU

CAC CAC CAU CAU CAC
Transition 1→2: Binding of a fresh tRNA-EF-Tu complex to site A;
\[ \frac{dn}{dt} = \omega_{h2} P(N)Q(N) + \omega_{p} \left( \prod_{s=1}^{i} (1 - p(s)) \right) + \omega_{p} P_{i}(1) \]

Transition 2→3: GTP part of EF-Tu hydrolized to GDP;
\[ \frac{dn}{dt} = \omega_{h2} P_{i}(i-1)Q(i-1) + \omega_{p} P_{i}(i) - \omega_{p} P_{i}(i) \]

Transition 3→4: Phosphate group, a product of the hydrolysis, leaves, and releases the EF-Tu;
\[ \frac{dp}{dt} = \omega_{p} P_{i}(i) - k_{2} P_{i}(i) \]

Transition 4→5: Shift of tRNA, and site A occupied by EF-G in the GTP bound form;
\[ \frac{dp}{dt} = k_{2} P_{i}(i) - k_{2} P_{i}(i) \]

Transition 5→6: Hydrolysis of GTP to GDP and release;
\[ \frac{dp}{dt} = k_{2} P_{i}(i) - k_{2} P_{i}(i) \]

Transition 6→7→: Shift of ribosome.
Significance
Measeuring RiPs

\[
\lim_{\Delta t \to \infty} \text{RiPs}(t, \Delta t) = \frac{P(t + \Delta t) - P(t)}{(R(t + \Delta t) + R(t))/2}
\]

\[
\text{RiPs}(t) = \frac{dP}{dt} \frac{1}{R(t)}
\]
Protocol

1. Inoculation
   - Inoculate E.coli with PSB1A2 (BBa_J04450 inserted)
   - Get medium from LB every 15mins
   - Centrifugation and Wash the sediment

2. Target RNA extraction and measurement
   - Extract total RNA with Takara Trizol extraction protocol
   - Use 1ug RNA for reverse transcription
   - Real-time PCR

3. Target protein extraction and measurement
   - Suspend the sediment and lysate
   - Heat in 100
   - SDS-PAGE with standard curve to quantify
averageRiPs(\Delta t) = \left( \int_{t_0}^{t_0+\Delta t} \frac{dP}{R(t)} \right) / \Delta t
\( \text{RiPs}(t) = \frac{dP}{dt} \frac{1}{R(t)} \)

- OD value
- temperature
- pH
- RBS strength
- medium
- copy number of plasmid
- ect.