

Estimation of clones numbers needed for Library

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Estimation of number of clones needed for Library

Clark & Carbon equation 1976

$$N = \frac{\ln(1-P)}{\ln(I-n)}$$

حيث إن :

$N =$ عدد الكلونات التي يجب اختيارها لتمثيل معظم مورثات المجين في المكتبة .

$n =$ نسبة حجم قطع الحامض النووي المهندسة وراثياً الى مجين الكائن .

$p =$ احتمالية وجود أي مورث في المكتبة .

Example: ✖

Library size needed for mouse genomic Library: ✖

- size of DNA fragments 20 Kb ✖
- Mice genome 30000000000 pb ✖
- Probability (P) 95% ✖

$$\text{Ln} (1-P) \quad 20 \text{ ✖}$$

$$N = \frac{\text{Ln} (1-n)}{\text{Ln} (1-P)} = \frac{30000000000 \text{ ✖}}{20 \text{ ✖}} = 1.4 \times 10^{10} \text{ ✖}$$

$$\text{Ln} (1- 0.95) \text{ ✖}$$

$$N = \frac{\text{Ln} (1- 1.4 \times 10^{10}) \text{ ✖}}{\text{Ln} (1- 0.95) \text{ ✖}} = 4.2 \times 10^{10} \text{ Cells ✖}$$

How can you calculate your library size? ✖

After transformation assay: ✖

1. Make 10^{-1} ; 10^{-2} ,..... 10^{-10} dilutions by adding 100 ul from the library to 900 ul from LB or agar broth to have the dilution 10^{-1} then make the rest dilutions from this stock.
2. Chose the dilutions from 10^{-7} to 10^{-10} . ✖
3. Take 100 ul from each dilution and plate it on LB or Agar ✖
plate – 3 plates each-.
4. Incubate plate at 37.5C for 24 hrs. ✖
5. Copy all plates colonies and re-plate them on AMP LB or ✖
Agar plates.
6. Incubate the plates at 37.5C for 24 hrs. ✖
7. Calculate the AMP resist colonies, compare these colonies ✖
with those in steps 4-5.
8. The number of AMP sensitive colonies will refer to positive ✖
clones = Library size

| | |
|---------------------------------|-----------------|
| <i>Haemophilus influenzae</i> | 1,830,135 × |
| <i>Helicobacter pylori</i> | 1,667,867 × |
| <i>Bacillus subtilis</i> | 4,214,814 × |
| <i>Mycoplasma genitalium</i> | 580,073 × |
| <i>Archaeoglobus fulgidus</i> | 2,178,400 × |
| Eukaryotes × | |
| <i>Saccharomyces cerevisiae</i> | 12,069,313 × |
| <i>Caenorhabditis elegans</i> | 97,000,000 × |
| <i>Drosophila melanogaster</i> | 180,000,000 × |
| <i>Arabidopsis thaliana</i> | 115,500,000 × |
| <i>Homo sapiens</i> | 3,200,000,000 × |
| <i>Mus domesticus</i> | 3,000,000,000 × |

Thank you a lot for listening

