Generation of cDNA expression libraries enriched for in-frame sequences

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Objectives

Goal: Generate cDNA libraries containing high percentage of open, in-frame clones

- Non directional cloning: single restriction enzyme used to digest DNA insert and vector.

  -Results in around 8% correct protein sequences (Claytus et al., 1996)

- Directional cloning: two different restriction enzymes allows DNA insert to ligate to vector in specific orientation.

  -Correct protein sequences increases by factor of 2. (Claytus et al.,1996)

Approaches

- Directional cloning of oligo(dT)-primed cDNA fragments

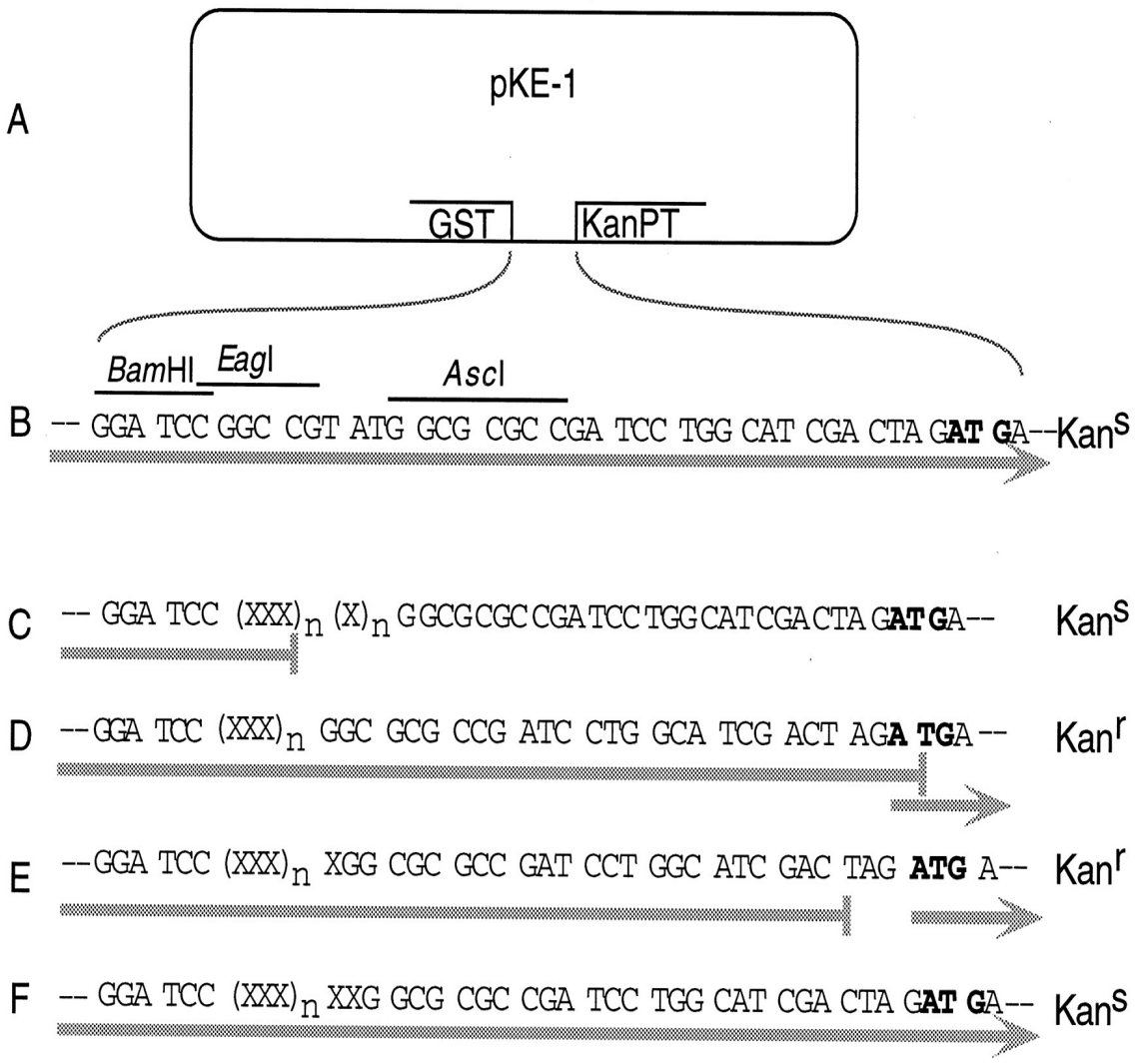
  -only forward reading frames expressed
- pORF vector

  -out of frame B-galactosidase coding sequence
- pFLAG-Shift$_{12}$ and IBI vectors

  -contain cloned sequences in all three reading frames

"However, there is no control over which frame of the cDNA is translated, because translation of the cDNA must be initiated on vector sequence." (Claytus et al., 1996)

pKE-1



A. Modified pGEX-2T vector containing kanamycin phosphotransferase coding region

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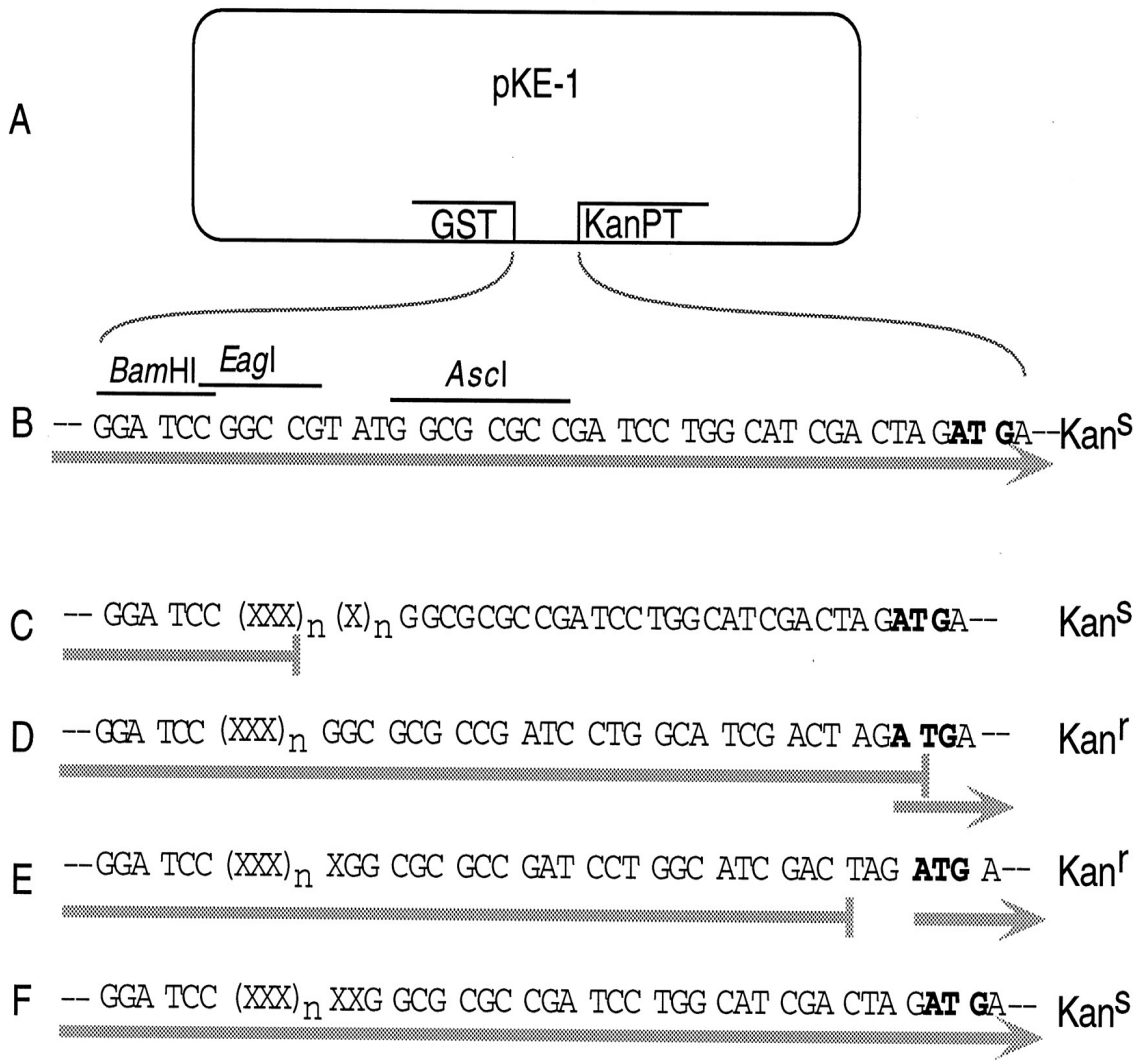
pKE-1



B. No inserted DNA. KanPT translated in wrong reading frame

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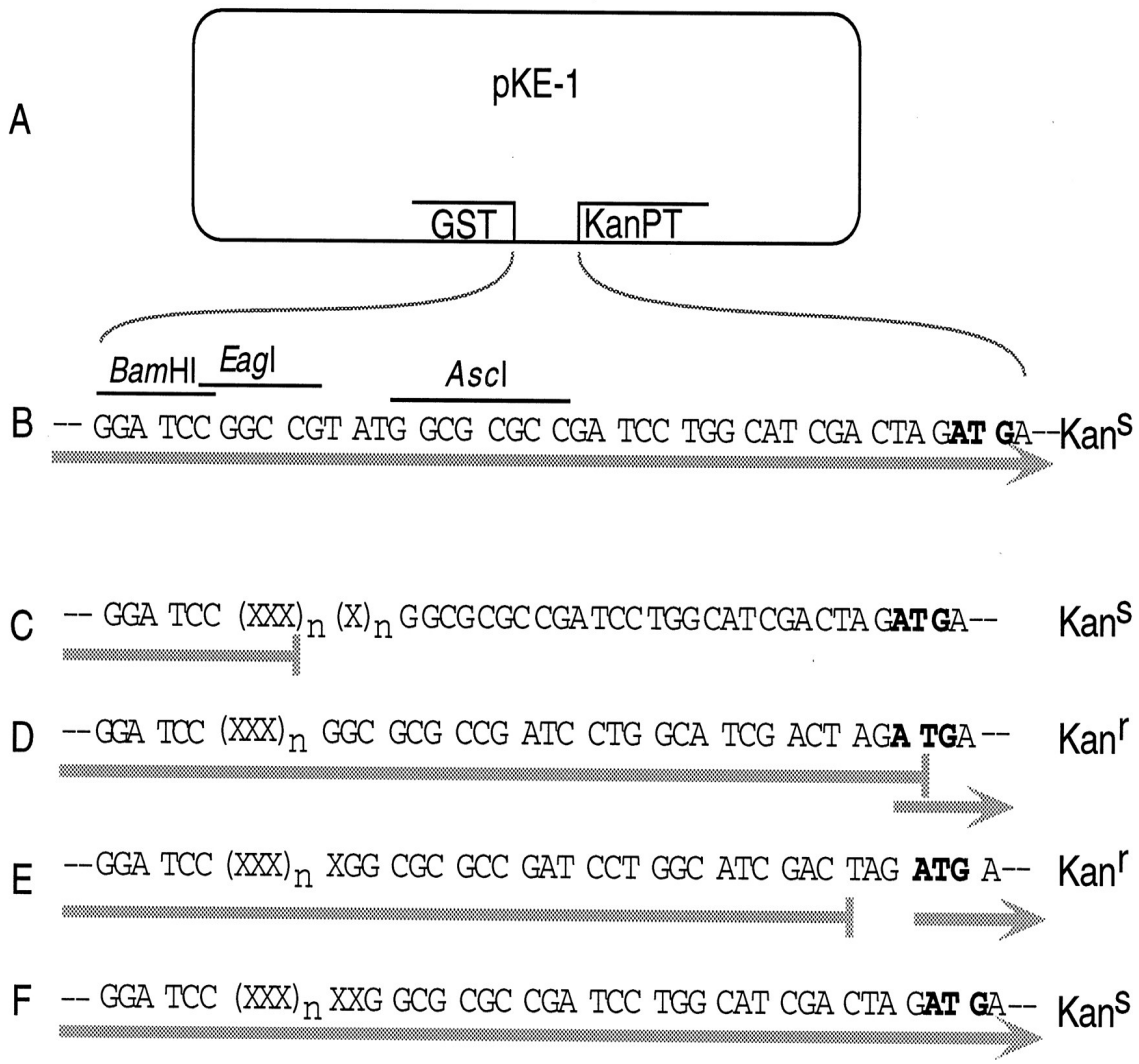
pKE-1



C. Insert contains in frame stop codon.

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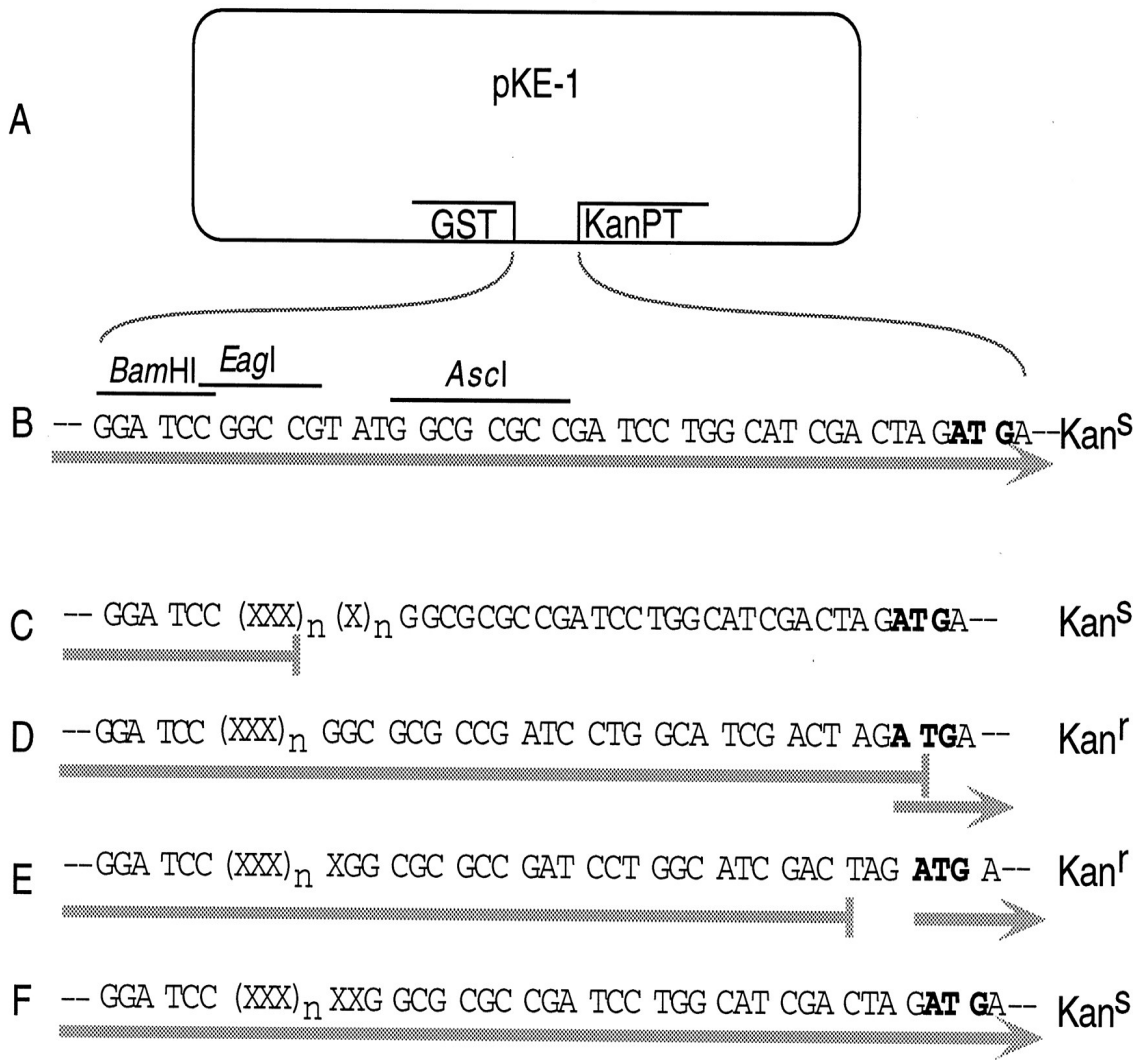
pKE-1



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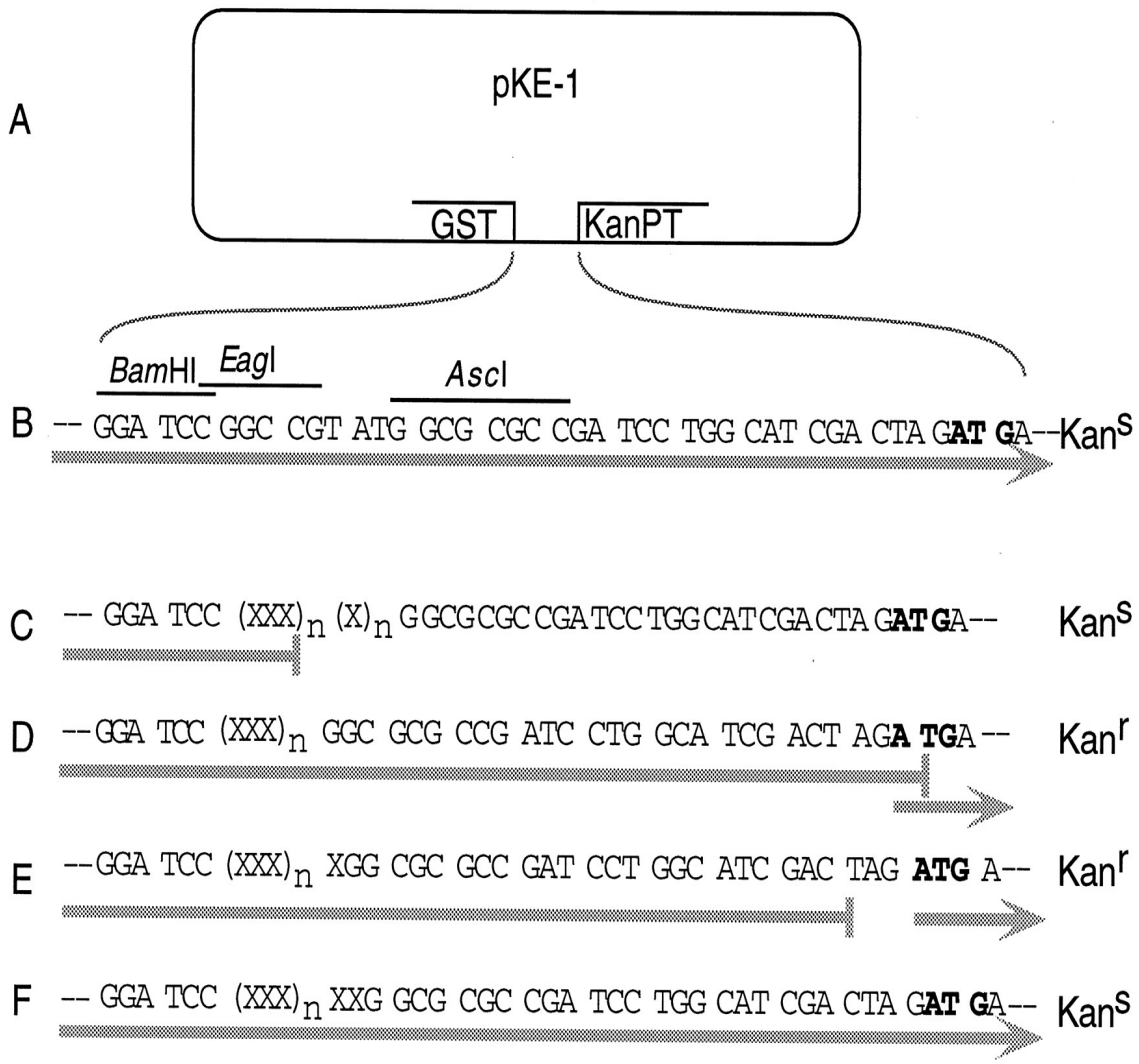
D. Insert length is in multiples of three. Translation reinitiates on overlapping KanPT start codon.

pKE-1



E. Insert length is in multiples of three, plus one.

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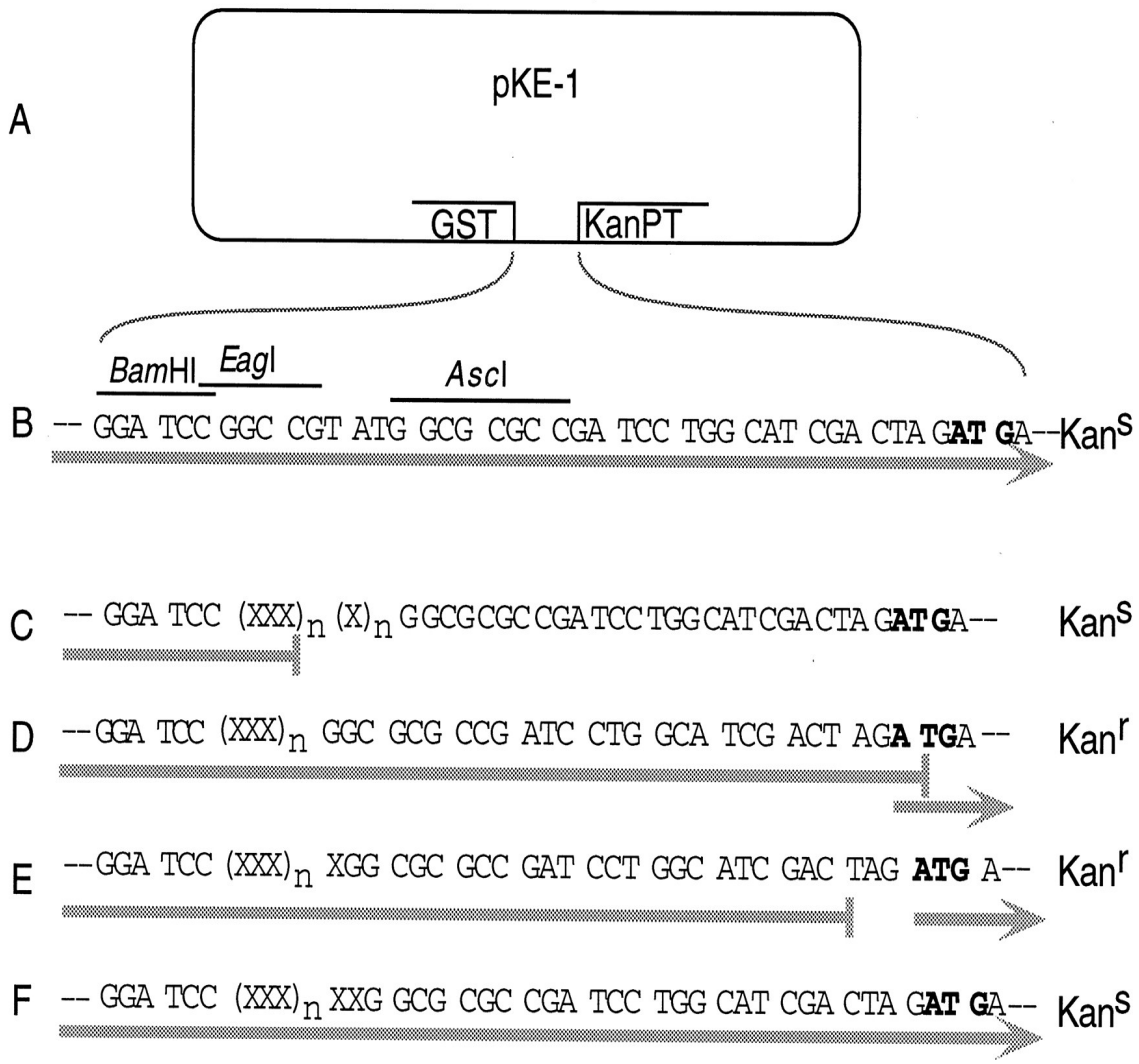
pKE-1



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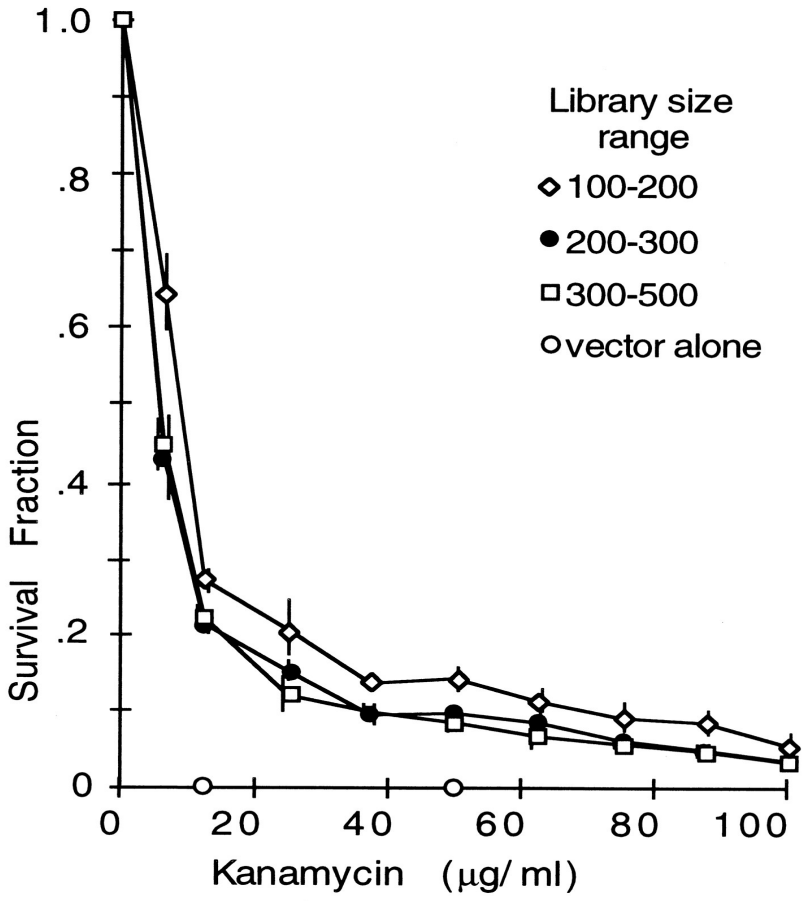
F. Insert length is in multiples of three, plus two. KanPT translated in wrong reading frame.

Response to Kanamycin Selection



- Libraries only containing pKE-1 had a very low survival fraction ($2x10^{-4}$ at 12.5μg/mL)
- Libraries containing cDNA inserts resulted in kanamycin resistant colonies

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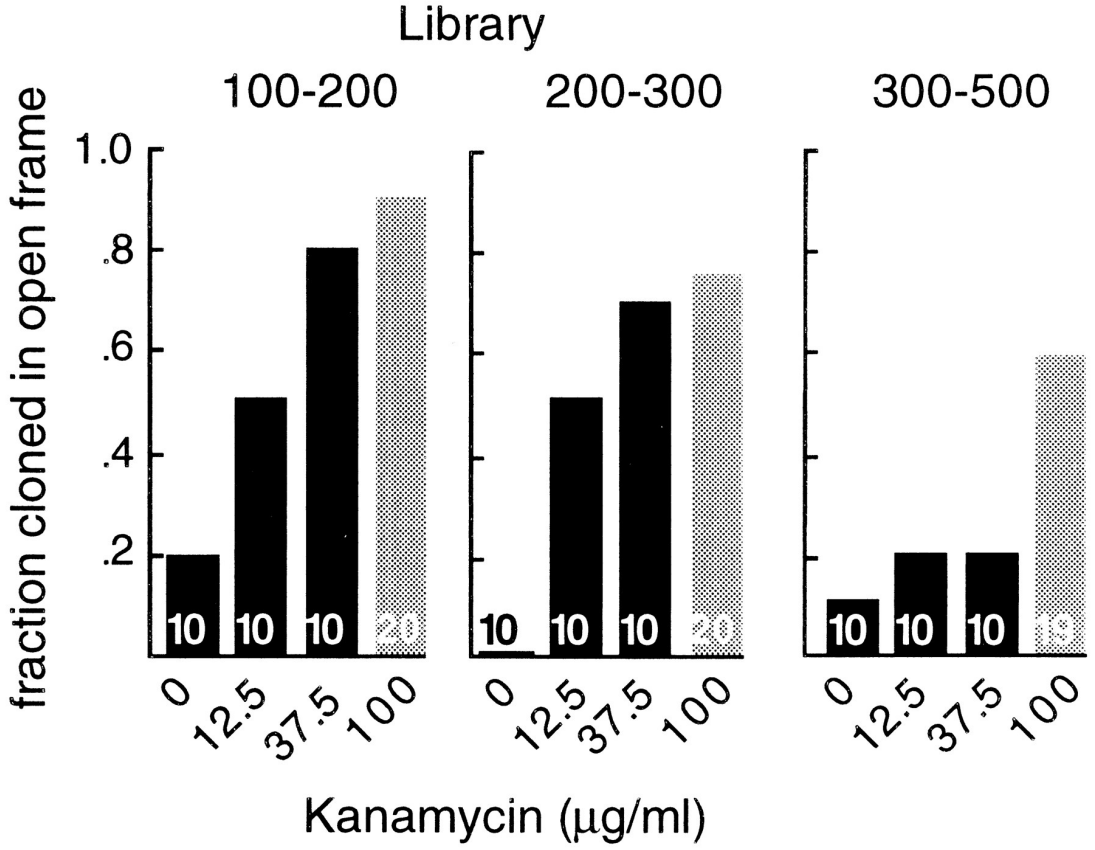
Survival of sized cDNA libraries after selection with kanamycin.

Selection for Cloning in ORFs

Fraction of sequences cloned in ORFs. Clones were picked at random from the different sized cDNA libraries that had been subjected to selection with 0, 12.5, 37.5, or 100 µg/ml kanamycin.



- Selection enriched for smaller sized sequences cloned in ORFs.
- *E. coli* XL1-Blue had higher fraction of clones in open frame compared to *E. coli* DH10B

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Selection for Cloning in the Correct Reading Frame

- Using calculations, an expected frequency of open but **incorrect** reading frames of 100 to 200 bp of randomly selected cDNA sequences is around 15% and less than 5% for larger sequences. (Claytus et al., 1996)

- Kanamycin selection resulted in ≈2-fold increase of **correct** reading frame sequences. (Claytus et al, 1996)

Findings

- Creation of pKE-1 vector
- Developed new methodology using kanamycin selection for making cDNA libraries that contain 60-80% open, in frame clones.
- Sequences cloned in ORF is dependent sizes of cDNA
- E. coli XL1-Blue yielded a higher amount of cloned correct reading frame sequences compared to DH10B.

(Claytus et al.,1996)

Major Implications

- Genome projects
- cDNA expression library screens using antibody or ligand binding
- Generating authentic cDNA encoded protiens

(Claytus et al,1996)

Future Research

"Using the vector in a host optimized for the translation of eukarotic sequences could possibly improve the selection efficiency for larger size inserts."

(Claytus et al.,1996)

Methods

A Human cDNA Expression Library in Yeast Enriched for Open Reading Frames

Caterina Holz,[1] Angelika Lueking,[2] Lara Bovekamp,[2] Claudia Gutjahr,[2] Natalia Bolotina,[1] Hans Lehrach,[2] and Dolores J. Cahill[2,4]

[1]Technical University Berlin, Institute for Biotechnology, D-13355 Berlin, Germany; [2]Max Planck Institute of Molecular Genetics, D-14195 Berlin, Germany; [3]PROTAGEN, D-44801 Bochum, Germany

-Increased clone inserts in correct reading frame from 14% to 60%

-Used the yeast *Saccharomyces cerevisiae* as host

-Used inserts 200-3000bp long

(Holz et al.,2001)

Sources

1. Davis, Claytus A. and Seymour Benzer. “Generation of cDNA expression libraries enriched for in-frame sequences.” *The National Academy of Sciences of the USA, vol. 94, 1997, pp. 2128-2132.*

2. Holz, Caterina. Lueking, Angelika. Bovekamp, Lara. Gutjahr, Claudia. Bolotina, Natalia. Lehrach, Hans. Cahill, Dolores J. “A Human cDNA Expression Library in Yeast Enriched for Open Reading Frames.” *Genome Res.* 2001 11: 1730-1735.