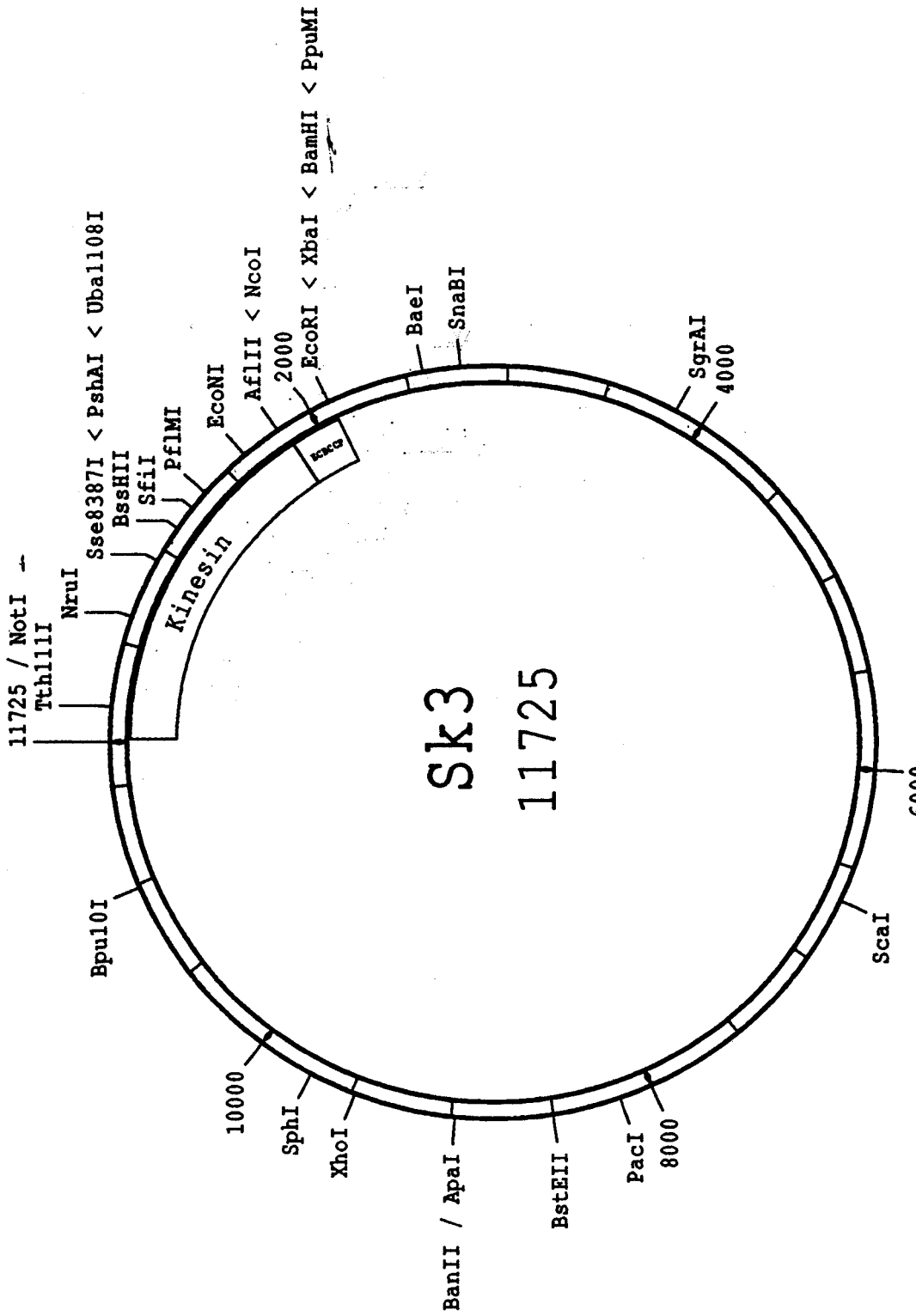


Plasmid Information

Plasmid name	pSK3
Contents (What are the segments that the plasmid contains?)	<p>Dras. Kinesin H.C. 1-612</p> <p>E.C. BCCP (last 82)</p> <p>baculovirus promoter</p>
Purpose (Why was it made? What is it to be used for?)	<p>Baculovirus expression</p> <p>R612-BIO</p>
Source (List all DNA segments from which plasmid was assembled, or person/company obtained from and date)	<p>pSK1 2123 bp Not I / Bam HI</p> <p>pVL1392 9602 bp Not I / Bam HI</p>
Lab notebook reference (Number and page of lab notebook that describes plasmid assembly or first use)	<p>See Berliner et al</p> <p>JBC <u>269</u> 8610-8615 (1994)</p>
Plasmid prepared or obtained (Date? By whom?)	
Vector (Source of plasmid origin of replication)	pVL1392
Antibiotic resistance genes	amp ^R
Bacterial strain used for plasmid preparation	JC10289
Computer file and directory	[gellies] SK3. seq
This sheet filled out (Date? By whom?)	JG 6/24/94



ASSEMBLE September 10, 1991 09:46

Symbols: 1 to: 2123 from: sk1.seg ck: 2955, 2243 to: 4365

ASSEMBLE July 9, 1990 16:29

PLASMIDMAP of: Sk3.Seq check: 6478 from: 1 to: 11725

Mahtani September 10, 1991 10:18

MATERIALS AND METHODS

Plasmids—We prepared plasmids for expressing K612-BIO in *E. coli* and in the baculovirus expression vector system by sub-cloning a fragment of the *Drosophila* kinesin α subunit cDNA (clone 1, described by Yang *et al.* (Ref. 19), gift of L. S. B. Goldstein, Harvard University) and a fragment of the *E. coli* BCCP sequence from plasmid pCY142. The DNA sequence in the pCY142 fragment is derived from the *E. coli* chromosomal *KpnI/PstI* fragment encoding BCCP residues 70–133 (20) and a synthetic oligonucleotide encoding amino acids 134–156. To construct the bacterial expression plasmid pSK4, we ligated the 1830-bp *KspI/NcoI* fragment from clone 1, the synthetic oligonucleotide 5'-AGCTTGCGGC-CGCATATGTCCGC-3'/3'-ACGCCGGCGTATACAGG-5', and the 2370-bp *HindIII/NcoI* fragment from pCY142 to produce an intermediate plasmid, pSK2. The 2123-bp *NdeI/SalI* fragment of this intermediate was subcloned into the polylinker of the pT7-7 expression vector (Ref. 21, gift of S. Tabor, Harvard Medical School) to give pSK4. To construct the baculovirus expression plasmid pSK3, we ligated the 1830-bp *KspI/NcoI* fragment from clone 1, the synthetic oligonucleotide 5'-AGCTTGCGGCC-CGCATGTCCGC-3'/3'-ACGCCGGCGGTACAGG-5', and the 2370-bp *HindIII/NcoI* fragment of pCY142 to produce an intermediate plasmid, pSK1. The 2123-bp *NotI/BamHI* fragment of the intermediate was isolated and ligated to the 9602-bp *NotI/BamHI* fragment of pVL1392 (Invitrogen) to give pSK3. The validity of all plasmid constructs was tested by restriction mapping using the overlapping restriction sites in the synthetic oligonucleotides.