Rajeev,
Here is the processivity protocol.
please place copy in protocol book
for lab. Tks, Ede

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In vitro Processivity Assay

1. end-label $d(T)_{16}$ with [γ-32P]-ATP (X 2 tubes)
   - 30μl oligo $d(T)_{16}$ (0.1μg/μl stock, 3μg)
     5μl 10X T4 PNK buffer
     10μl [γ-32P]-ATP (3000Ci/mmol, 10mCi/ml)
     1μl 10U/μl T4 PNK
     4μl ddH_2O
   - 50μl total

T32-C221
Micro Bio-Spin 6 Columns

2. incubate at 37^0C for 30min
3. stop reaction by adding 2μl 0.5M EDTA
4. transfer 52μl into a Bio-Rad P-6 micro spin-column, cf 4min
5. pool 2 flow-through together, store at -20^0C
6. mix 25μl poly(dA) (25μg) with 50μl labeled oligo$d(T)_{16}$ 75
7. heat at 70^0C, 5min, xfer to RT, cool for 1/2hr
8. pass annealed poly(dA) template thru 2 P-30 micro spin-columns (aliquot template into 2 columns so that centrifuge is balanced)
9. set up TNT samples and incubate on ice for 30min

sample

Pol Assay Soln.

	stock	(10X) 41 μl
100mM $(NH_4)_2SO_4$	2M	12.5μl
20mM Tris-HCl (pH7.5)	1M	5
3mM $MgCl_2$	100mM	7.5
0.1mM EDTA	10mM	2.5
0.5mM DTT	50mM	2.5
4% glycerol	100% 50%	10 20
40μg/ml BSA	10μg/μl	1μl
		51 μl (5X)

10. add 3μl poly(dA):oligo(dT) and incubate for 10min at RT
11. add 15μl Pol Assay Soln. (4.1μl) + 1mM dTTP(2.5μl) (final conc.=100μM) + ddH_2O (8.4μl)
12. incubate for 1hr, 37^0C

13. add 25µl ddH_2O and 50µl phenol:chloroform:isoamylalcohol (25:24:1), mix, cf, xfer aqueous phase to new tube
14. extract with 50µl chloroform:isoamylalcohol (24:1)
15. add 20µl urea-PAGE loading dye
16. pre-run 15% Urea-PAGE at 300V for 30min
17. transfer 10µl sample into new tubes
18. flush wells with 1X TBE to get rid of urea
19. while flushing the wells, heat sample (10µl) at 98^0C for 5min, immediately load onto gel
20. resolve samples in 15% Urea-PAGE at 300V for 2hrs and 45min (regular size) or at 500V for 8hrs (long gel)

<u>15% Urea-PAGE (regular size)</u>

	40ml
40% acry/bis (19:1)	15ml
7M urea	16.8g
10X TBE	4ml
ddH_2O	7.77ml
10% APS	300µl
TEMED	30µl

- dissolve urea in acry/bis, TBE, and ddH_2O at 42^0C
- cool gel soln at RT for 0.5 hr, add TEMED and APS last
- gel starts polymerizing after 5min, and let gel set for at least 15min

<u>15% Urea-PAGE (61 cm long)</u>

	80ml
40% acry/bis (19:1)	30ml
7M urea	33.6g
10X TBE	8ml
ddH_2O	15.65ml
10% APS	500µl
TEMED	50µl

- dissolve urea in acry/bis, TBE, and ddH_2O at 42^0C
- cool gel soln at RT for 0.5 hr, add TEMED and APS last
- pour gel soln. with 25ml pipet in 5min, gel starts polymerizing after 5min
- let gel set for at least 15min

- if not dry gel, put gel on filter paper and wrap gel with saran wrap
- bands start diffusing after 1 day if gel is not dried
- for long gel, expose overnight with blue film (Fuji film)
- for regular-size gel, expose for 2hrs with Kodak X-omat

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denaturing PAGE loading dye (10ml)

9ml deionized formamide
2ml 0.5M EDTA (pH8) (final=10mM)
0.1g bromophenol blue (0.1% final)
0.1g xylene cyanol (0.1% final)

10X TBE

890mM Tris base	108g
890mM boric acid	55g
20mM EDTA (stock=0.5M)	40ml
add ddH2O to 1L	

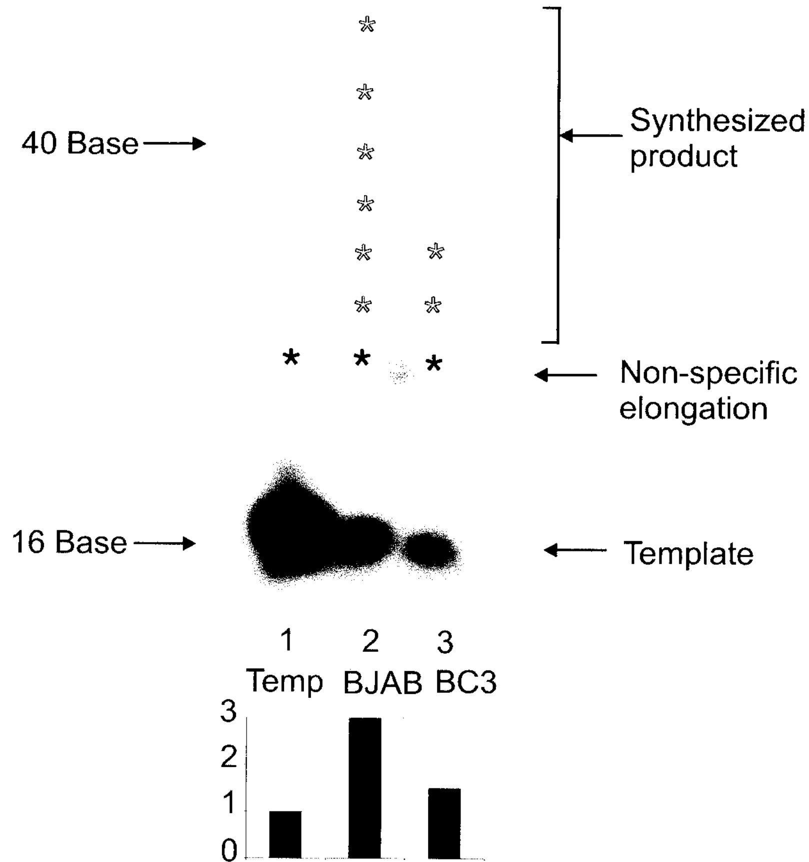

Fig.: Processivity assay showing the efficiency of replication machineryof BC3 and BJAB cells. The nuclear extracts from these cells were incubated with poly(dA):oligo$(dT)_{16}$ for 30 minutes (lanes 2 and 3 respectively). DNA products were fractioned by 7M urea-15% PAGE. Poly(dA):oligo$(dT)_{16}$ template was electrophoresed in lane 1 to show the size of primers (16 base). Nonspecific DNA products are labelled by closed asterik symbol and specific are marked by open asterik symbol.