

# Antigen Receptor Gene Rearrangement Studies by PCR in Lymphoma Diagnosis

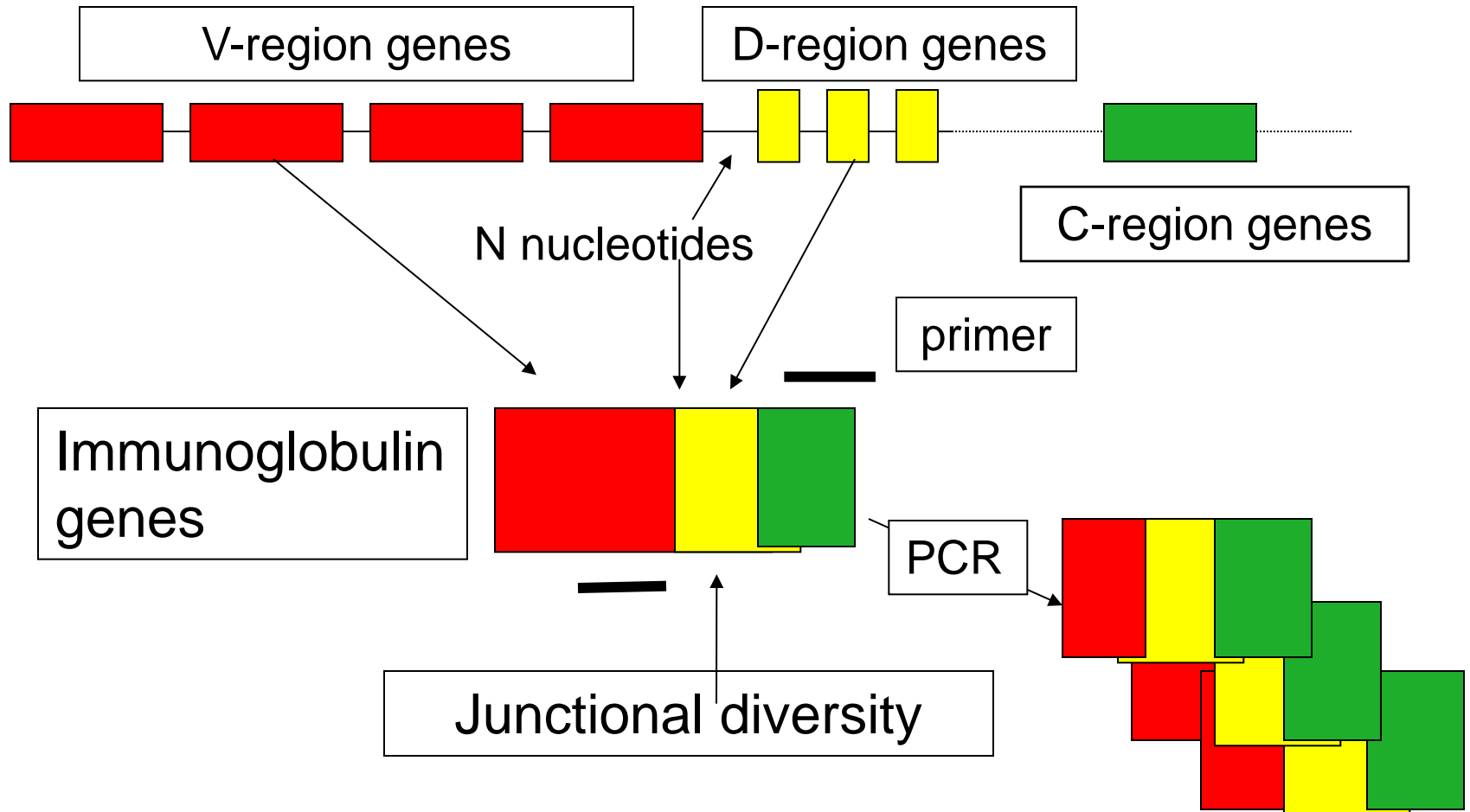
Bridget S Wilkins

BLPG/BDIAP Meeting May 2014

# Biomed-2 Concerted Action

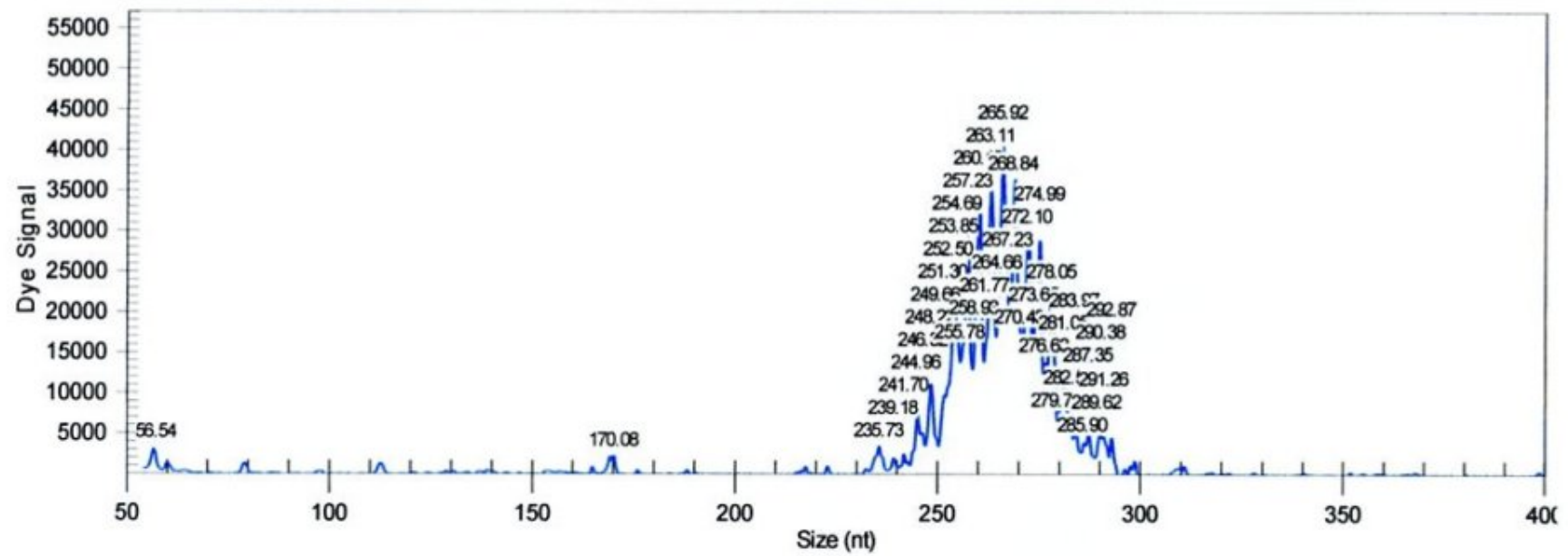
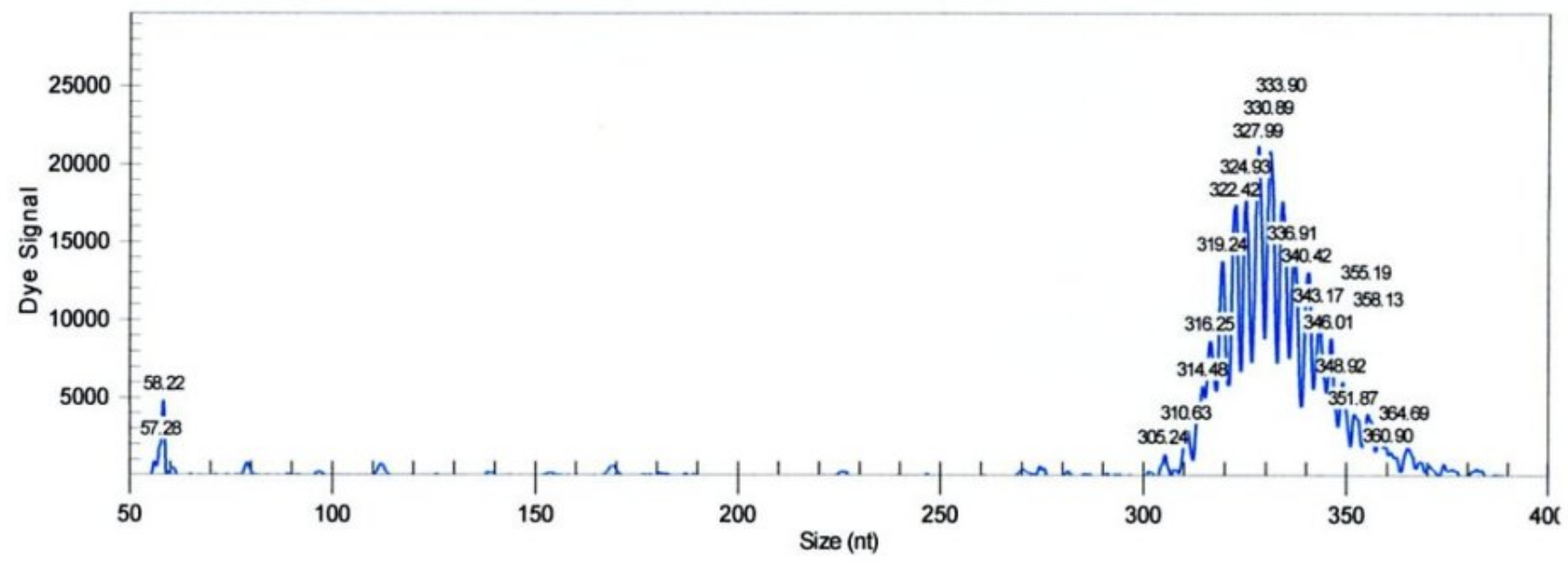
- 1998-2002; major European collaboration to optimise PCR to detect immunoglobulin (heavy and light chains) and T-cell receptor gene rearrangements
- Primer design and protocol optimisation
- Use in FFPE tissue was one (small) workstream
- Provided an international gold standard of immense practical utility

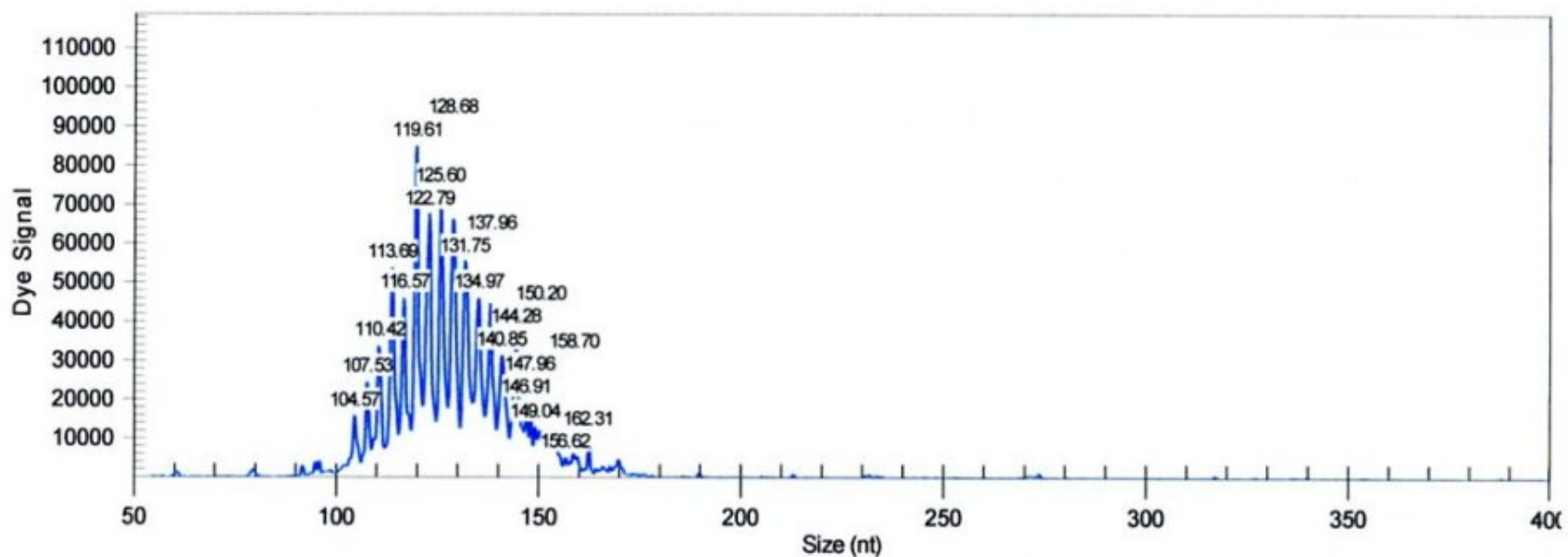
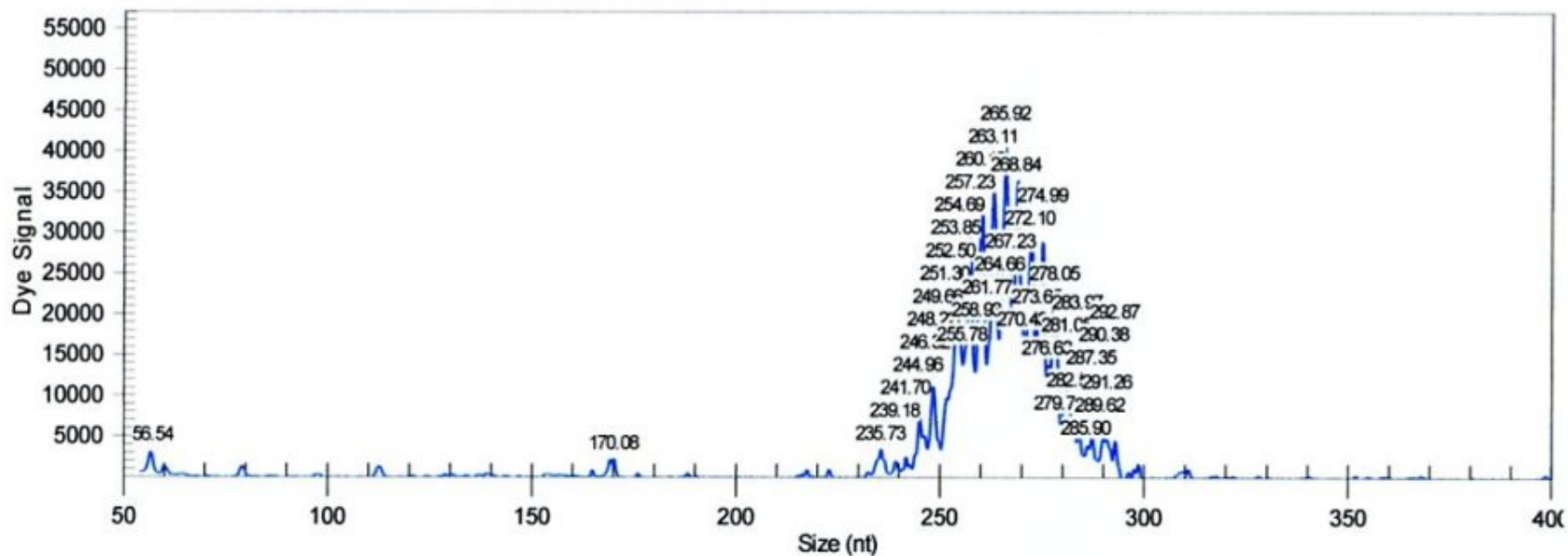
# Model of Antigen Receptor Gene Diversity Generation and PCR



# IGH Primers

- FR1 ( $V_H1-J_H$ ):
  - products 310-360 nt
- FR2 ( $V_H2-J_H$ )
  - products 250-295 nt
- FR3 ( $V_H3-J_H$ )
  - Products 100-170 nt





# IGK and L Primers

- IGK (KA)  $V_K$ - $J_K$ : 8 primer combinations
  - 120-160 nt
  - 190-210 nt
  - 260-300 nt
- IGK (KB)  $V_K$ - $K_{de}$ : 8 primer combinations
  - 120-160 nt
  - 190-210 nt
  - 260-300 nt
- IGL  $V_L$ - $J_L$ : 2 primer combinations
  - 140-165 nt

# TCR-Beta Primers

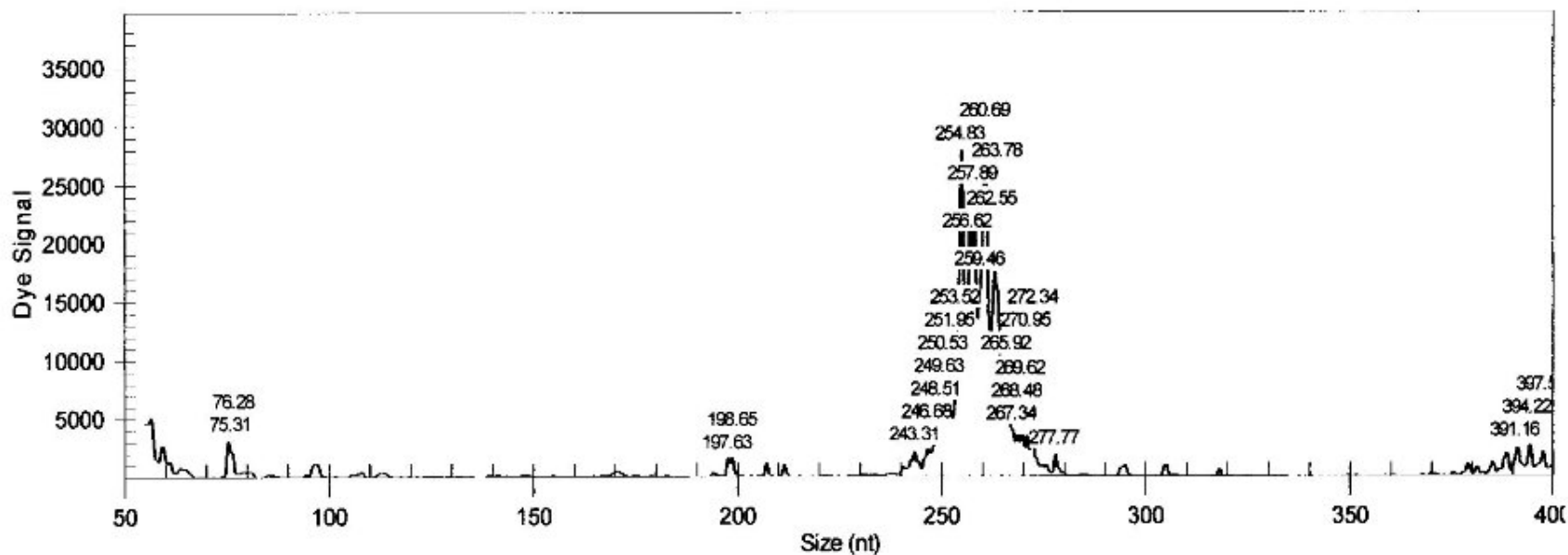
- Two  $V_B$ - $J_B$  multiplexes:
  - BA (15 combinations) gives products 254-286 nt
  - (BB: 11 combinations) gives products 258-293 nt
- $D_B$ - $J_B$  multiplex (run in 1 or 2 tubes)
  - BC1 (8 combinations)
    - 304-320 nt
  - BC2 (3 combinations)
    - 182-191 nt



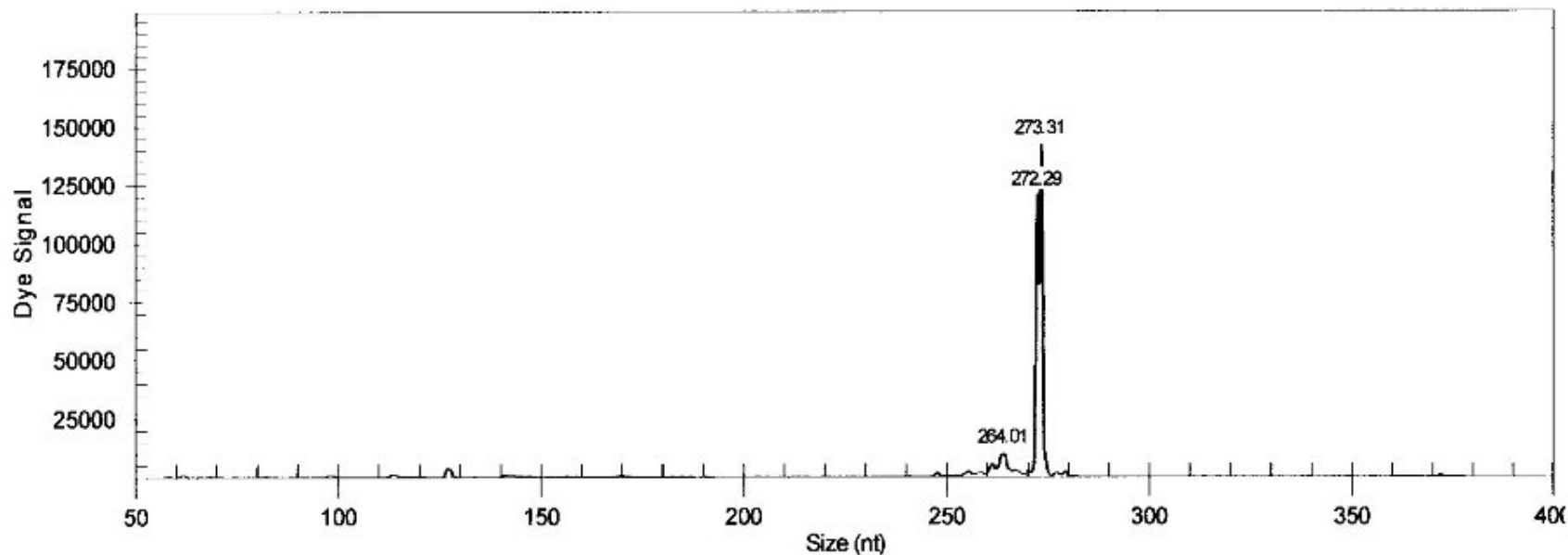
TCCLON

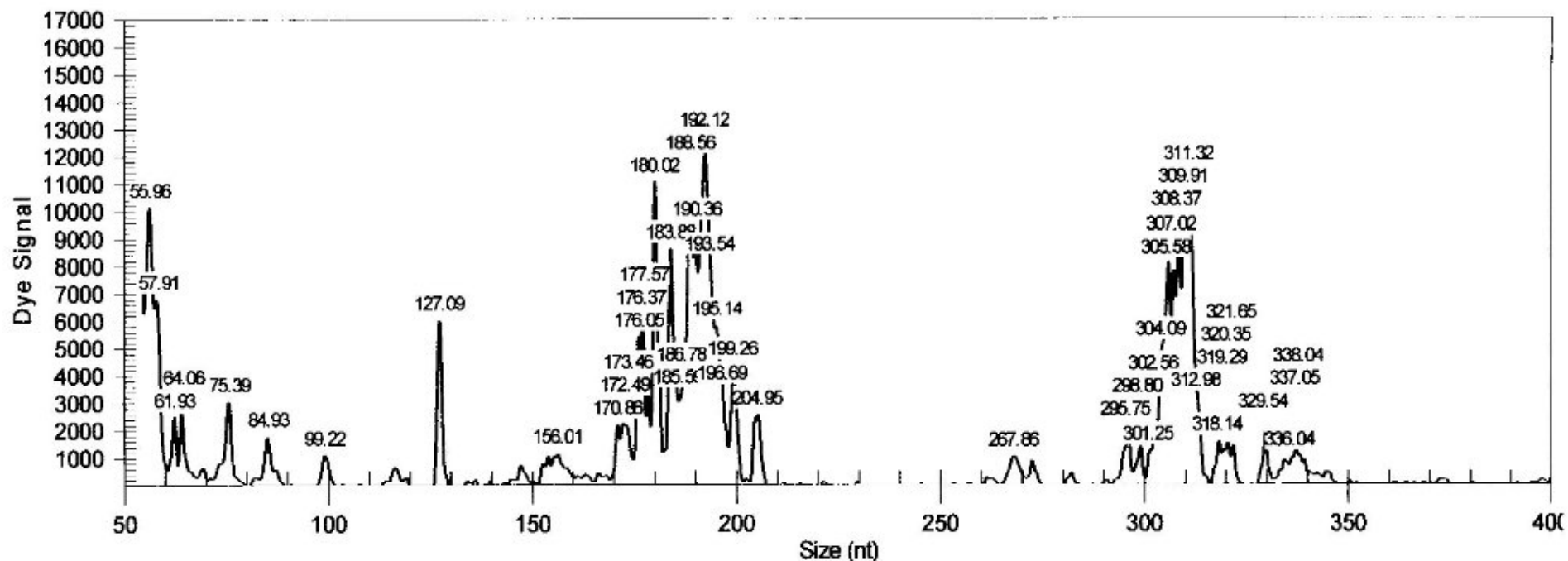
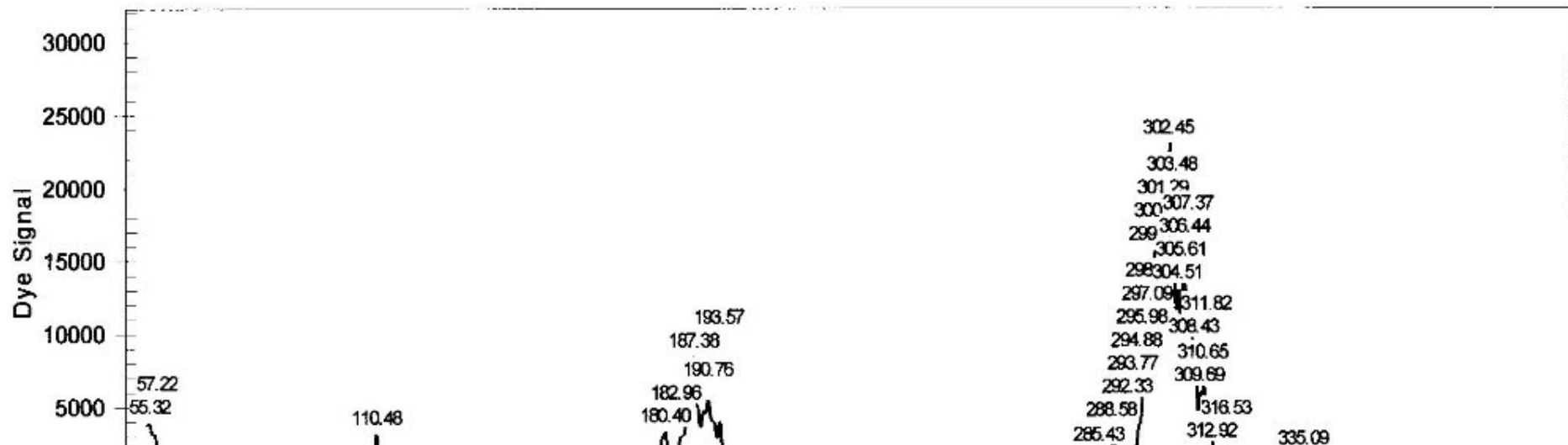
ELA014\_W4730\_BA.A08\_10100108YR with dye4 **Polyclonal**

SP10-16093



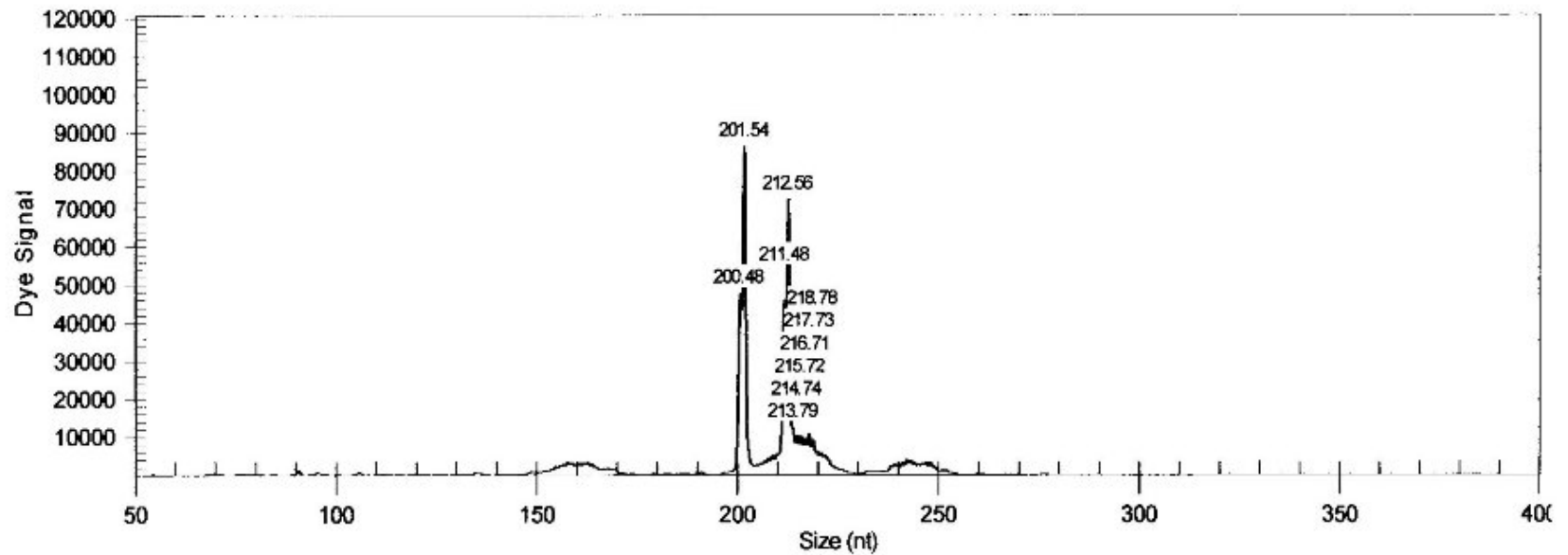
ELA014\_W4730\_BB.B08\_10100108YS with dye4 **Clonal**



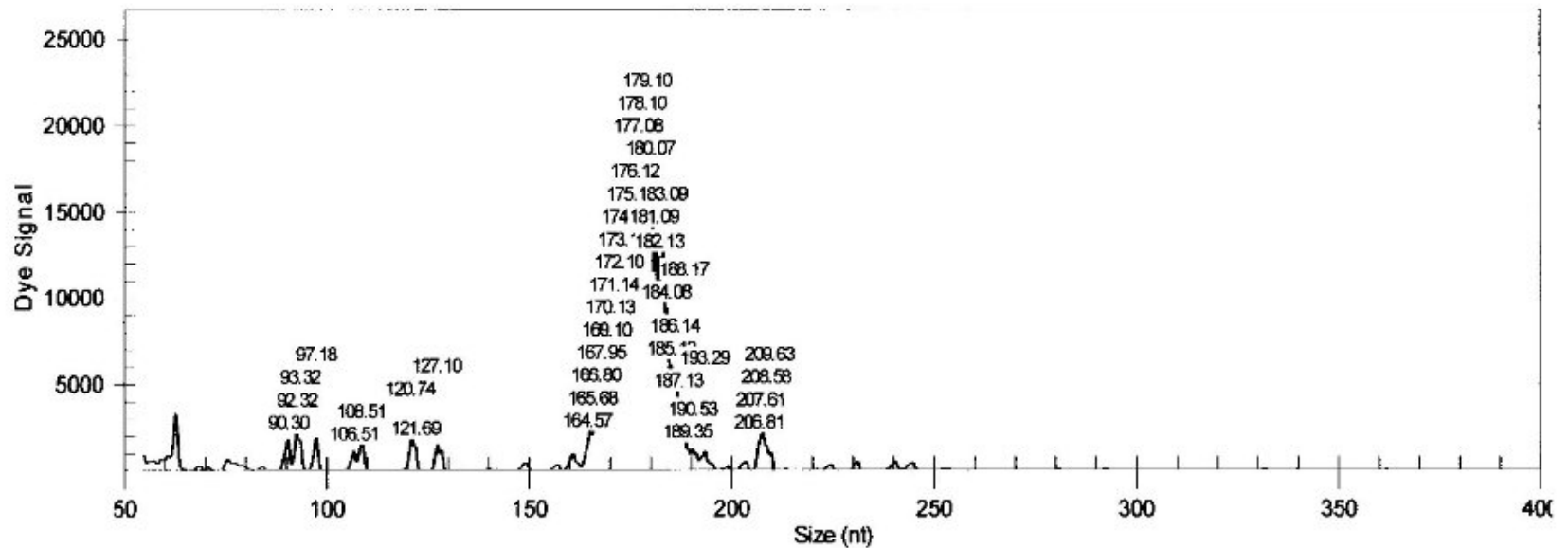


# TCR-Gamma Primers

- Two  $V_G$ - $J_G$  multiplexes:
  - (GA) products
    - 145-175nt
    - 175-195 nt
    - 195-230 nt \*\*
    - 230-255 nt \*\*
  - (GB) products
    - 80-110 nt
    - 110-140 nt
    - 160-195 nt \*\*
    - 195-220 nt



ELA014\_W4730\_GB.F08\_10100108Z0 with dye4 **Polyclonal**



# Present and Future...

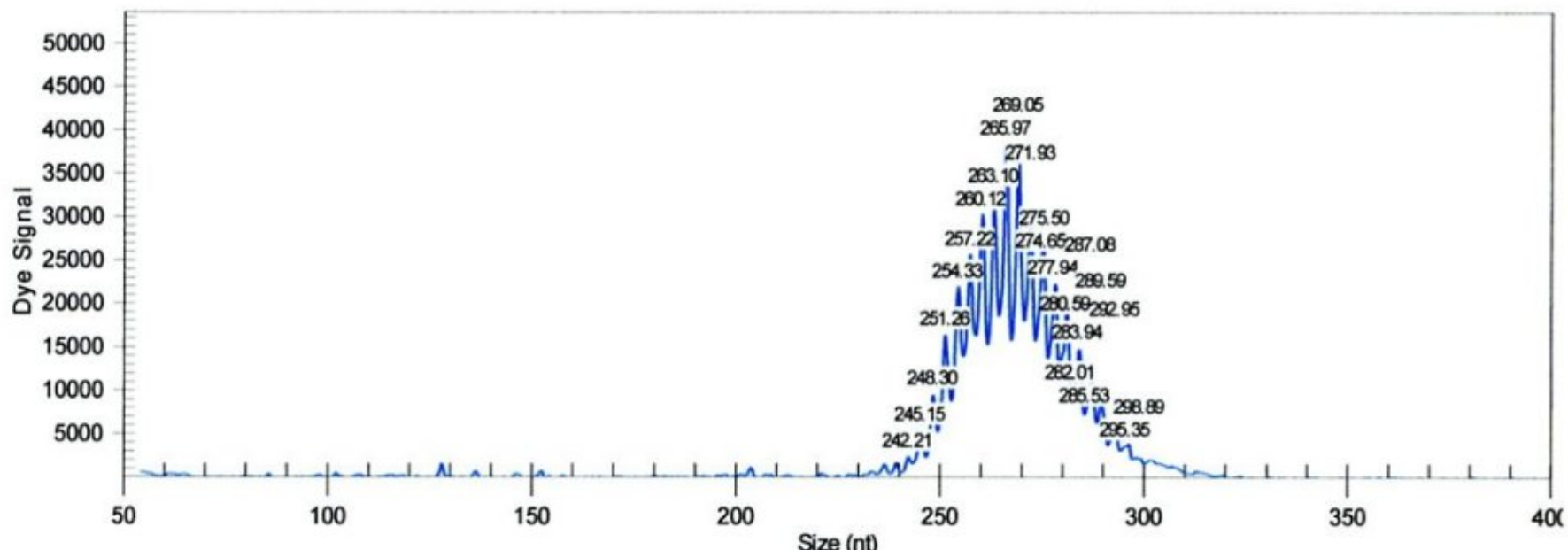
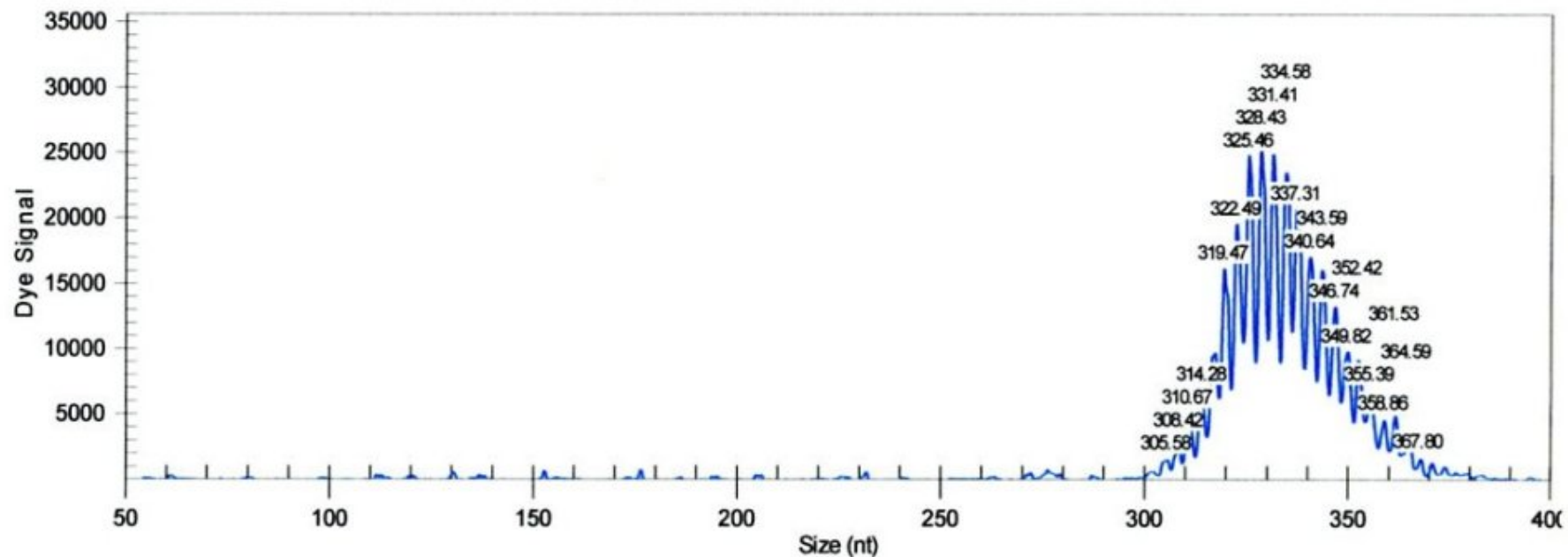
- Biomed-2 consortium members have an ongoing remit to improve quality and reliability
- Hold highly successful annual Euroclonality Workshops for paired molecular scientists and haematopathologists
  - ([workshop@euroclonality.org](mailto:workshop@euroclonality.org))
- Euroclonality Consortium to develop next-generation sequencing for IG and TCR immunogenetic analysis

# Next-generation sequencing for clonality analysis, repertoire, MRD

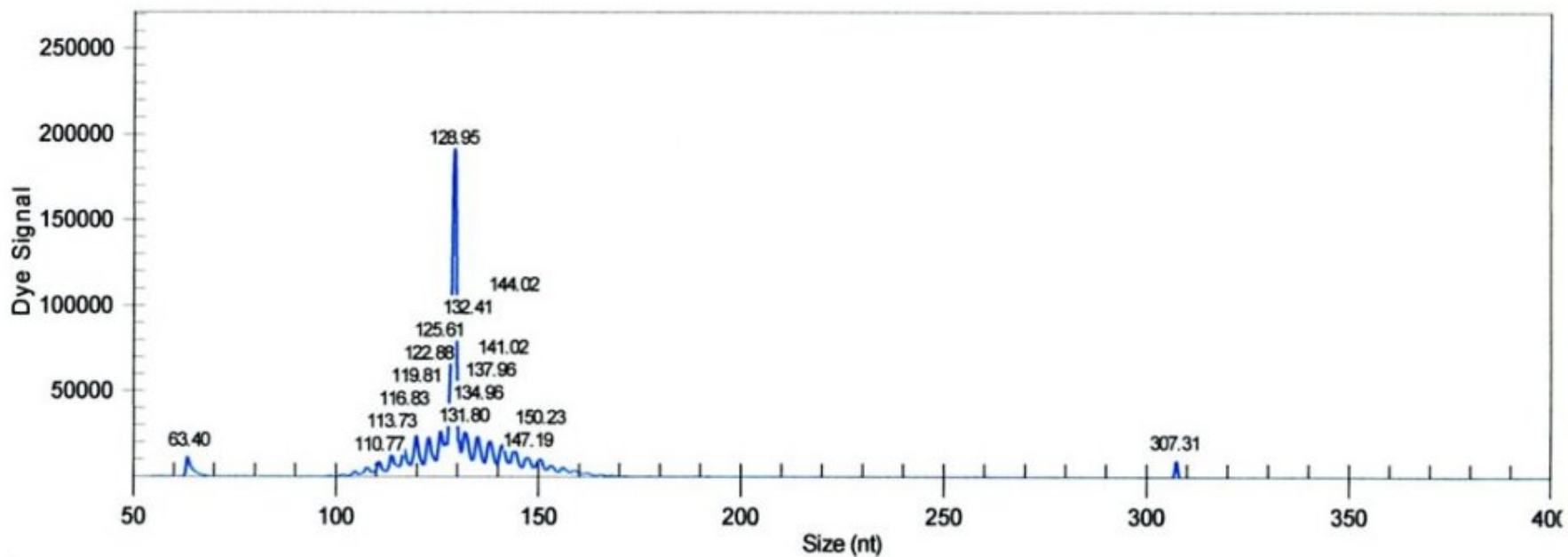
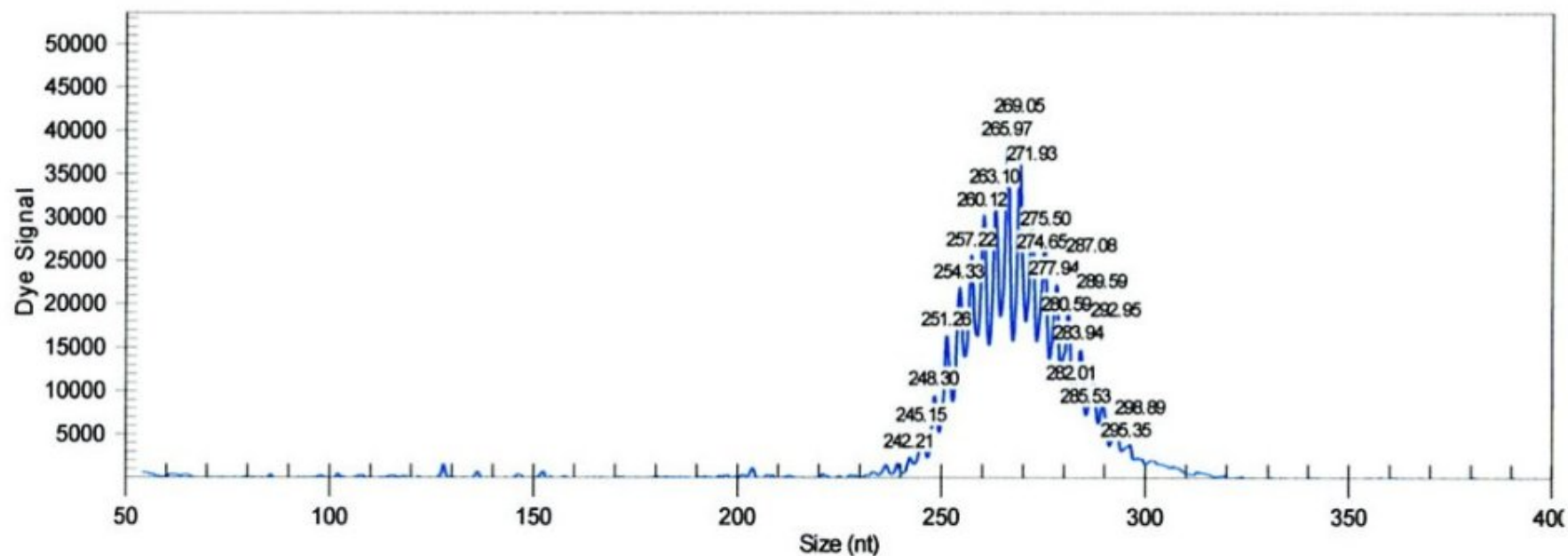
- Considerations:
  - Choice of platform: Roche 454, MiSeq, Ion Torrent
  - Complete redesign of primers to yield short amplicons (100-150 nt)
  - Artefacts: formalin-induced deamination, PCR errors, detection of normal variation
- [www.euroclonality.org](http://www.euroclonality.org)

# SP10-9765

- Lymphoid tissue mass at tongue base
- Morphology and immunohistochemistry equivocal - ?reactive or B-NHL
- PCR for *IGH* rearrangements

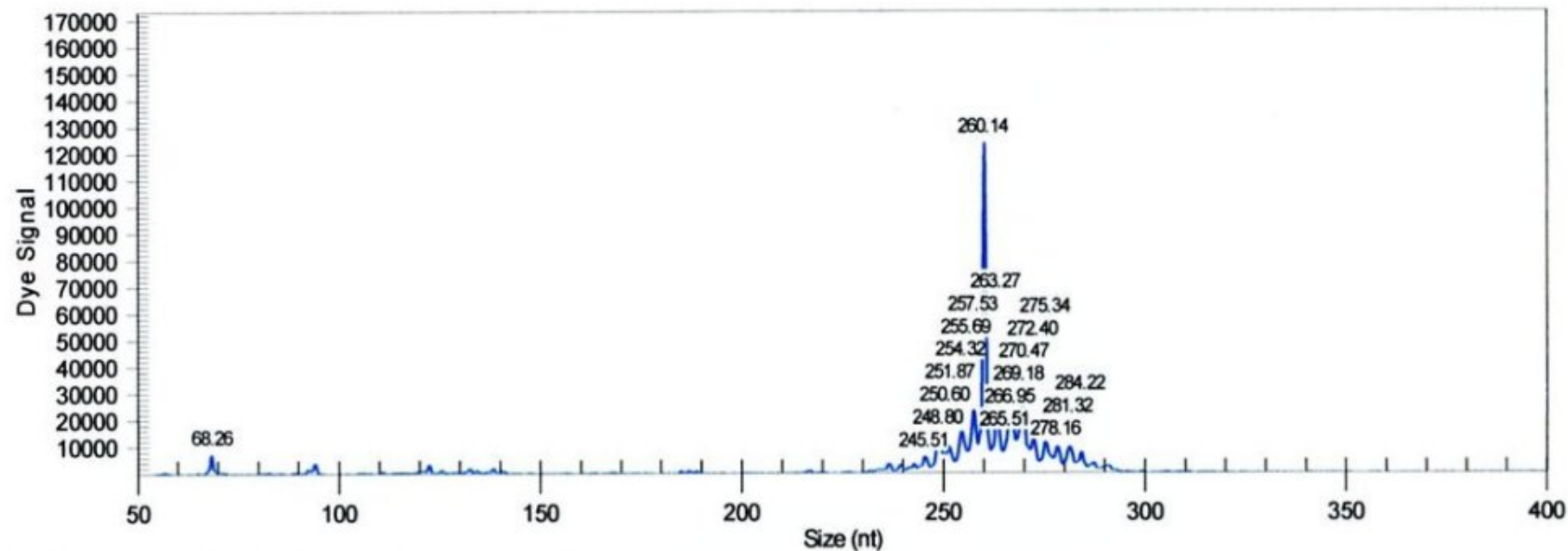
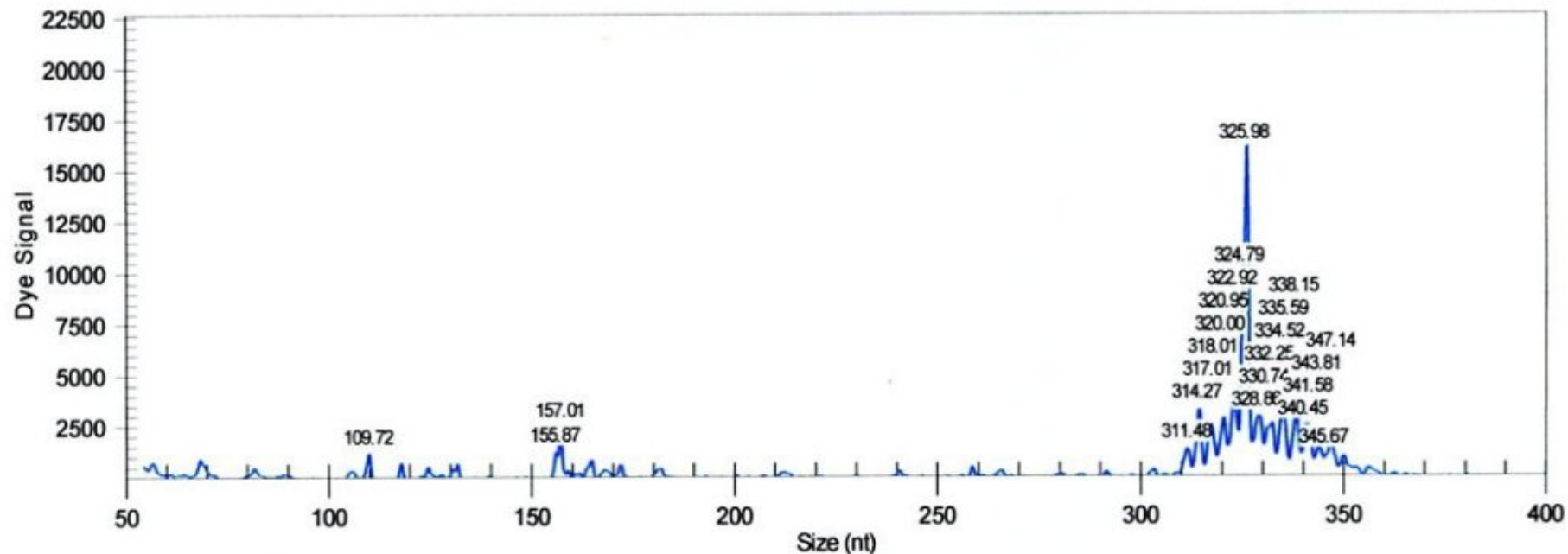


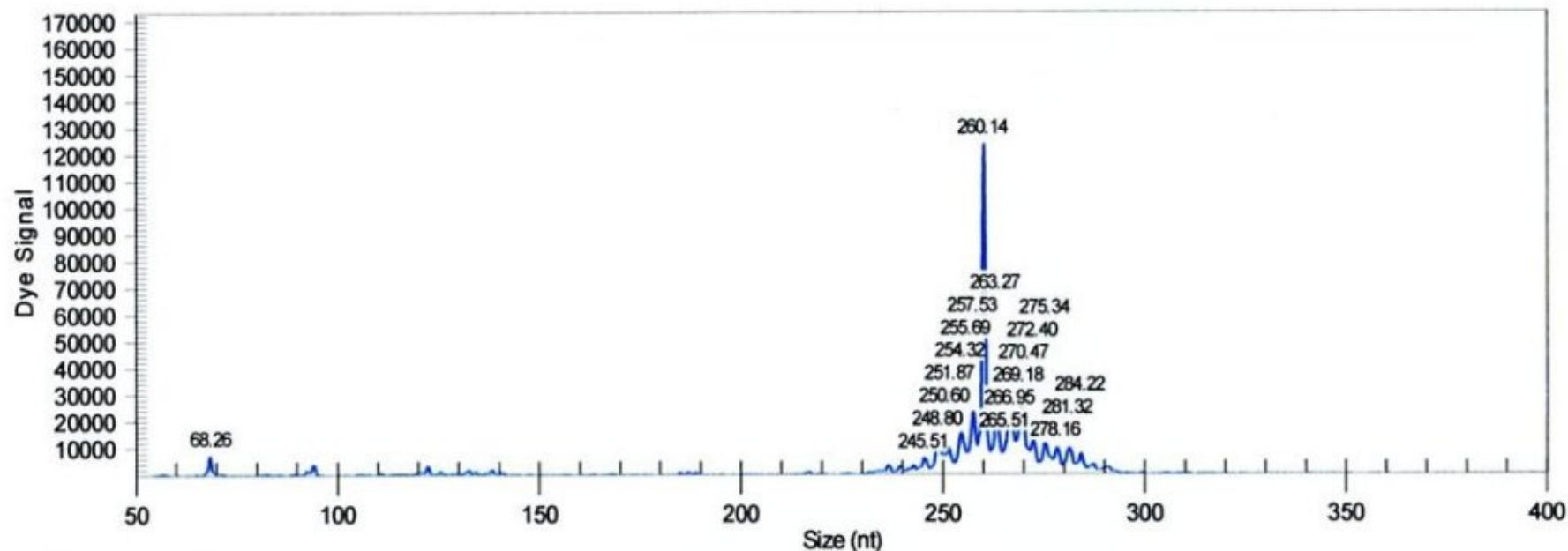




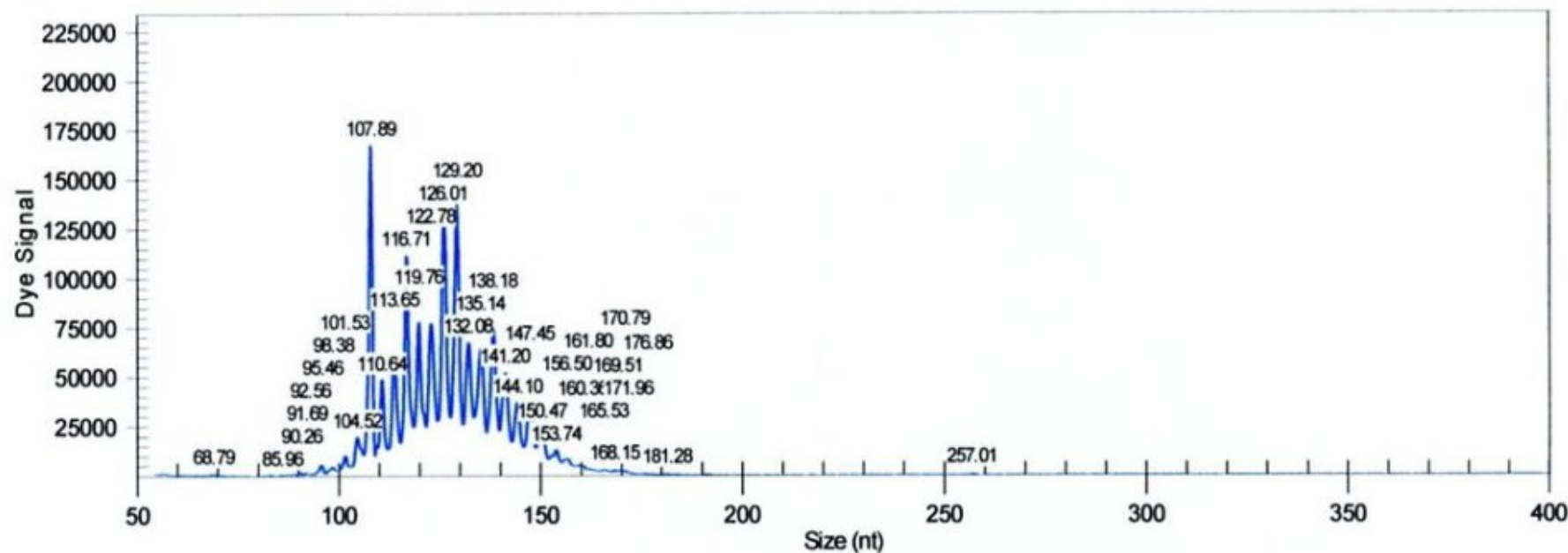
# SP07-3417

- Retrospective analysis of FFPE lung specimen
- In 2010, patient was diagnosed with DLBCL
- Lymphoid infiltrate in lung in 2007 reviewed- ?reactive or low grade B-NHL
- PCR for *IGH* rearrangements



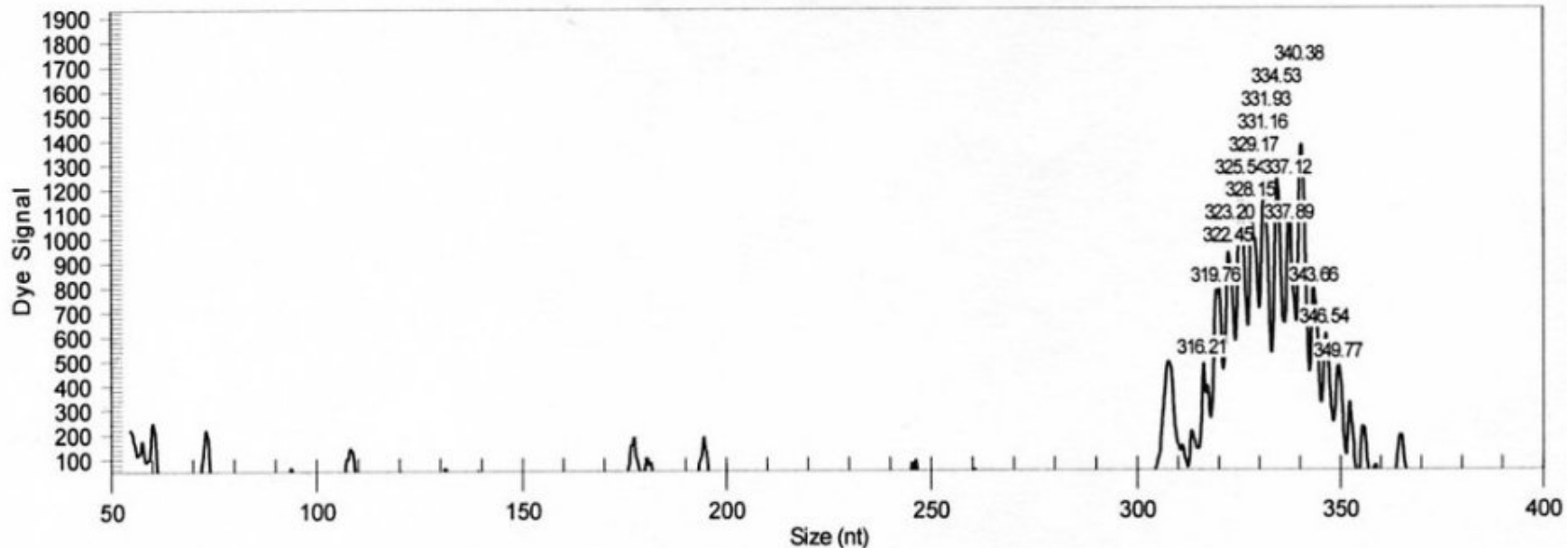


4687 FR3\_.H04\_10090209QU with dye4 Emerging Clone

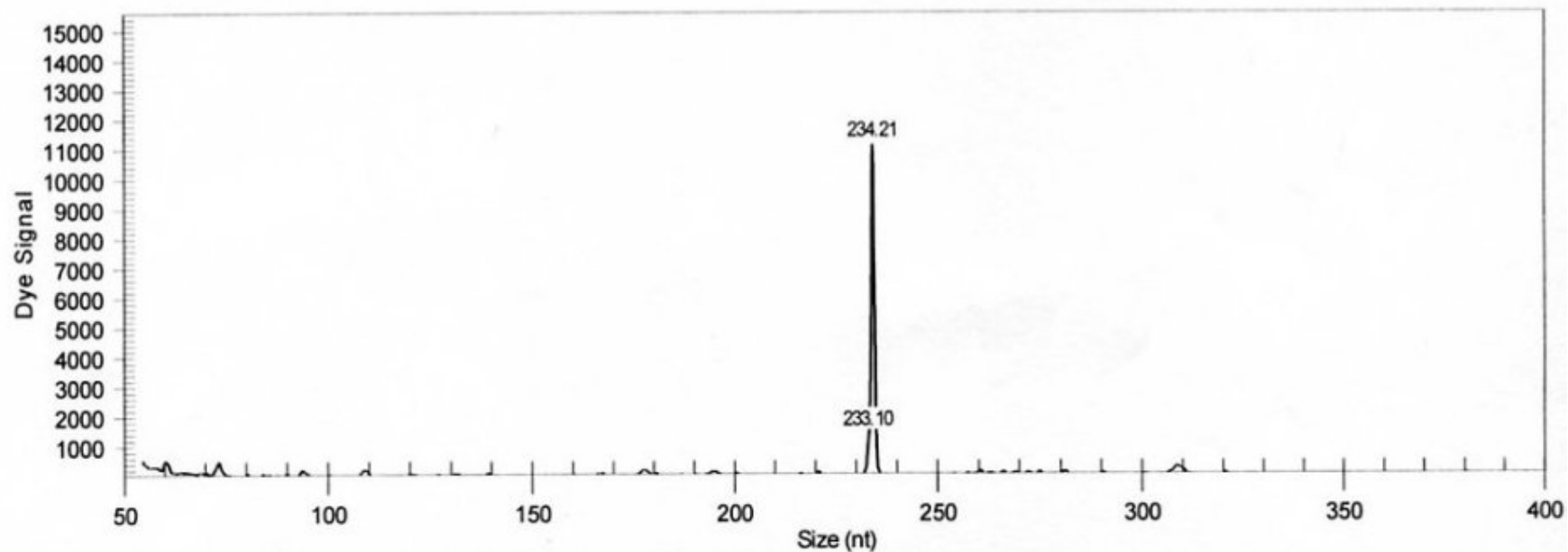


# OC10-11907

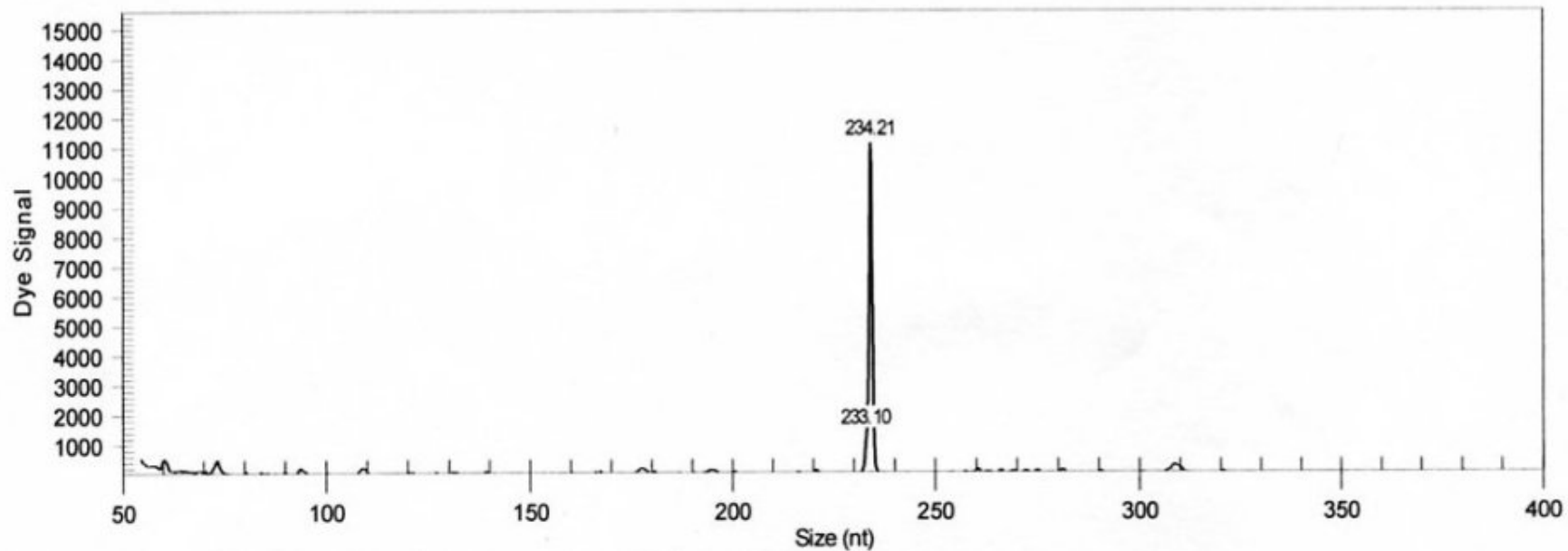
- FFPE Lymph Node
- Morphology and immunohistochemistry raise suspicion of ?follicular lymphoma obscured by reactive lymphocytes
- PCR for *IGH* rearrangements



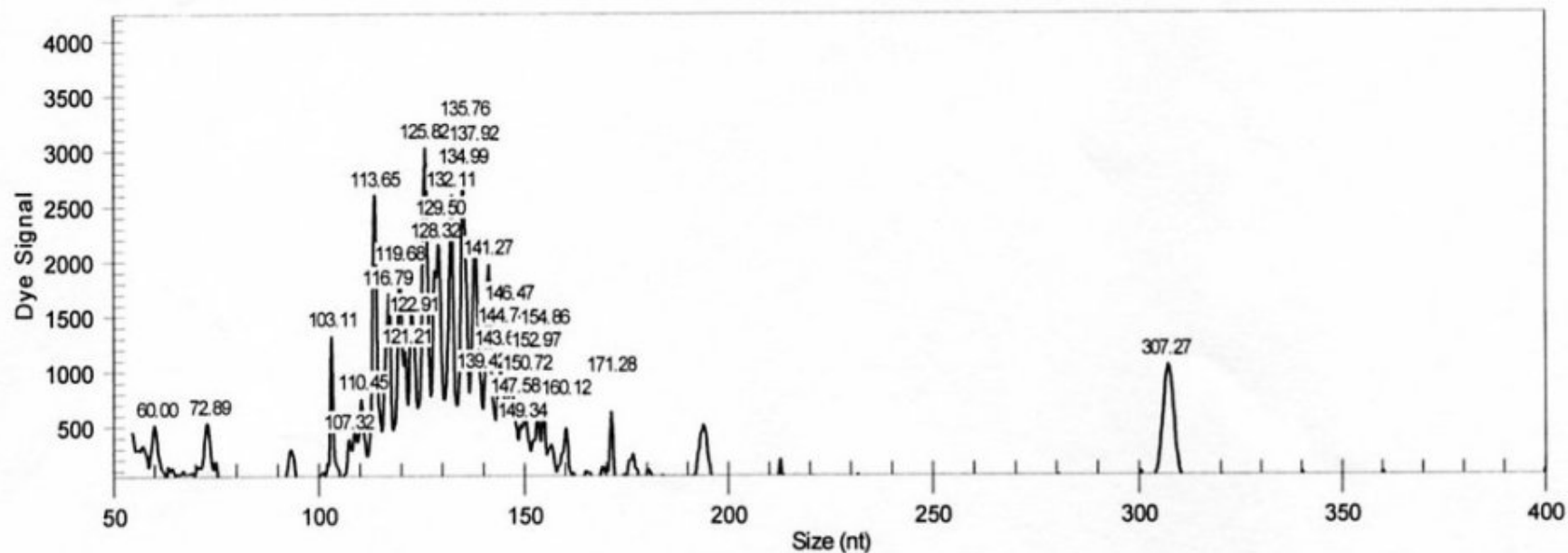
ELA006\_W4626\_FR2.B03\_100729134O with dye4 Clonal (fragment size out of expected range 250-295)



ELA006\_W4626\_FR2.B03\_100729134O with dye4 Clonal (fragment size out of expected range 250-295) OC10-11907



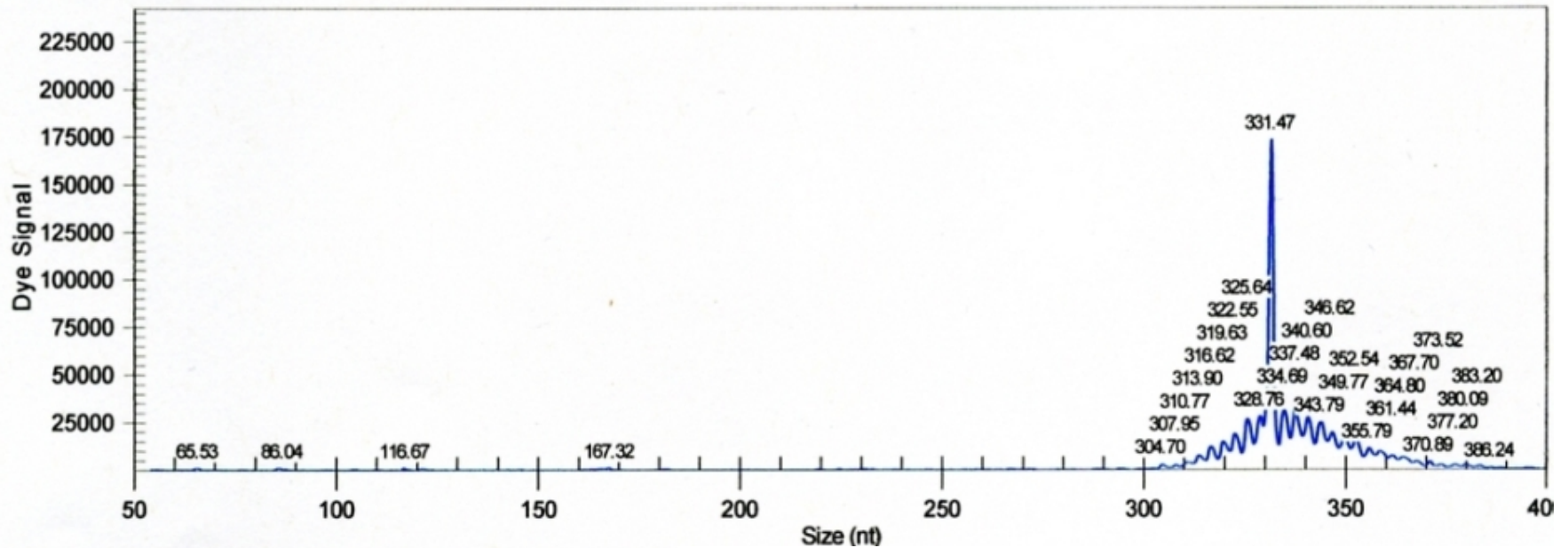
ELA006\_W4626\_FR3.C03\_100729134Q with dye4 Polyclonal



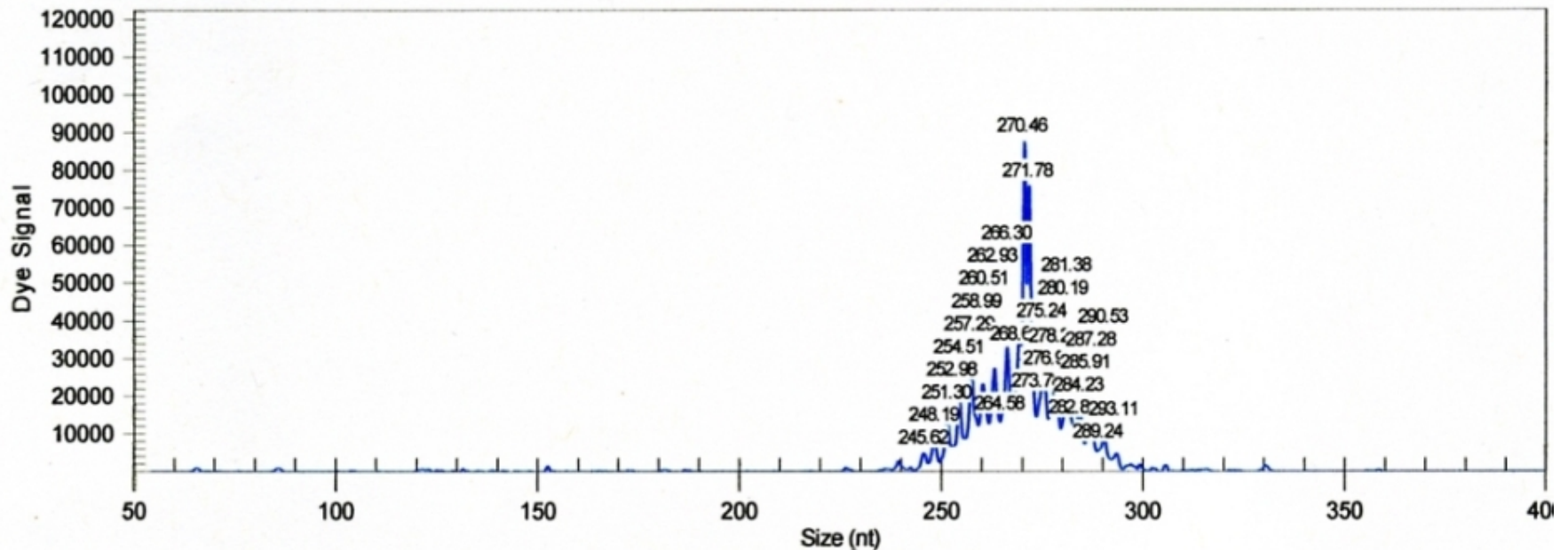
# SP11-9578

- Sjogren's syndrome; recent unilateral progression of parotid enlargement
- Histology (and immunophenotype) suggest development of MALT lymphoma
- PCR for *IGH* rearrangements





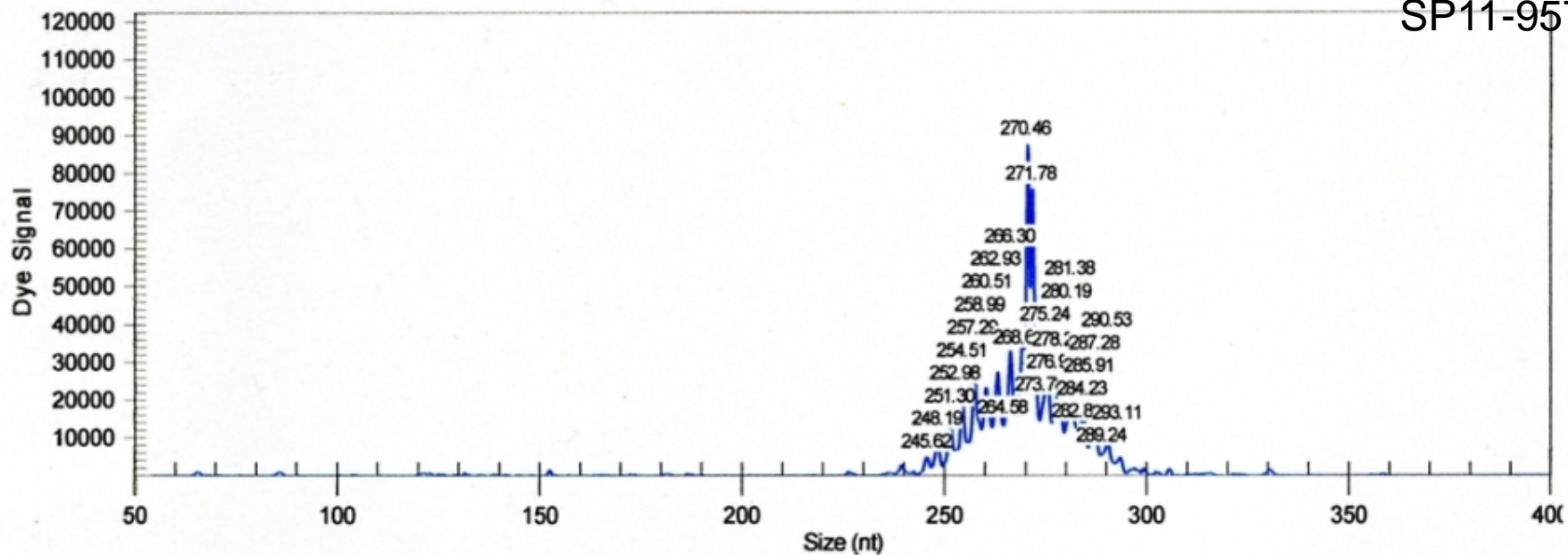
5229\_FR2.G04\_11060909A8 with dye4 **Dominant clone in polyclonal background**



5229\_FR2.G04\_11060909A8 with dye4 background

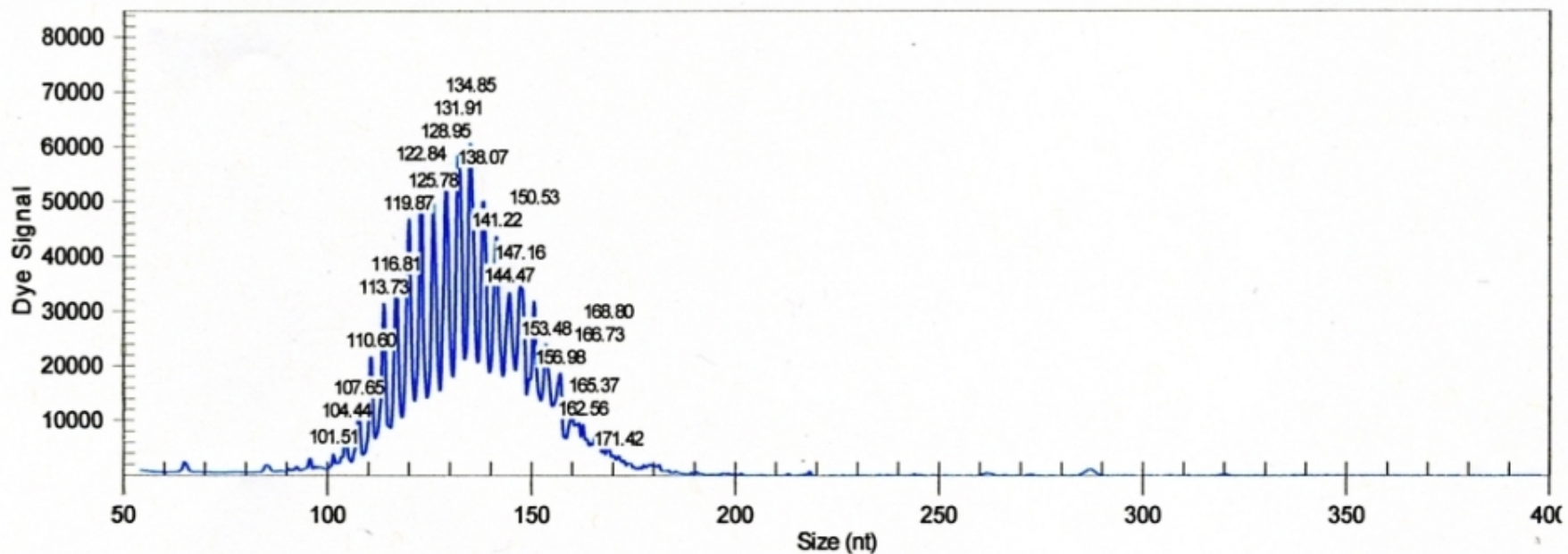
Dominant clone in polyclonal background

SP11-9578



5229\_FR3.H04\_11060909AA with dye4

Polyclonal



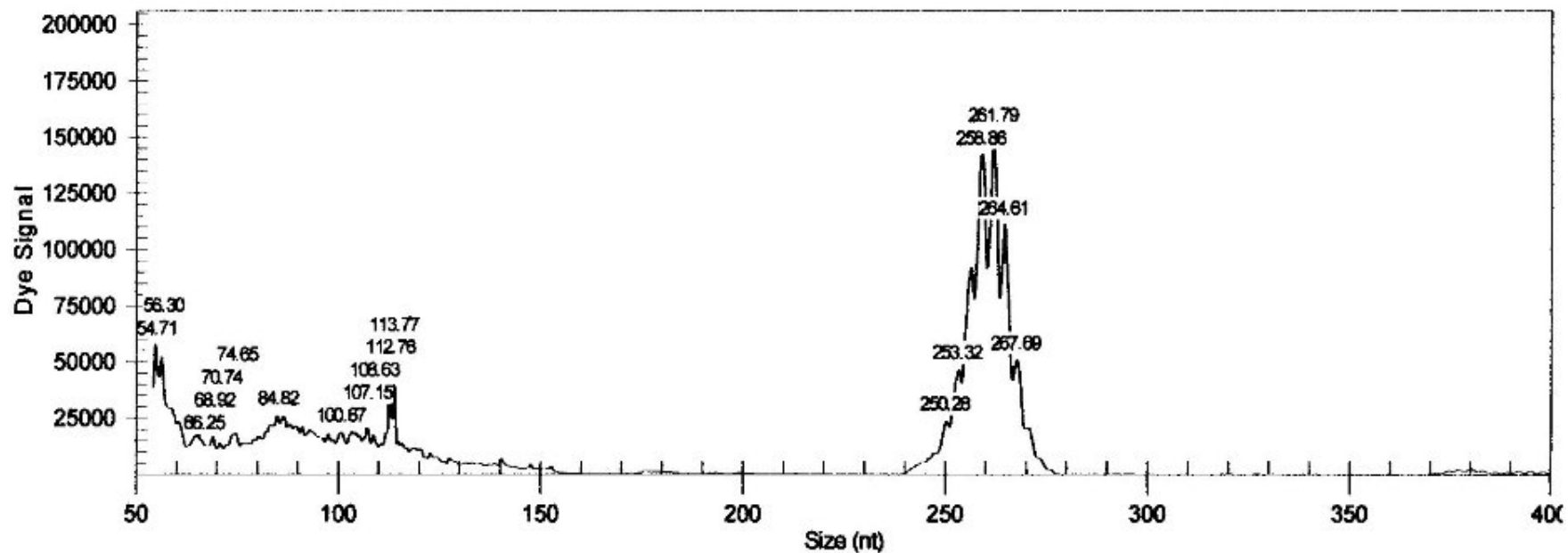
# SP14-8502

- FFPE enlarged lymph node in patient with previous adult T-cell lymphoma/leukaemia
- In remission for several years; new 8mm cervical lymph node
- Histology and immunohistochemistry indicate dermatopathic but scattered CD25+ve T-cells; ?reactive/neoplastic
- PCR for *TCR* rearrangements

ELA322\_W7516\_BA.A04\_14050808KQ with dye4

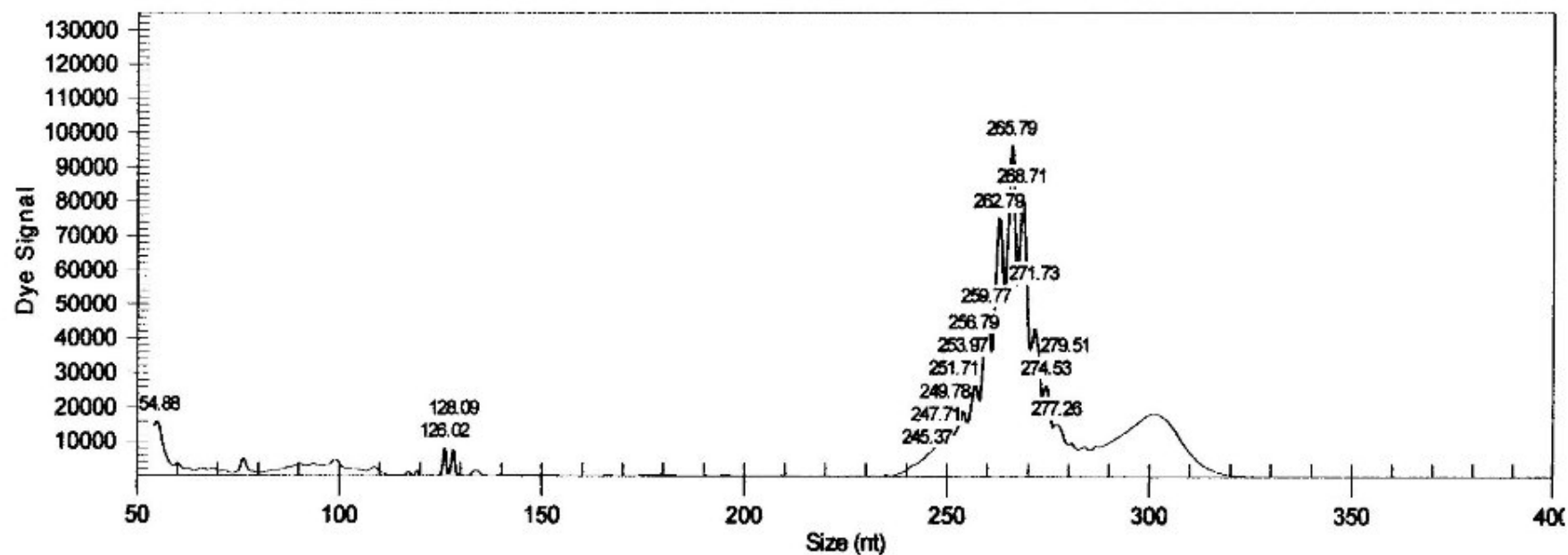
Polyclonal

SP14-8502



ELA322\_W7516\_BB.B04\_14050808KV with dye4

Polyclonal

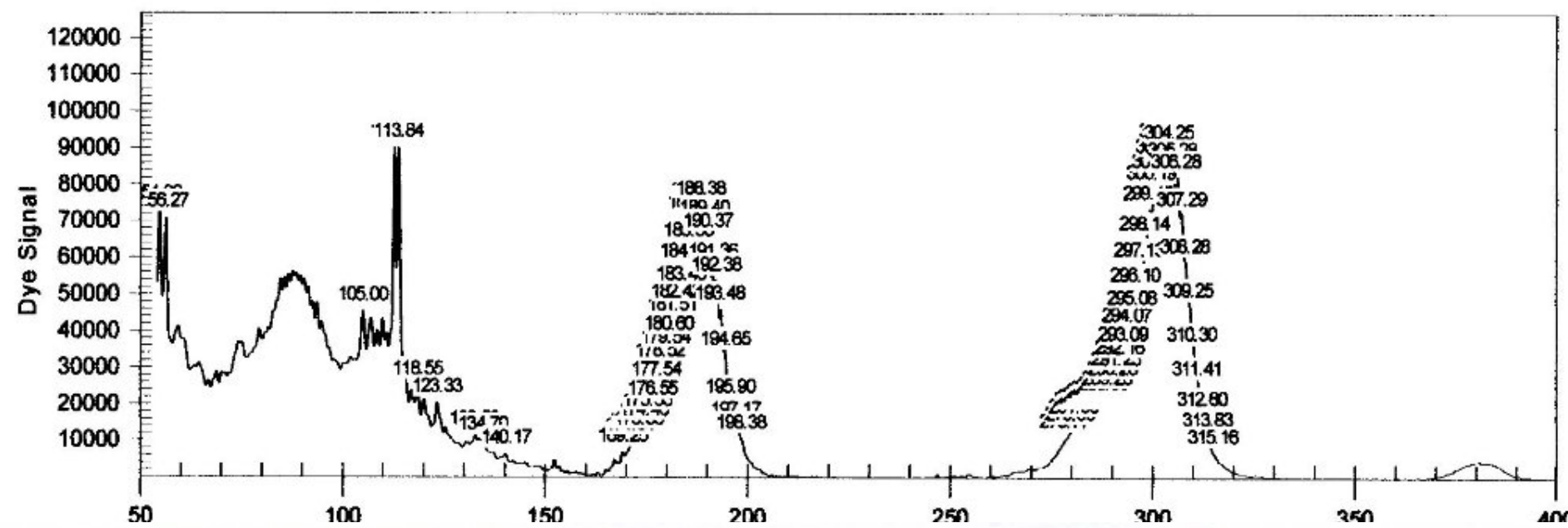




ELA322\_W7516\_BC1.C04\_14050808KX with dye4

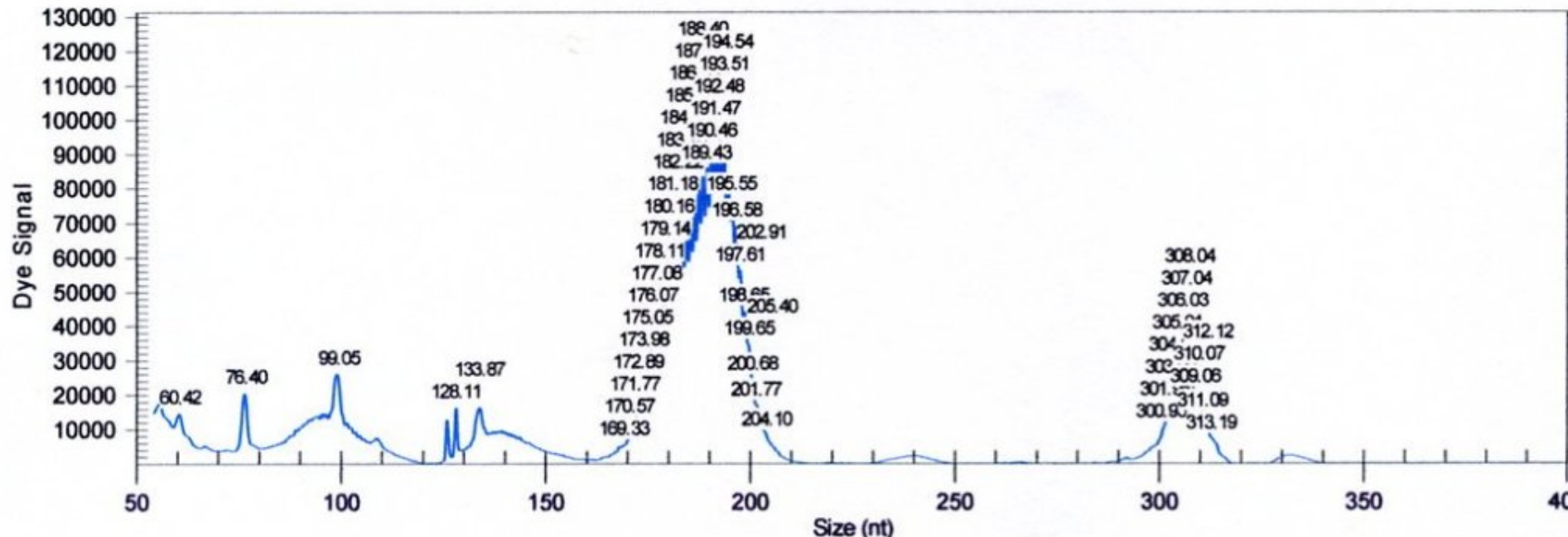
Polyclonal

SP14-8502



ELA322\_W7516\_BC2.D04\_14050808L4 with dye4

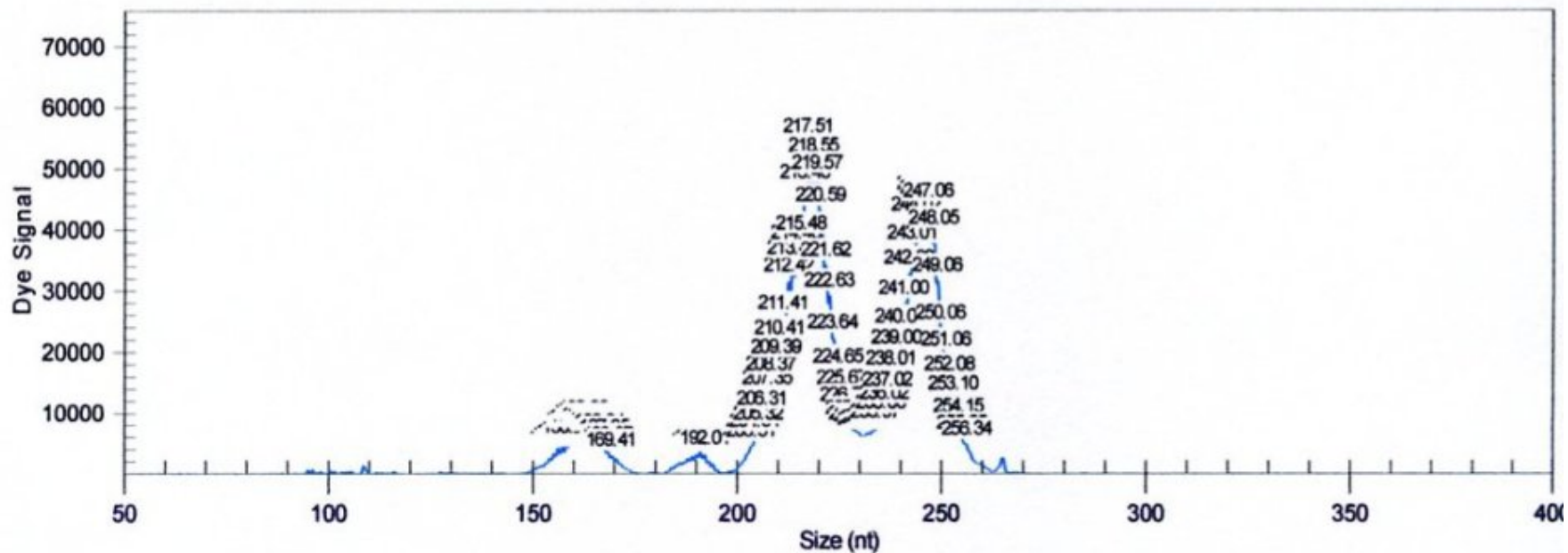
Polyclonal



ELA322\_W7516\_GA.E04\_14050808L6 with dye4

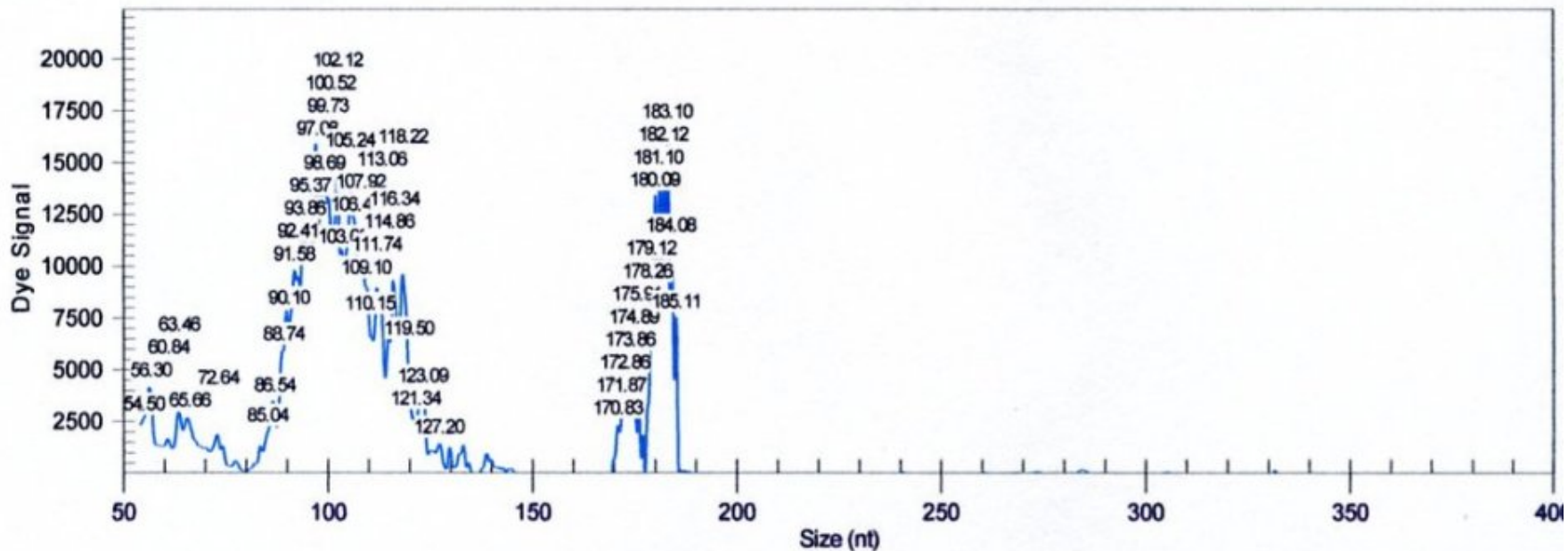
Polyclonal

SP14-7100



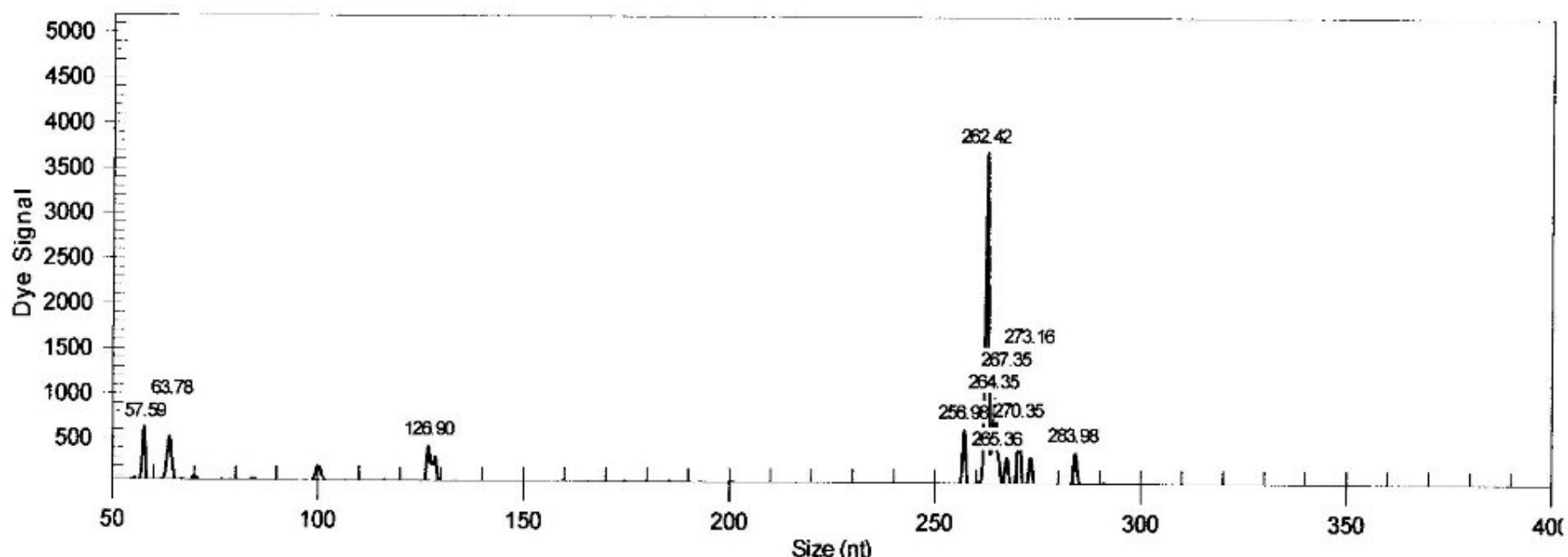
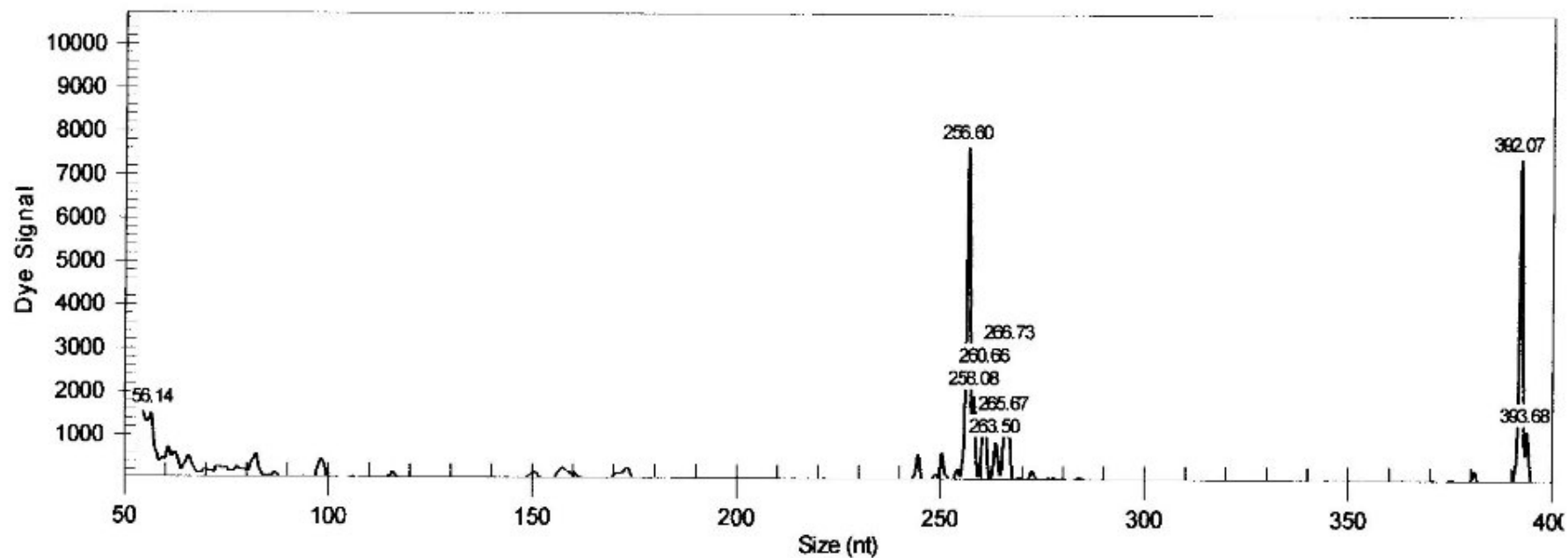
ELA322\_W7516\_GB.F04\_14050808L9 with dye4

Polyclonal

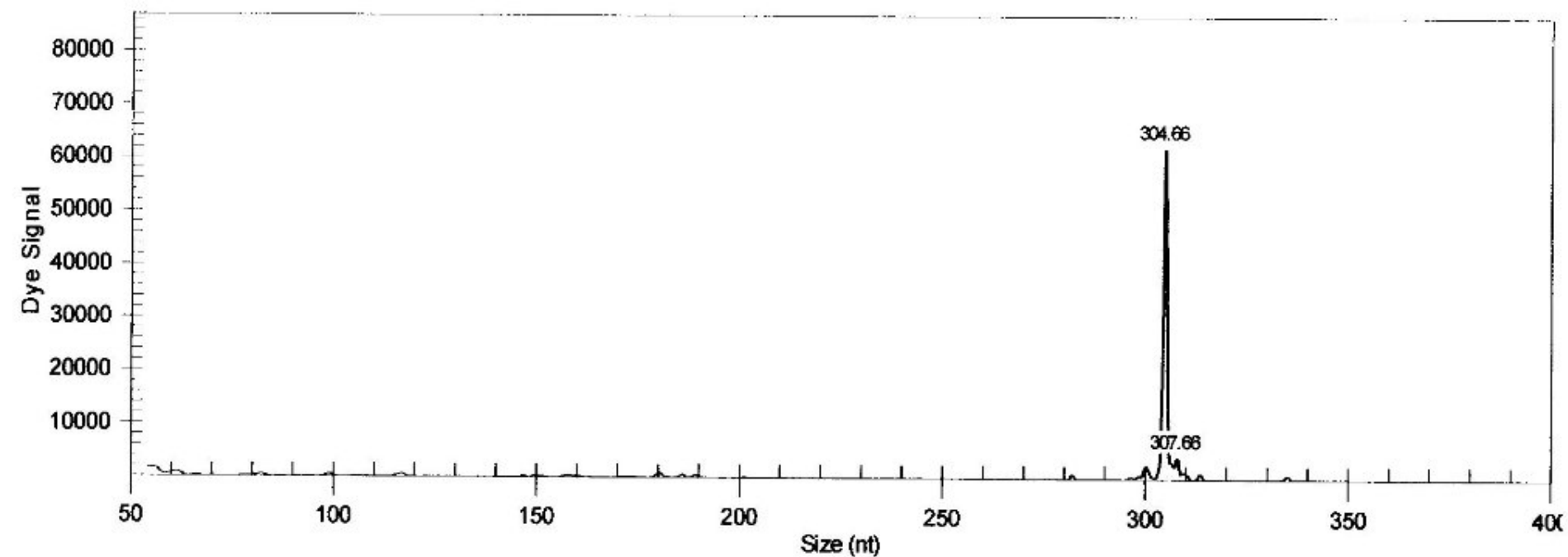
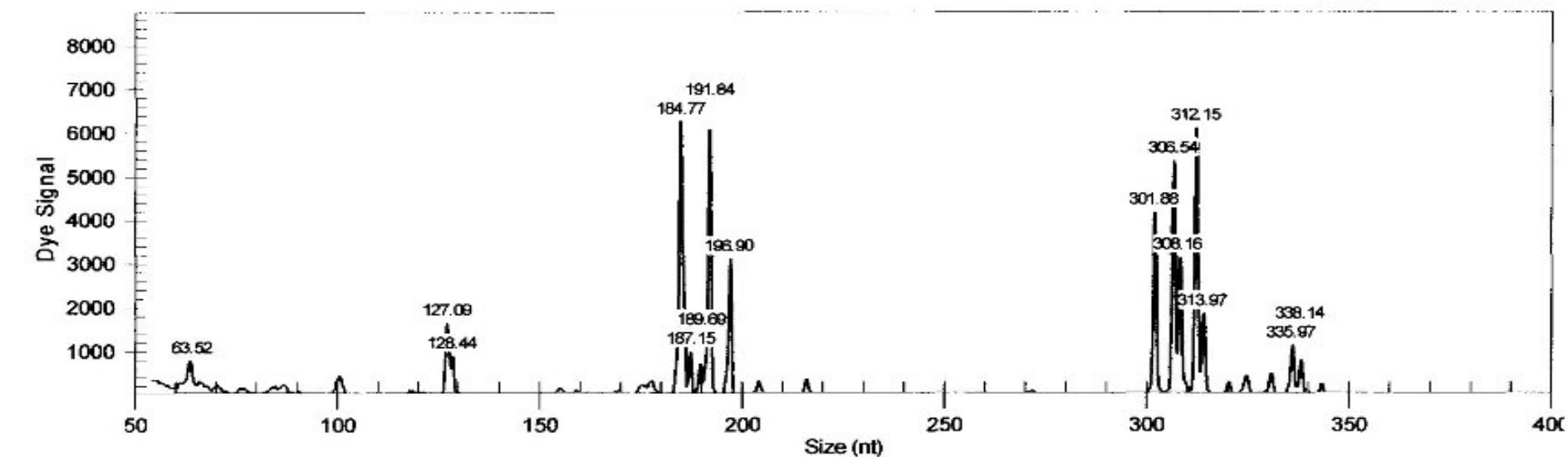


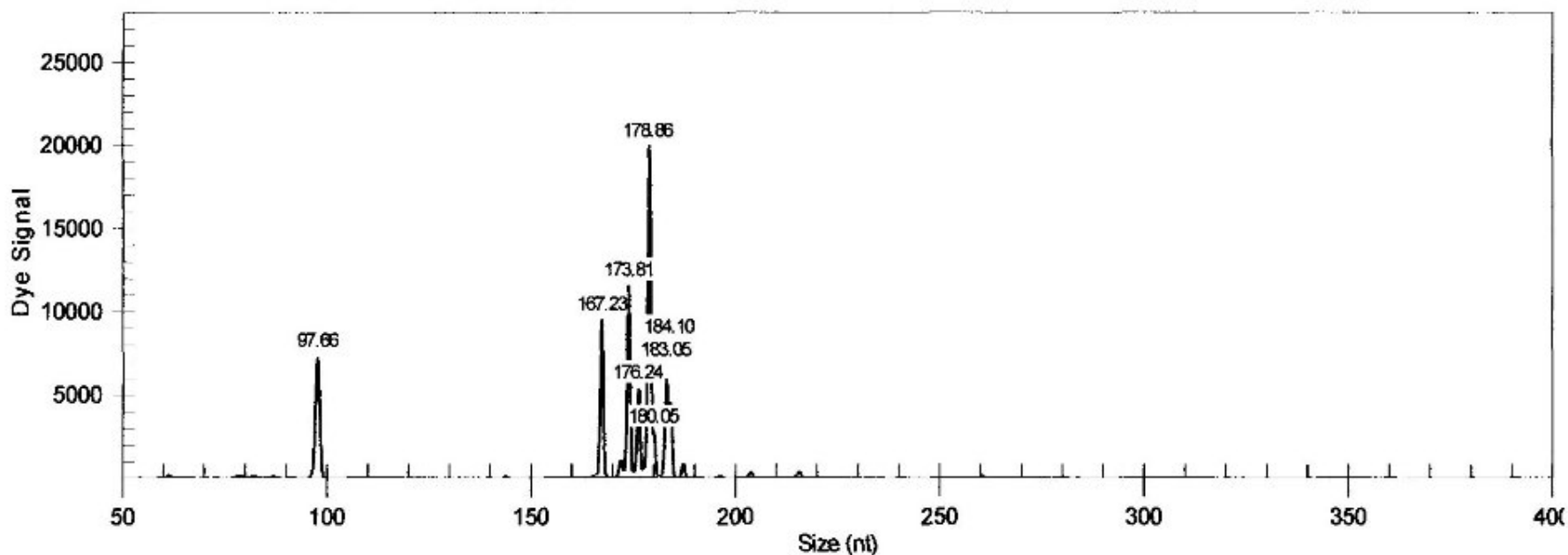
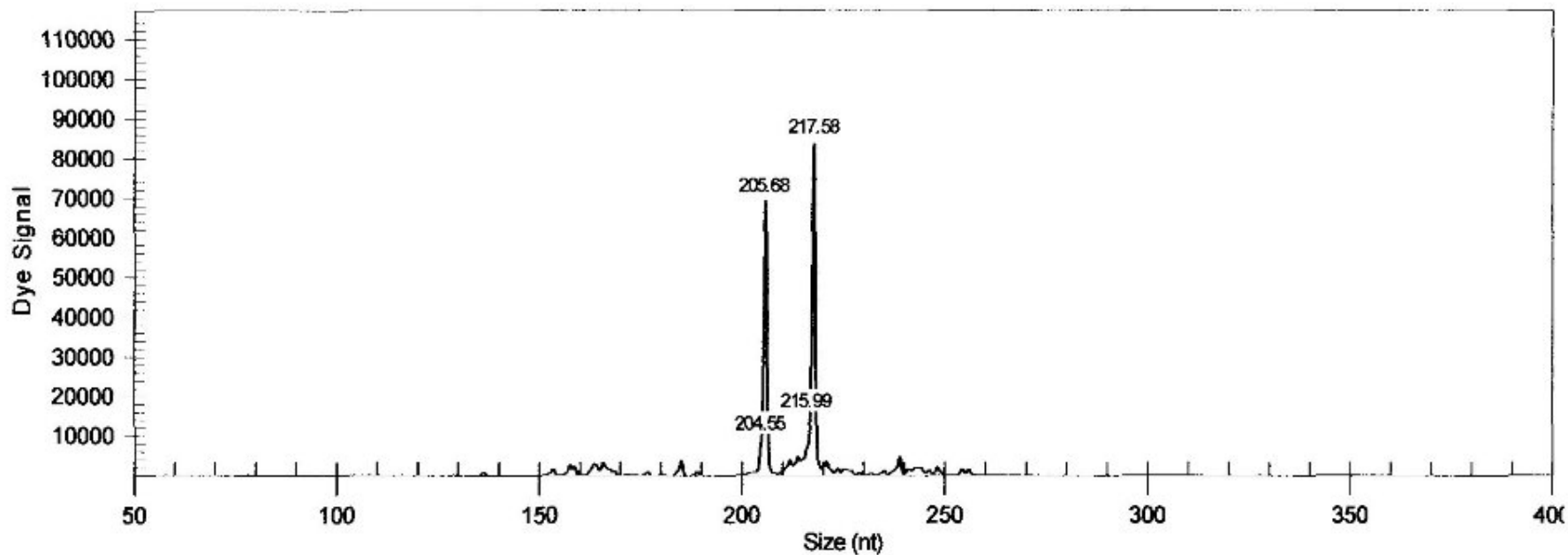
# SP10-9772

- FFPE, EDTA-decalcified bone marrow trephine core
- Known previous lymphoplasmacytic lymphoma
- Morphology and immunohistochemistry indicate recurrence but also prominent and cytologically atypical T-cell infiltrate
- PCR for *TCR* rearrangements



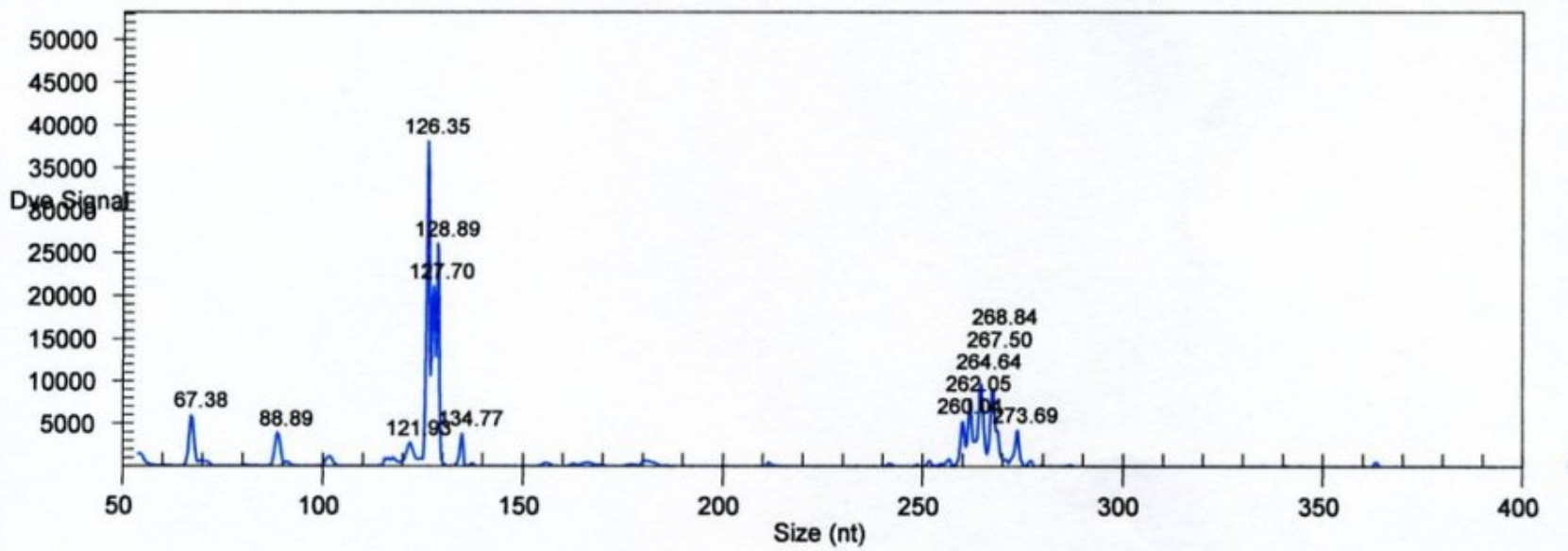
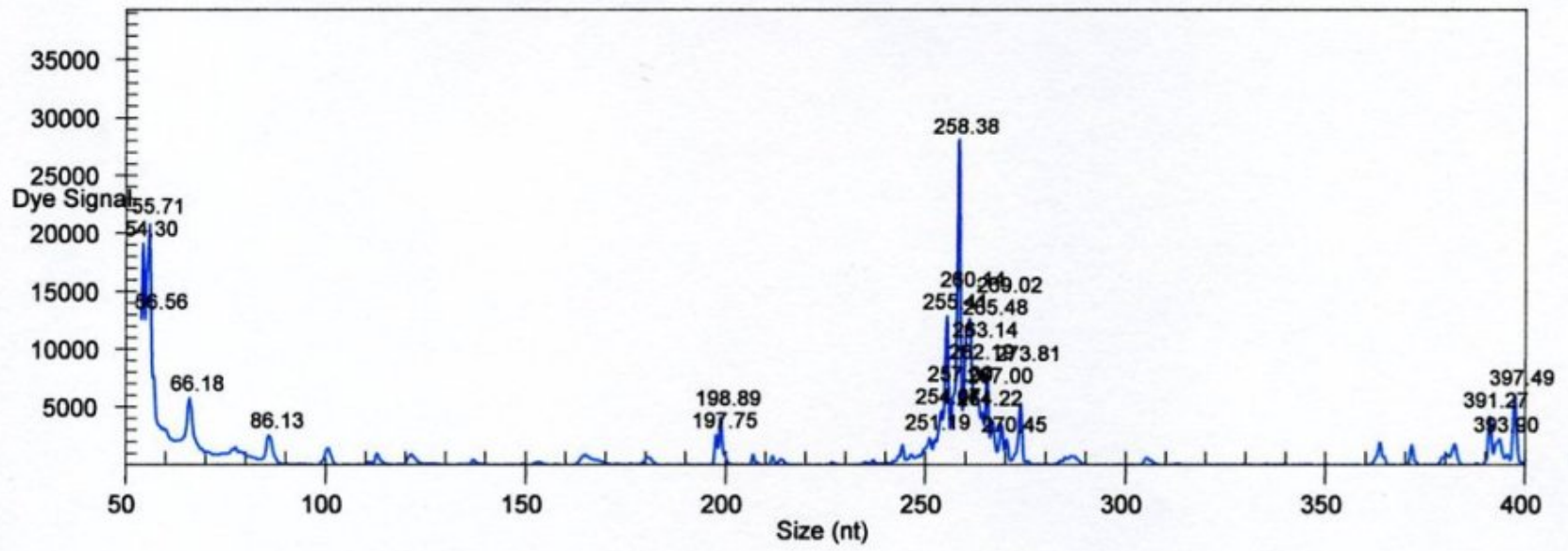


4544\_BC1.C10\_10062110Q5 with dye4 **Clonal**4544\_BC2.D10\_10062110Q6 with dye4 **Polyclonal - ?oligoclonal**



# SP14-7100

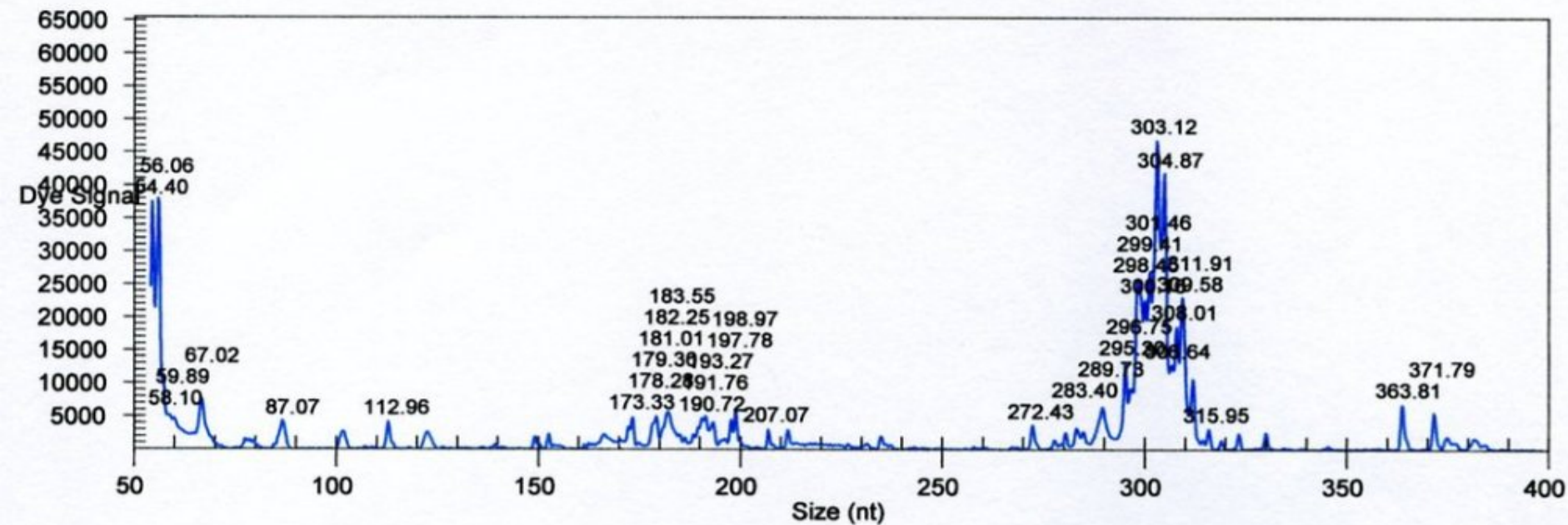
- FFPE, EDTA-decalcified bone marrow trephine core
- Thrombocytopenia - ?ITP
- Histology and immunophenotype; T-cell lymphocytosis in marrow otherwise c/w ITP. No cytological atypia
- PCR for *TCR* rearrangements



ELA320\_W7455\_BC1.C07\_14041707W1 with dye4

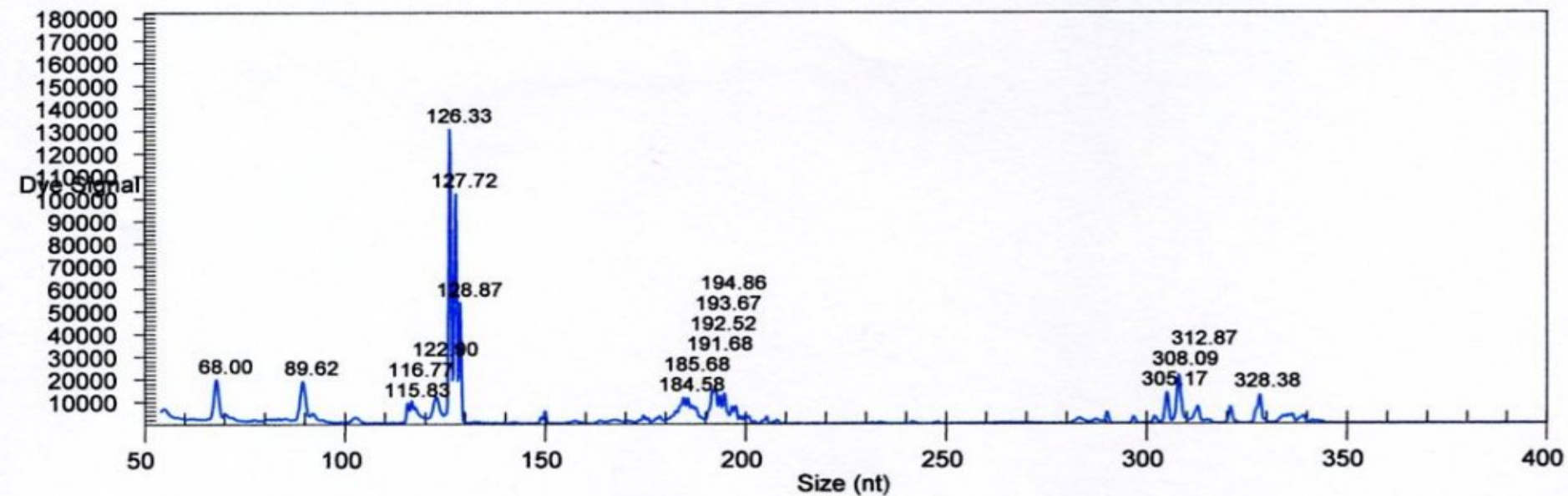
Polyclonal

SP14-7100

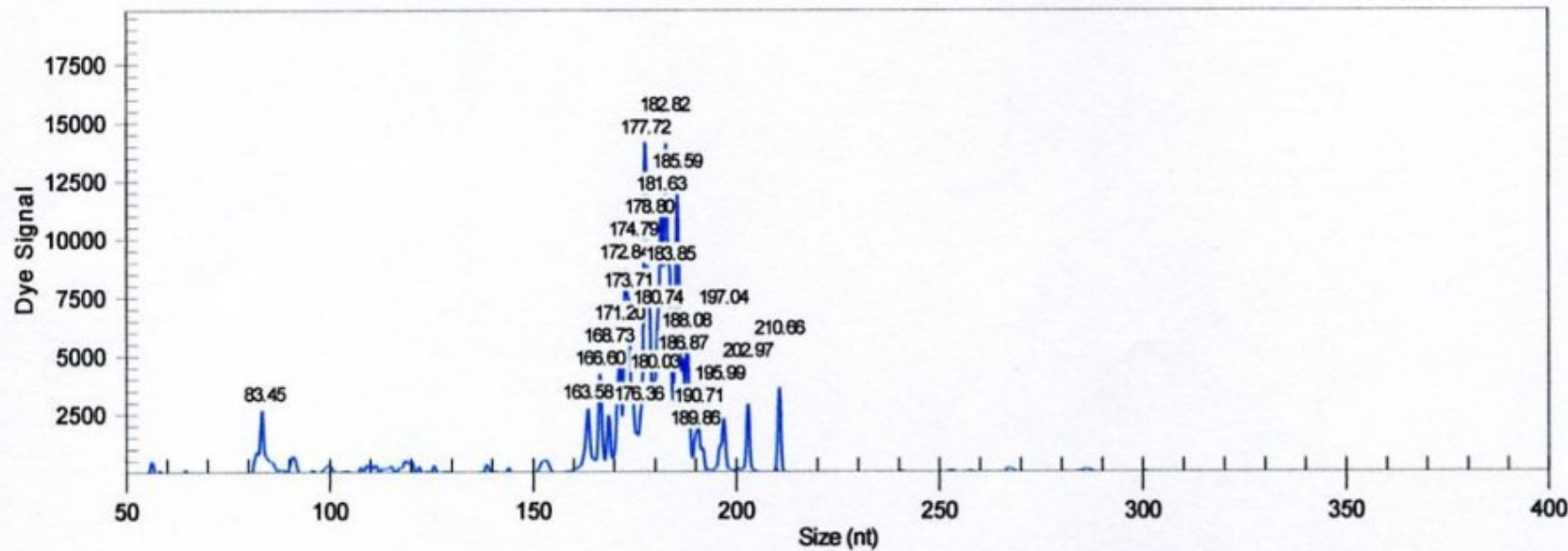
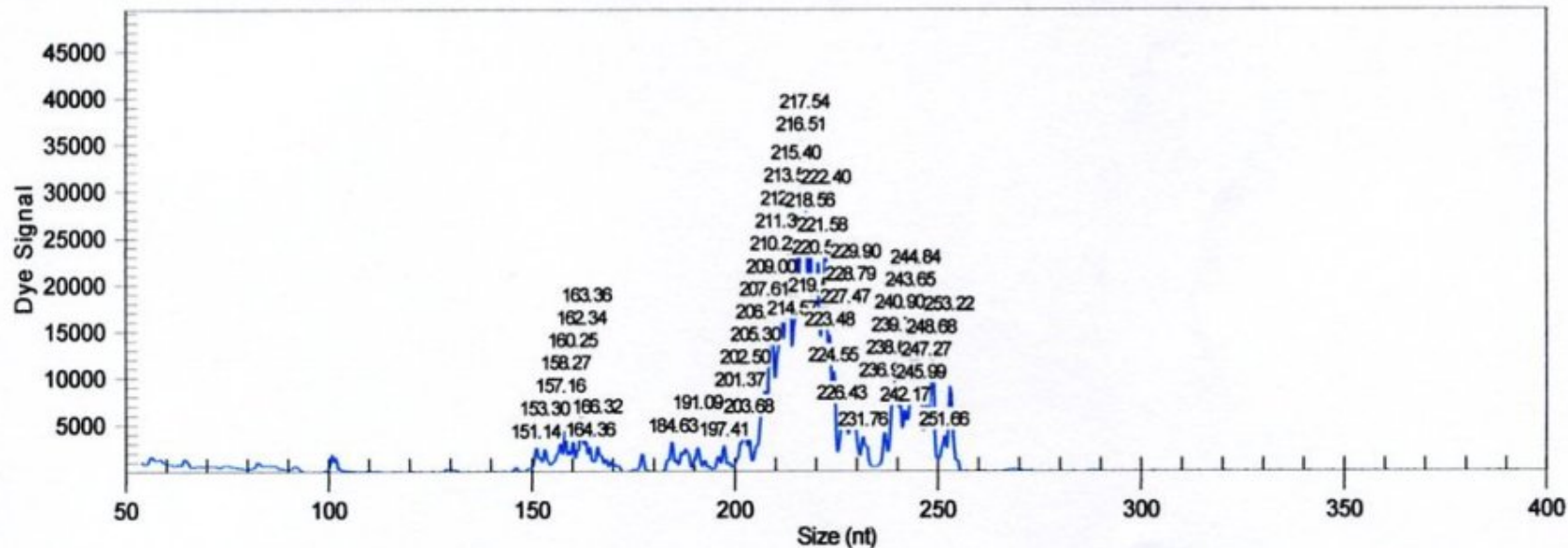


ELA320\_W7455\_BC2.D07\_14041707W3 with dye4

Polyclonal





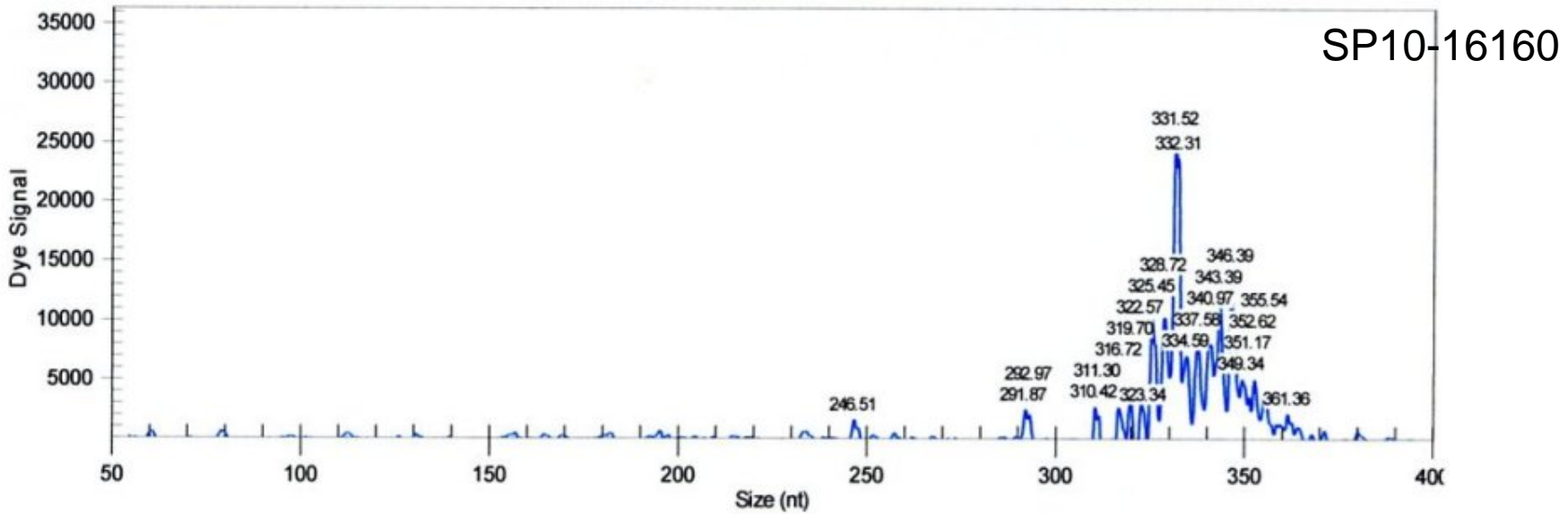


# SP10-16160

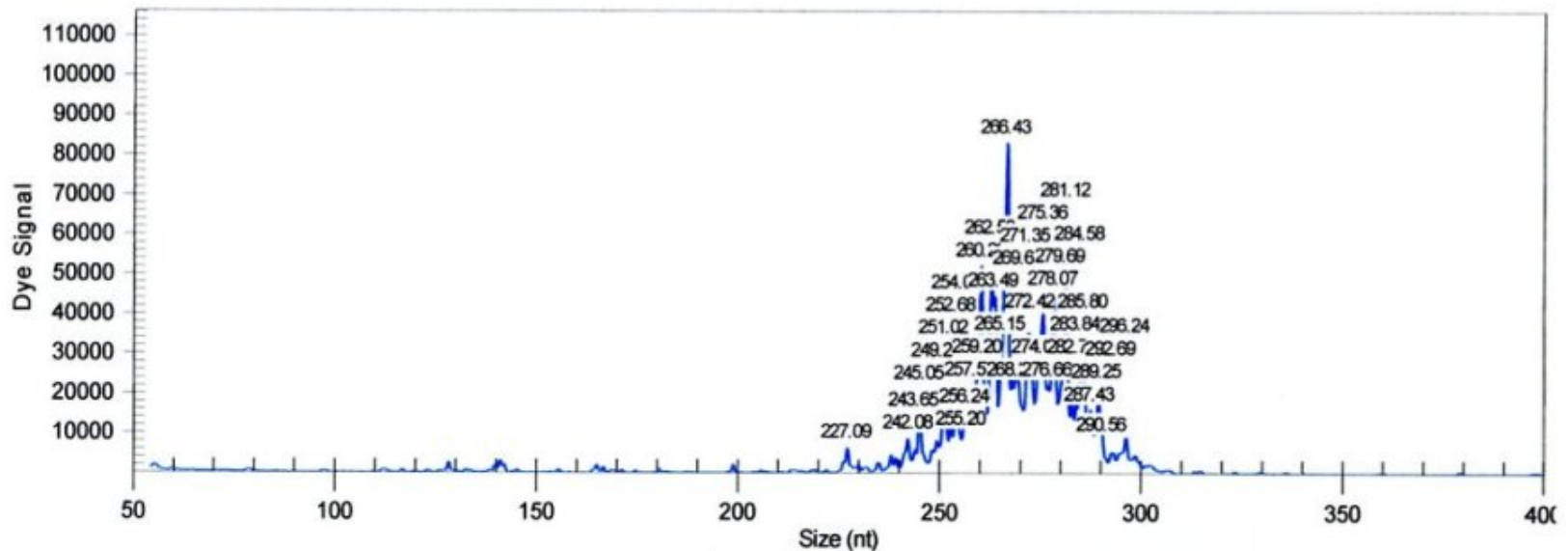
- FFPE lymph node
- Histology and immunohistochemistry suggest angio-immunoblastic T-cell lymphoma
- High content of EBER+ve large B-cells
- PCR for *IGH* and *TCR* rearrangements

BCCLON

ELA013\_W4720\_FR1.F03\_10092408KQ **Dominant clone in polyclonal background**

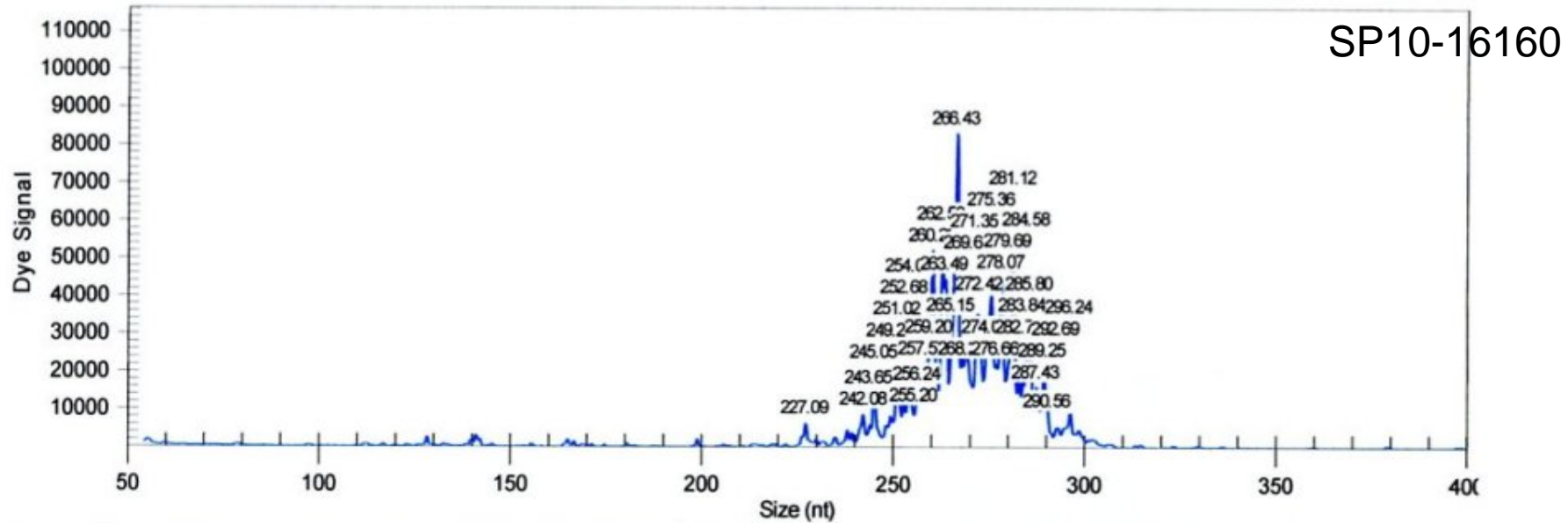


ELA013.W4720\_FR2.G03\_10092408KS **?Dominant clone in polyclonal background**

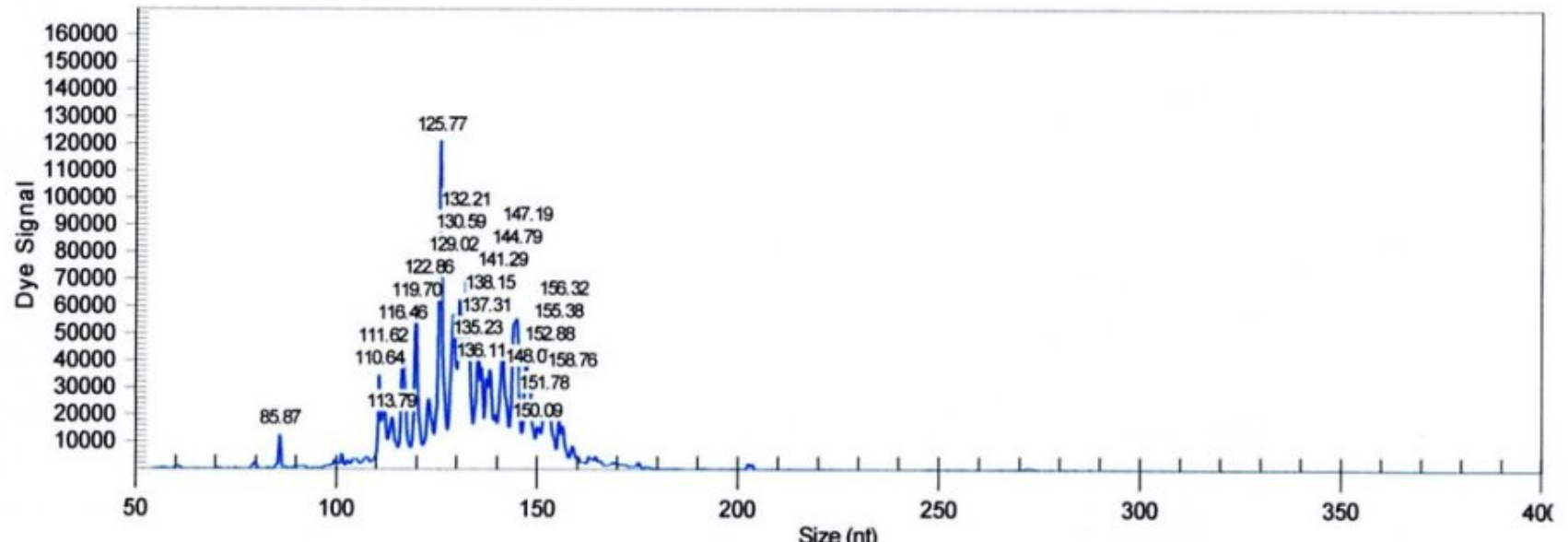




# ELA013.W4720\_FR2.G03\_10092408KS ?Dominant clone in polyclonal background

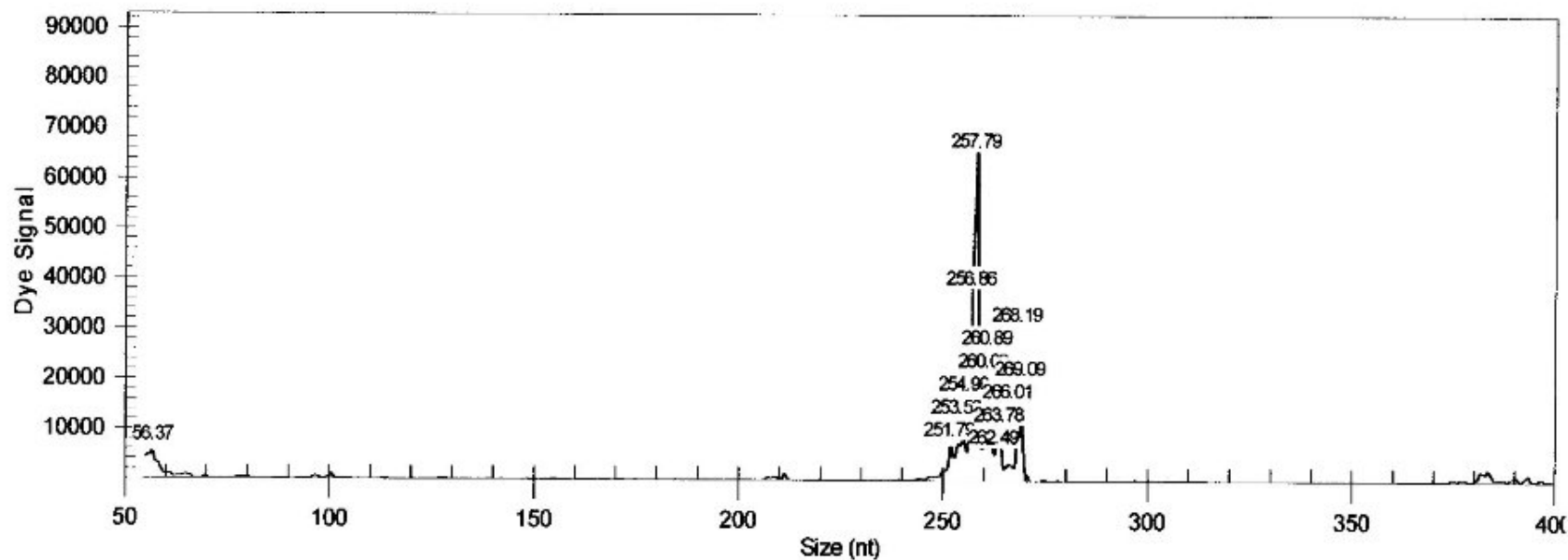


# ELA013\_W4720\_FR3.H03\_10092408KU with dye4 Polyclonal (??DC)



ELA013\_W4720\_BA.A08\_10092408TH with dye4

Clonal



ELA013\_W4720\_BB.B08\_10092408TI with dye4

Clonal?

