MAPPING HUMAN T-CELL LEUKEMIA VIRUS TYPE I INTEGRATION SITES IN THE HUMAN GENOME

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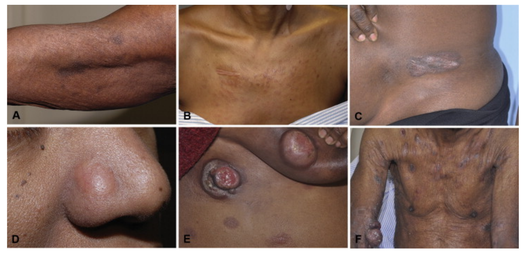
INTRODUCTION

- Human T-cell leukemia virus type 1 (HTLV-1) is a retrovirus that affects an estimated 10-20 million people worldwide. The virus is endemic in South America, intertropical Africa, Caribbean, and Southern Japan.
- HTLV-1 causes adult T-cell leukemia/lymphoma (ATLL) in about 5-10% of infected individuals. Aggressive ATLL has a very poor prognosis, with mean survival length of less than 1 year.
- ATLL has frequent cutaneous involvement, which can be highly variable among patients. Lesions can appear as nonspecific erythematous patches, nodular tumors, and erythroderma, or can manifest as infective dermatitis or crusted scabies.
- An estimated 1-3% of HTLV-1 infected individuals develop a chronic inflammatory disease of the CNS, known as HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP).
- Rapid and cost-effective diagnostic methods for HTLV-1 testing are not widely available, particularly in endemic regions. While CRISPR-based molecular sensors have been deployed as effective research tools in other viral diagnostic assays, HTLV-1 has not yet been explored as a target.



Marchetti MA, Pulitzer MP, Myskowski PL, et al. Cutaneous manifestations of human T-cell lymphotrophic virus type-1-associated adult T-cell leukemia/lymphoma: a single-center, retrospective study. Journal of the American Academy of Dermatology. 2015 Feb 1;72(2):293-301.

STUDY AIM

The primary purpose of this project is to map the integration sites of the HTLV-1 virus in the human genome for the purposes of HTLV-1 diagnostics.

METHODS AND RESULTS

Using CIRCLE-Seq and Cas9 to map integration sites

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Design of chromosomal primers flanking viral integration sites

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Design of HTLV-1 sequencing primers

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LongAmp PCR using chromosomal primers

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Gel extraction

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Cloning

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Plasmid purification

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Restriction digest and PCR

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Sanger sequencing

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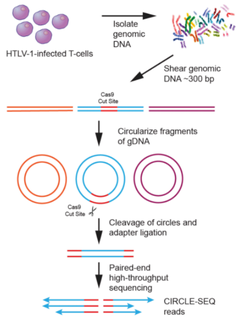
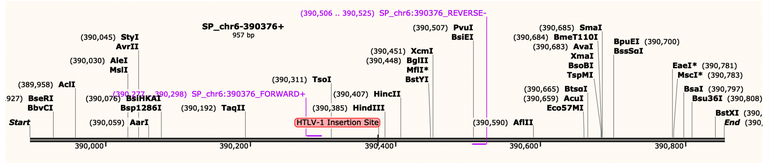
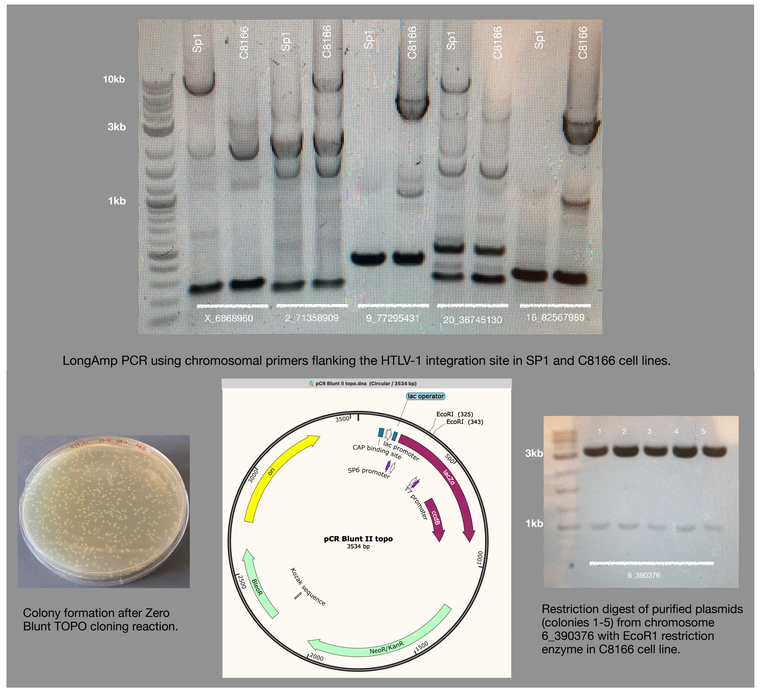
Configuration of aligned sequence



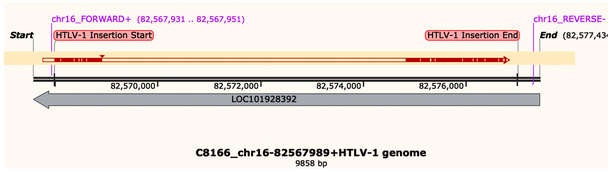
- Compile Genome (HTLV-I & Hg19)
- Map CIRCLE-Seq Reads with BWA
- Flag Chimeric Reads (HTSeq)
- Confirm Split Reads Mapping to HTLV-I (PCR/Sanger)
- Confirm Integration Sites (WGS)



Design of chromosomal primers flanking an HTLV-1 integration site on chromosome 6 of the human genome.



LongAmp PCR using chromosomal primers flanking the HTLV-1 integration site in SP1 and C8166 cell lines.



Construction of sequence for integrated HTLV-1 in chromosome 16.

DISCUSSION AND FUTURE DIRECTIONS

- In this project, we identified the sequence of integrated HTLV-1 in three different chromosomes of the human genome.
- Confirmation of HTLV-1 integration sites in the human genome lays the foundation for:
  1) design and testing of sensitive, specific, and cost-effective CRISPR-based diagnostics
  2) design of a CRISPR system that can target HTLV-1 in human cells for therapeutic purposes
- CRISPR-Cas12a/guide RNA ribonucleotides have been shown to function as molecular sensors in other viral diagnositic assays, with the potential for use in multiplexable, portable, rapid, and quantitative detection platform of nucleic acids. HTLV-1 can be leveraged as a target of these CRISPR-based diagnostic assays.
- The CRISPR/Cas9 system has been demonstrated to disrupt HIV-1 provirus by blocking its expression and removing internal viral genes from the host cell chromsome. A similar system can be designed that targets HTLV-1.
- Future work should be done to identify the sequences of integrated HTLV-1 in additional target chromosomes.

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