


Creating a Cancer-free World. One Person, One Discovery at a Time.

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# FuSpot: A Web-based Tool for Visual Evaluation of Fusion Candidates

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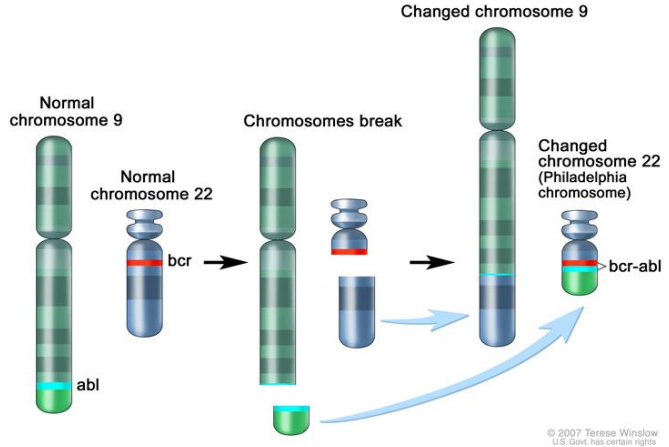
# Outline

- Fusions and Cancer
- Fusion Detection
- FuSpot
- Methodology
- Case Study

# Gene fusions are intimately linked with cancer.

- First genetic defect linked with cancer was t(9;22)
  - Philadelphia Chromosome → CML
- Created hybrid gene BCR-ABL
  - Dysregulated tyrosine kinase producer
  - Rapid cell division, oncogenic

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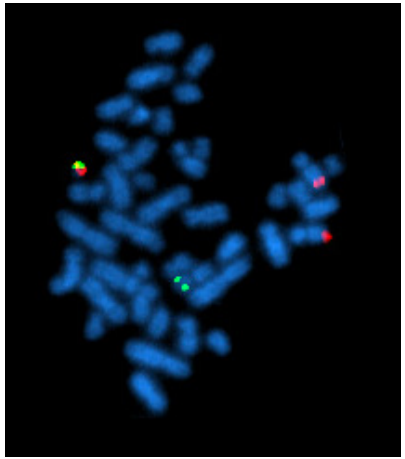
# Studying fusions leads to better therapies.

- Chemotherapy ineffective, transplants dangerous
- Developed BCR-ABL specific drug
  - Tyrosine kinase inhibitor – Imatinib
- Long term survival increased from 30% to ~90%[1]
  - 98% complete response, 17% relapse rate
- Also discovering prognostic markers
  - *PAX-FKHR* risk group stratification in ARMS[2]
  - Similar implications for *TMPRSS2-ERG* in Prostate Carcinoma[3]




1. Druker BJ, et al. New England Journal of Medicine 355, 2408–2417 (2006)
2. Sorensen PH, et al. J Clin Oncol. 2002 Jun 1;20(11):2672-9.
3. Berg KD. Dan Med J. 2016 Dec;63(12). pii: B5319.

# Many fusion detectors are available for download.

- Therapy implications + advent of RNA-seq led to development of many sequencing-based fusion detection tools (≥30 unique)[4,5]
- Some emphasize specificity, but sacrifice sensitivity
  - Trusted results, but miss true positives
- Others trade specificity to ensure good sensitivity
  - Large numbers of False Positives
  - Time consuming and costly PCR validation

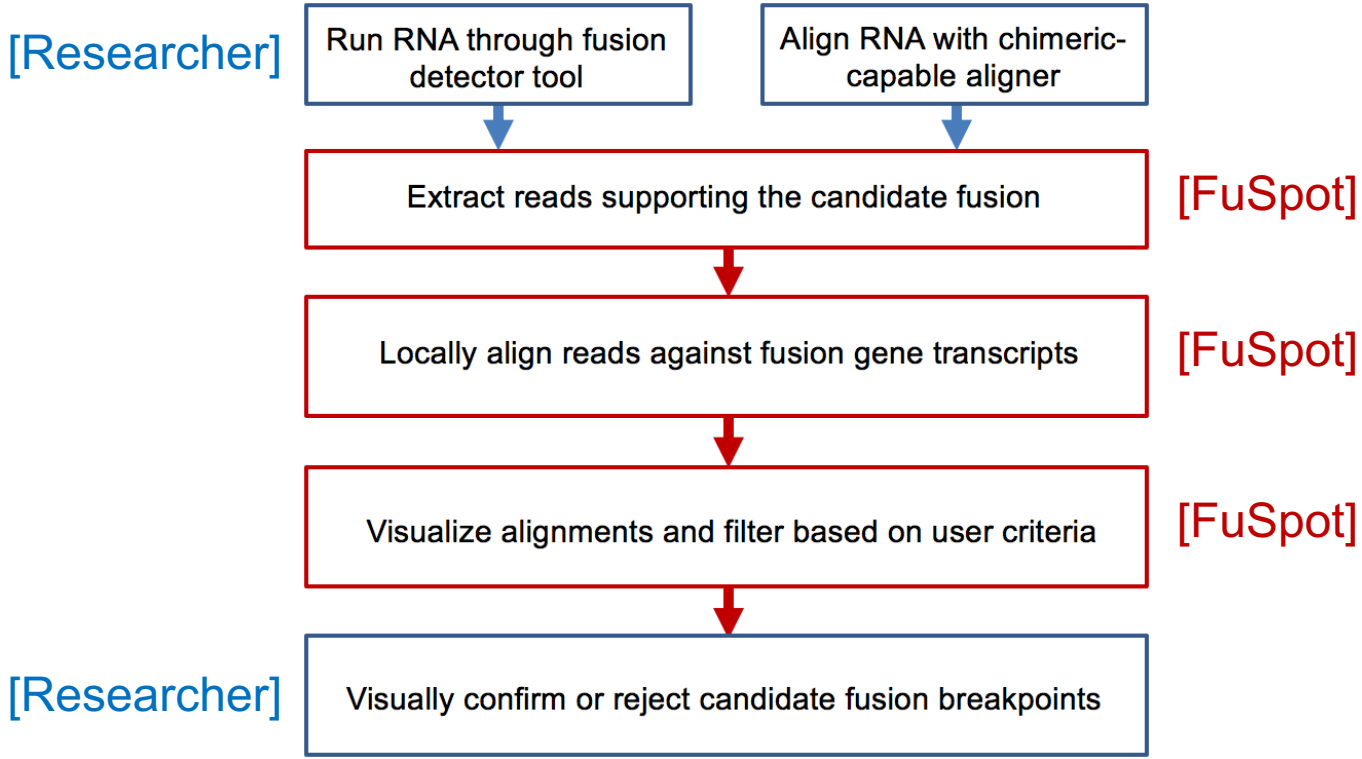

4. Liu et al. Nucleic Acids Res. 2016; doi:10.1093/nar/gkv1234.
5. Kumar et al. Wiley Interdiscip Rev RNA. 2016 Nov;7(6):811-823. doi: 10.1002/wrna.1382

# Our tool, FuSpot, accelerates discovery.

- **The Problem**: Difficult to choose tool that will get all true positives without overload of false candidates

- **Solution**: Use a sensitive tool + our tool **FuSpot** to filter out false positives with little time and no experimental cost

# FuSpot has an intuitive workflow.

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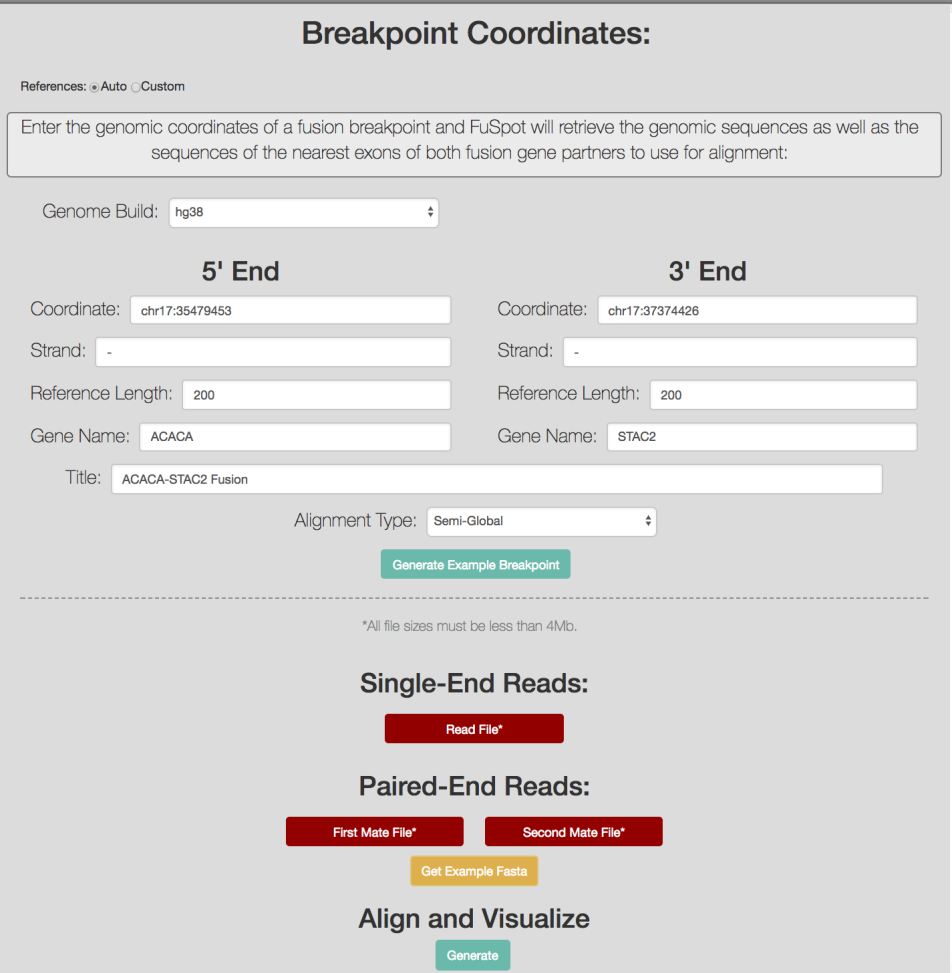


# FuSpot Interface

- Inputs

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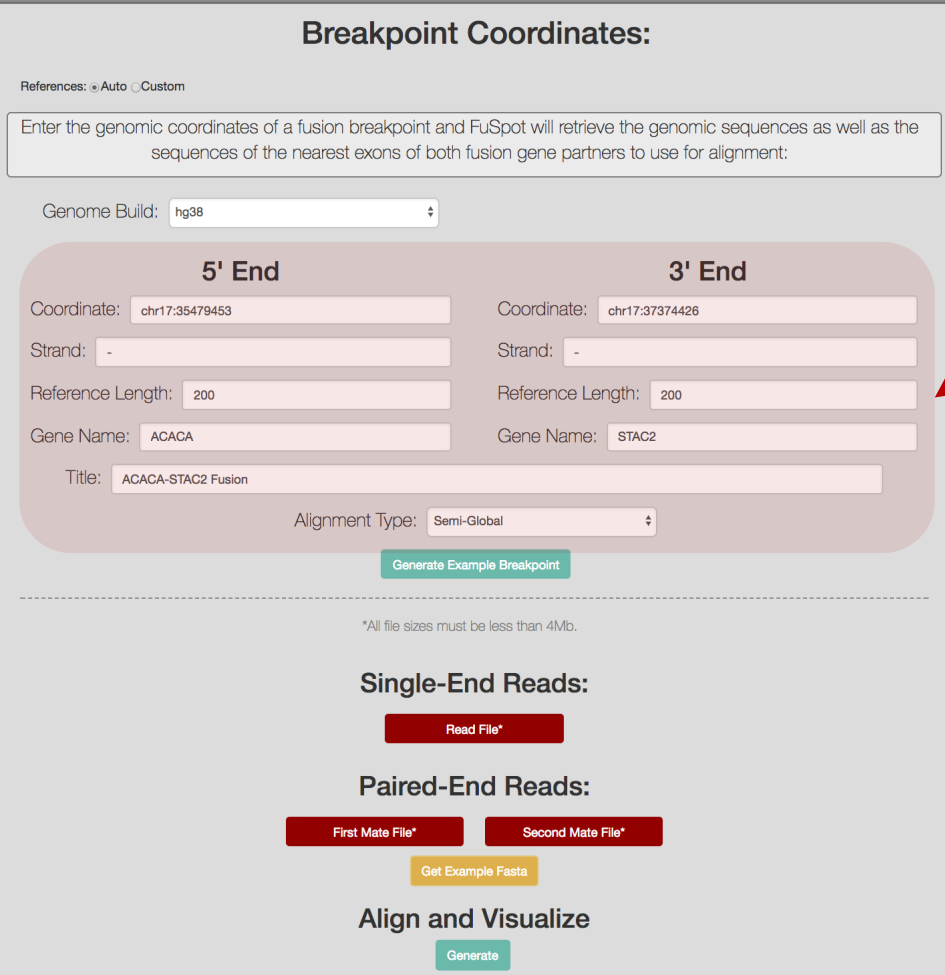


# FuSpot Interface

- Inputs
  1. Genomic Coordinates of Breakpoint

Ex.

Chr2:35479453 +

Chr17:27374426 -

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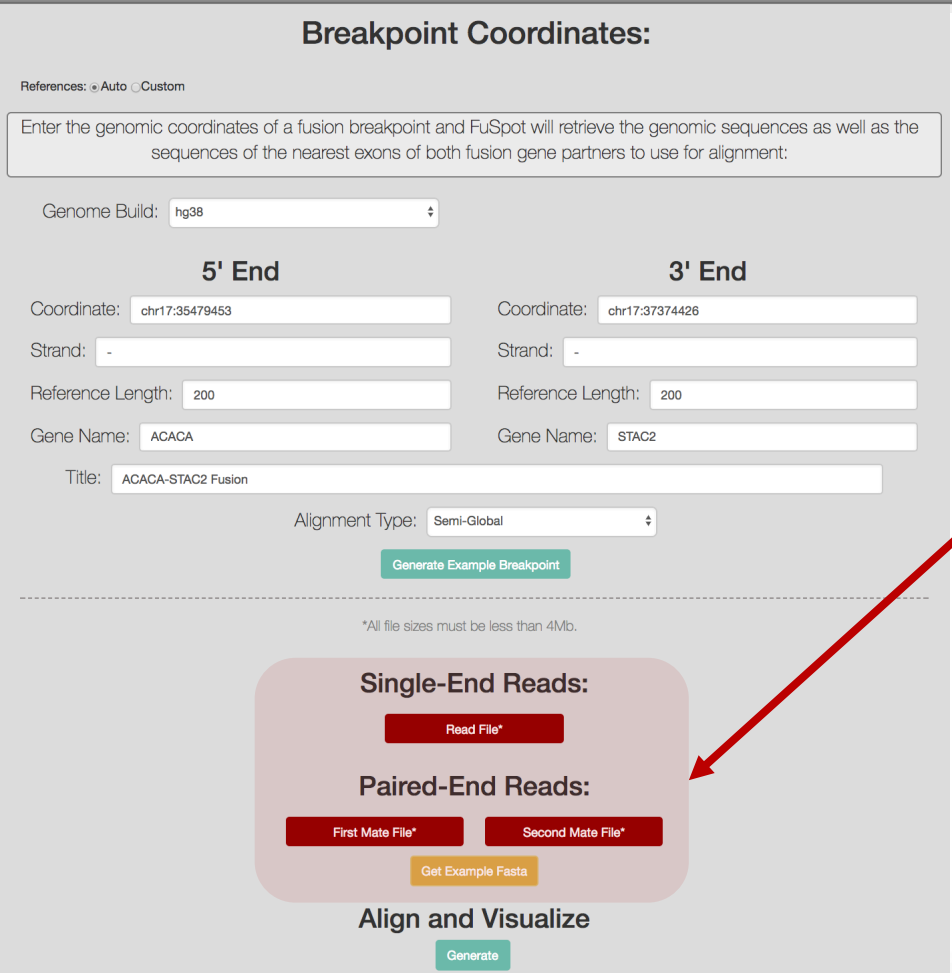


# FuSpot Interface

- Inputs
  1. Genomic Coordinates of Breakpoint
  2. Reads adjacent to the breakpoint*

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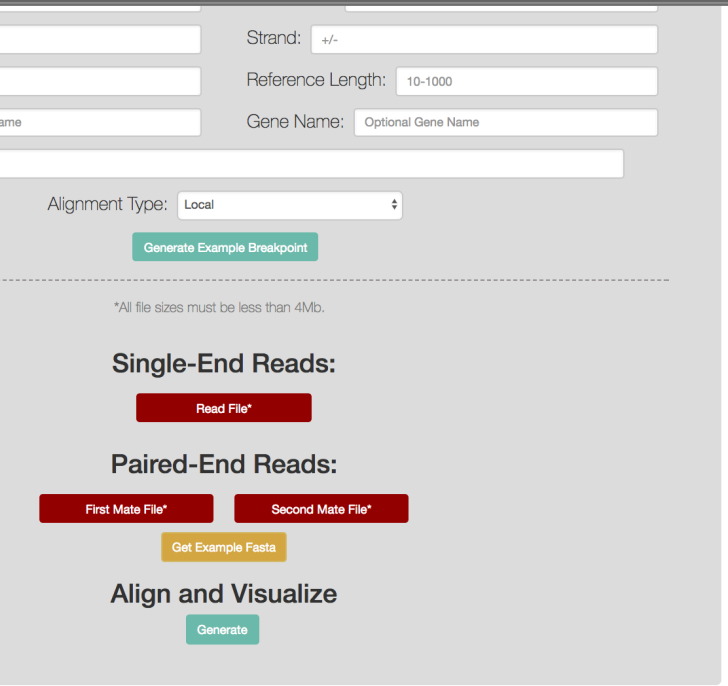


# FuSpot Interface

- Inputs
  1. Genomic Coordinates of Breakpoint
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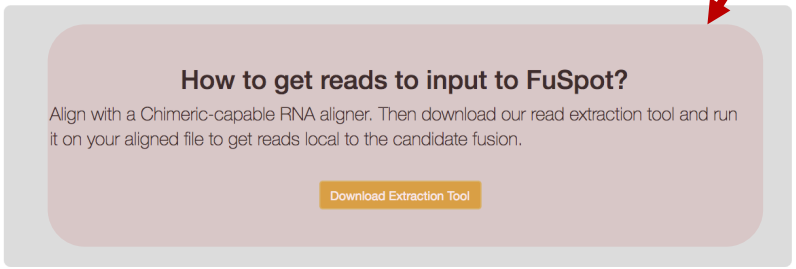
*FuSpot provides extraction tool

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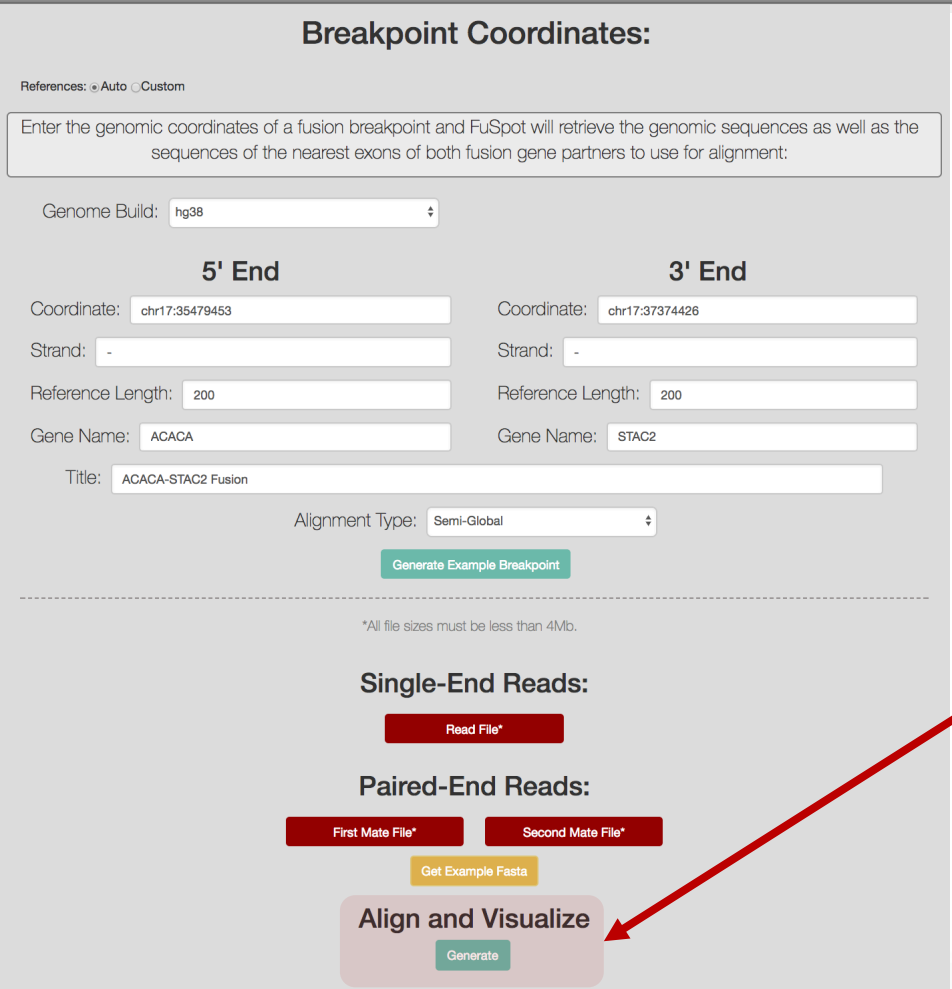


# FuSpot Interface

- Inputs
  1. Genomic Coordinates of Breakpoint
  2. Reads adjacent to the breakpoint*

*FuSpot provides extraction tool

- **Align and Visualize**

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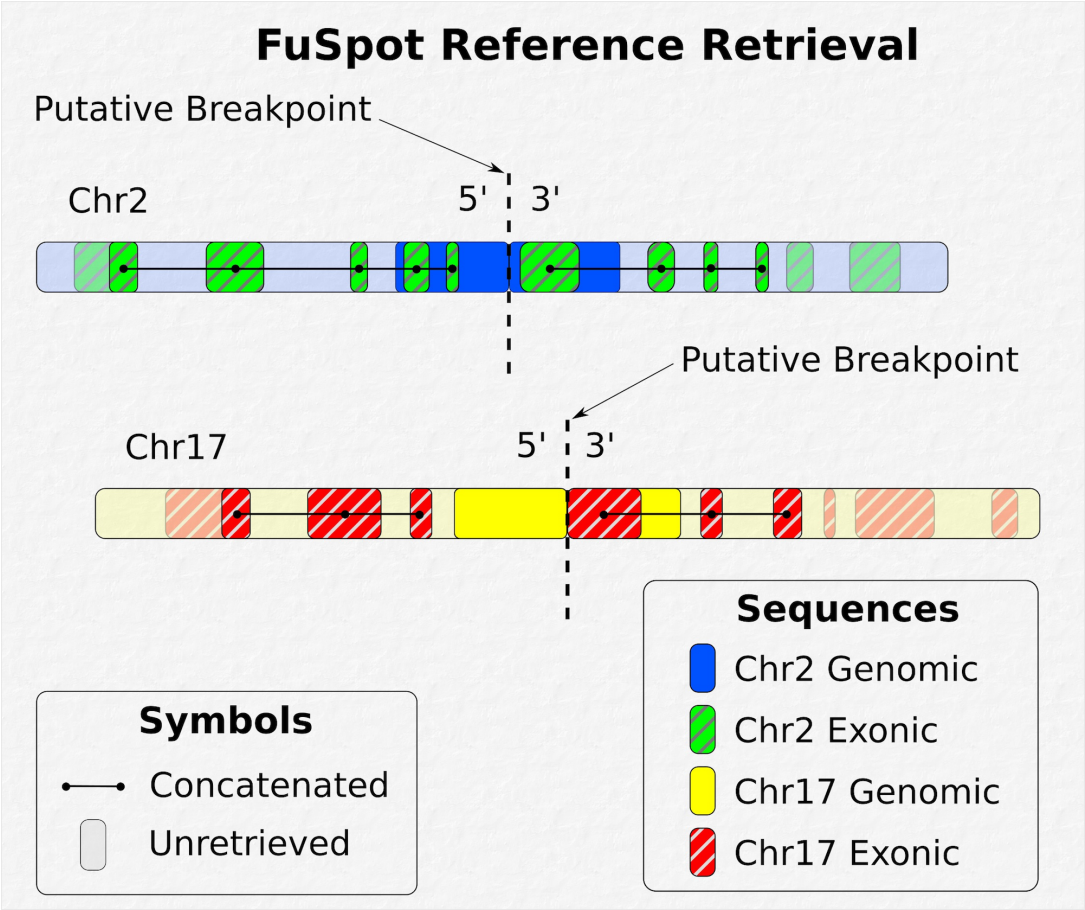
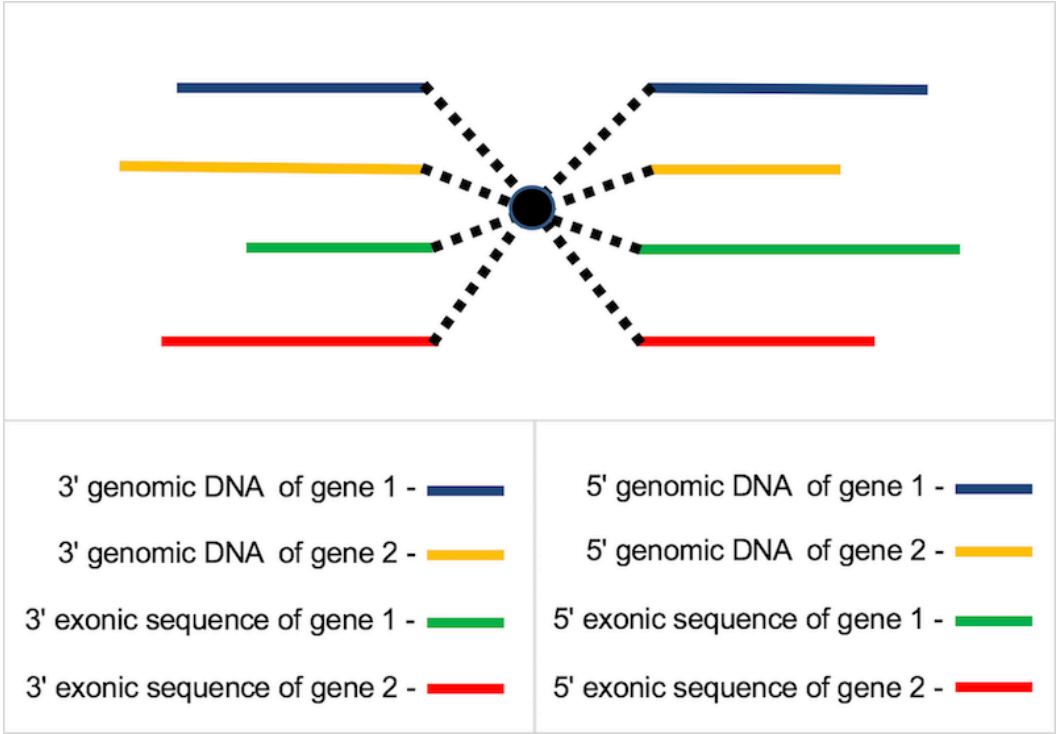
# Reference Extraction

- Gather sequences of each gene partner
- Collect bases outward in both directions from breakpoint
  - Genomic
  - Exonic
- UCSC canonical exons



# FuSpot Alignment

- References:
  - Automatic Retrieval
    - Human or Mouse
  - Custom
    - Any organism
    - Any combination of gene transcripts
- Simultaneous alignment to each reference
- Determine right-to-left connection (if any)

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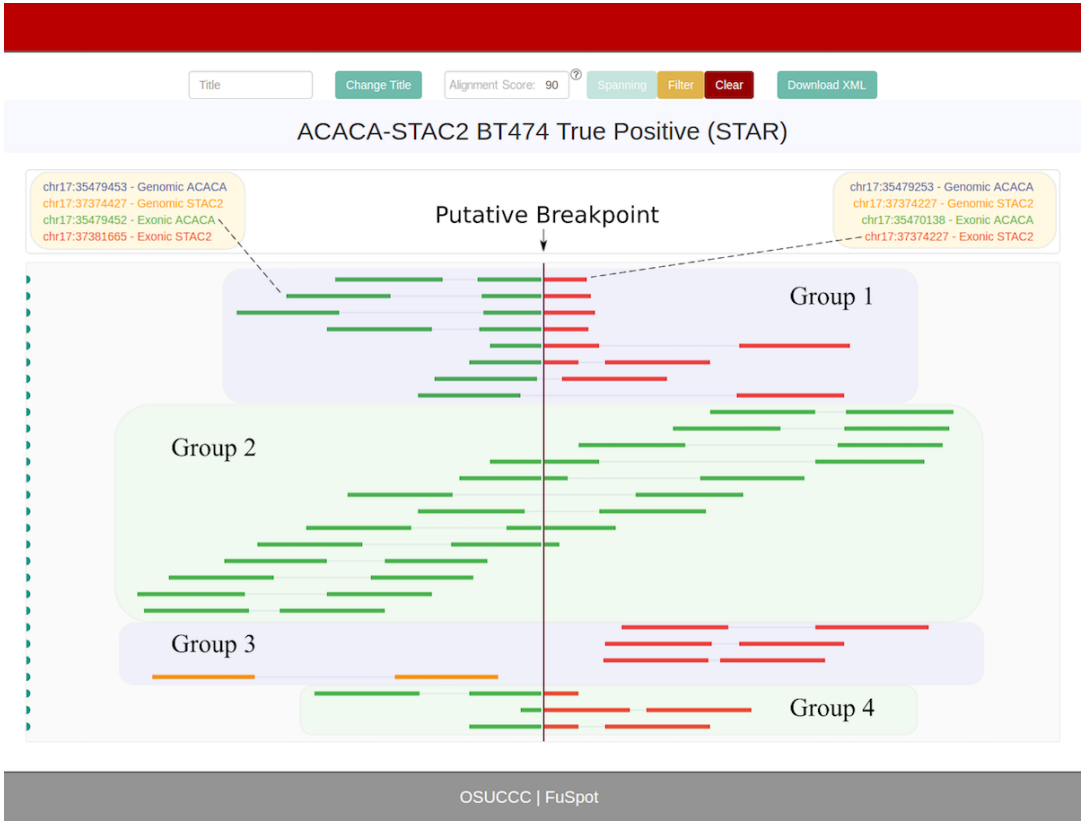
# Case Study: Edgren et al.[6] and BEERS[7]

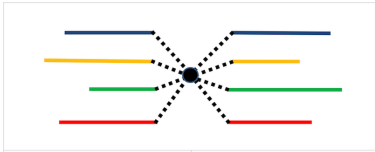
- Edgren et al. contains several well known fusions
  - True Positive case
- BEERS is synthetic dataset containing no fusions
  - False Positive case
- Ran FusionCatcher and FusionMap on both sets


6. Edgren et al. Genome Biology. 2011;12(1). doi:10.1186/gb-2011-12-1-r6.
7. Grant et al. Bioinformatics. 2011. doi:10.1093/bioinformatics/btr427.




# True Positive Case

- Reported by both tools
- Support breakpoint
  - Group 1+4
- Non-fusion Reads
  - Group 2+3
- Well supported breakpoint



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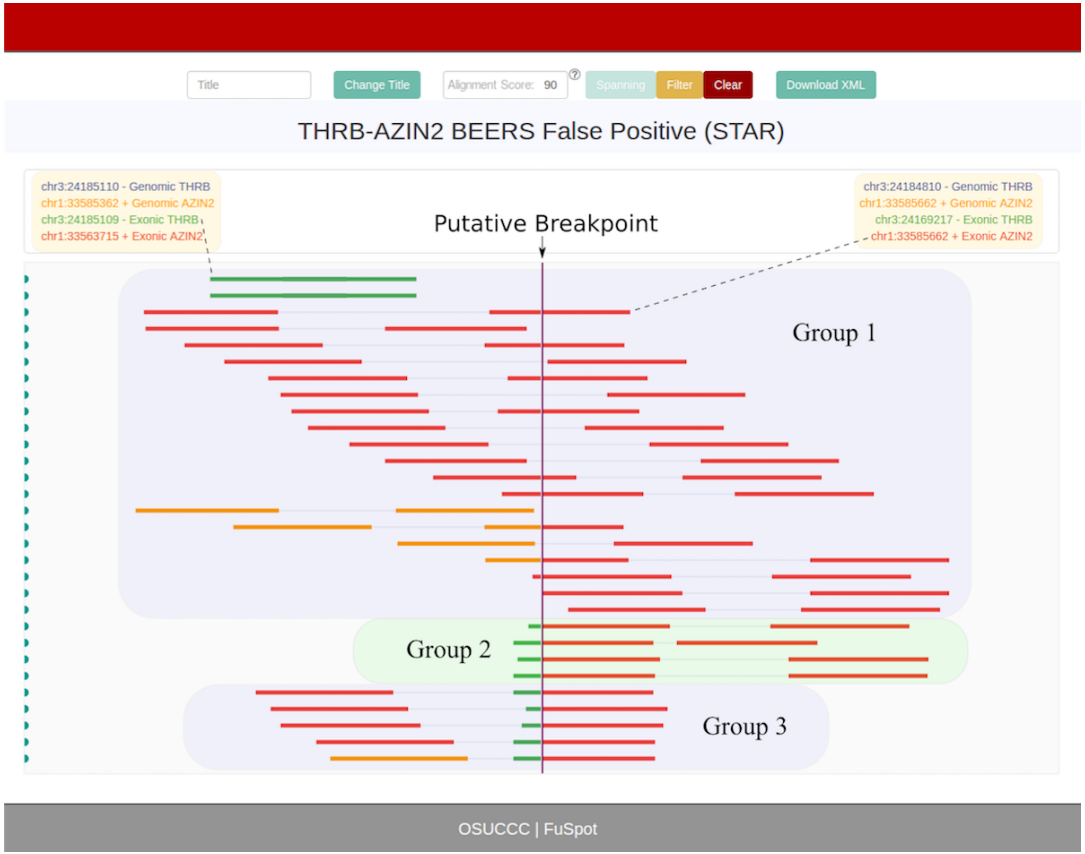
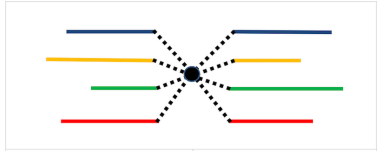



# False Positive Case

- Reported by FusionMap
  - Claims 9 supporting reads
- Non-fusion reads
  - Group 1
- Seem to support breakpoint
  - Group 2
- No biological sense
  - Group 3
  - Suggests homology between fusion partners



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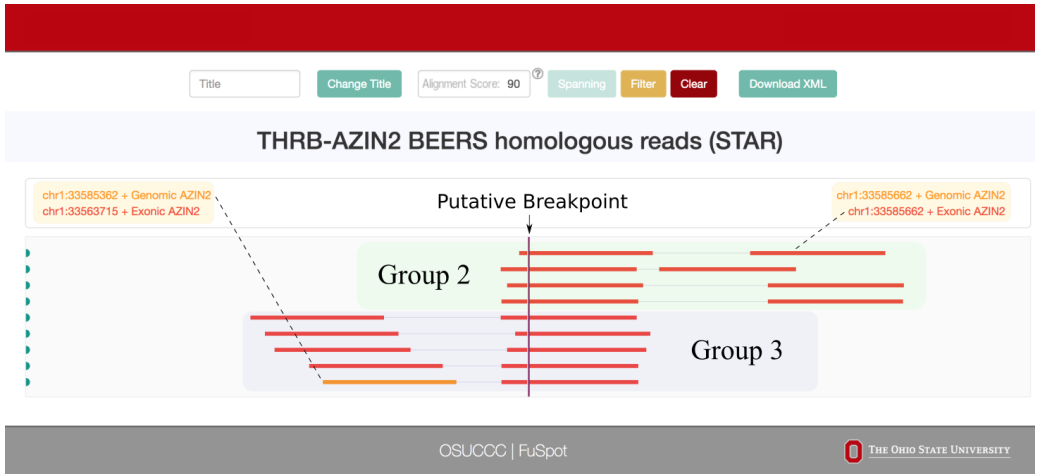
# Realign Groups 2+3




- Align using only 5' partner references
- All reads align → Sequence homology!
- This is what tricked the detector
- Confidently categorize as False Positive

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# FuSpot solves the tradeoff problem.

- Fusion Detectors vary in tradeoff between specificity/sensitivity
  - Risk missing important fusions
  - Or spend resources validating false positives
- FuSpot visualizes evidence in easily digestible form
- Enables convenient and rapid inspection by researcher

# FuSpot is online and facilitating research.

- FuSpot takes advantage of human reasoning to eliminate tedious post-processing
- FuSpot will result in more fusions to be identified at lower overall cost
- Already supporting research in The James
  - Dr. He and Dr. de la Chapelle
  - Dr. Cynthia Timmers, Director of The Solid Tumor Translational Science Shared Resource at OSUCCC

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# Thank You

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http://bioserv.mps.ohio-state.edu/FuSpot/

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