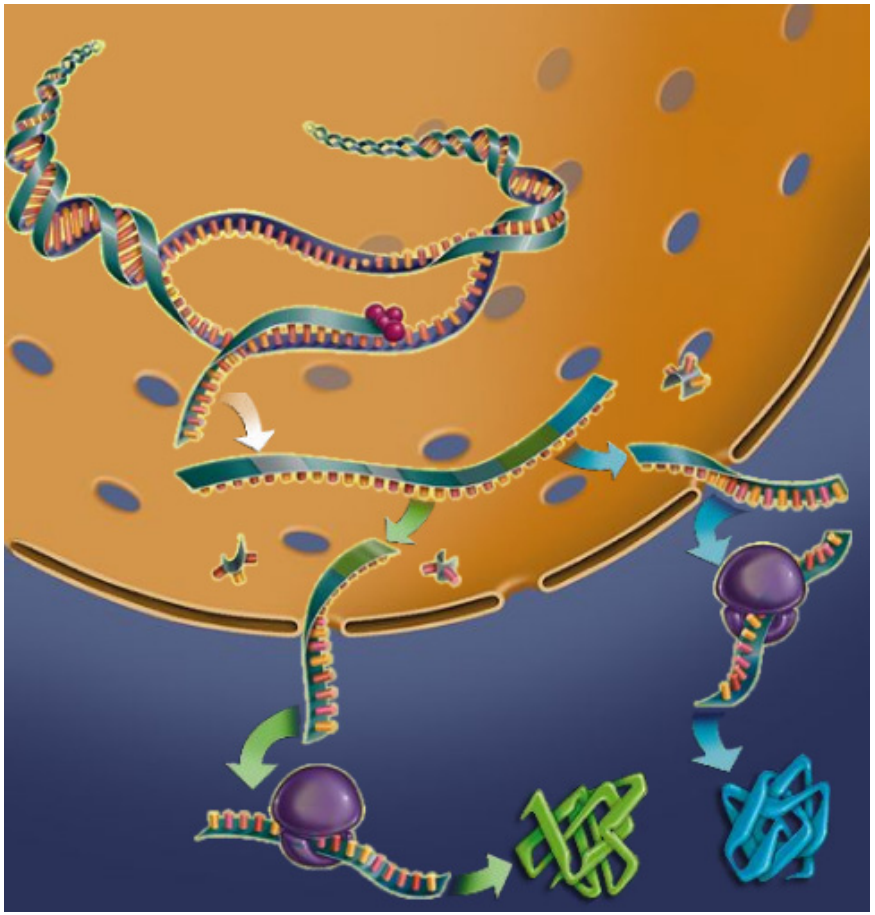
Microarray analysis of alternative transcription



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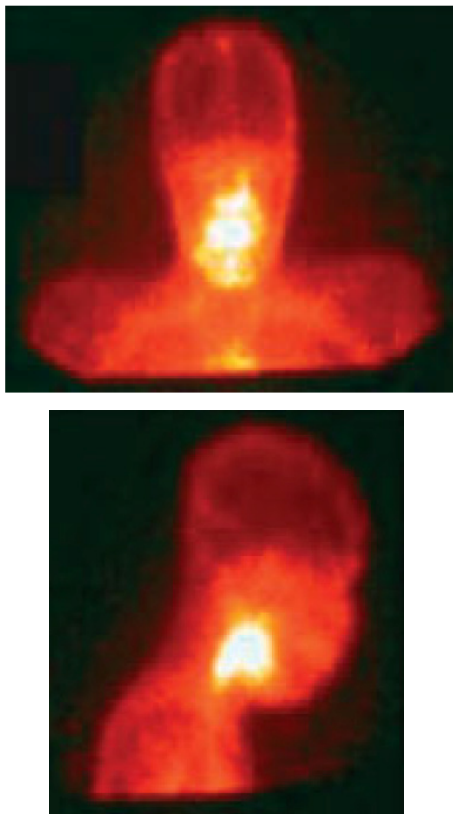
GSC Science Retreat – Nov. 2 2006

Background and rationale

- Alternative transcription generates multiple isoforms from most human loci
  - ~75% of human genes are alternatively spliced (Johnson 2003)
- Specific isoforms may represent useful therapeutic targets or diagnostic markers
- Recent developments in microarray technology allow the efficient detection of specific isoforms
  - A) Affymetrix exon microarrays
  - B) Custom splicing microarrays

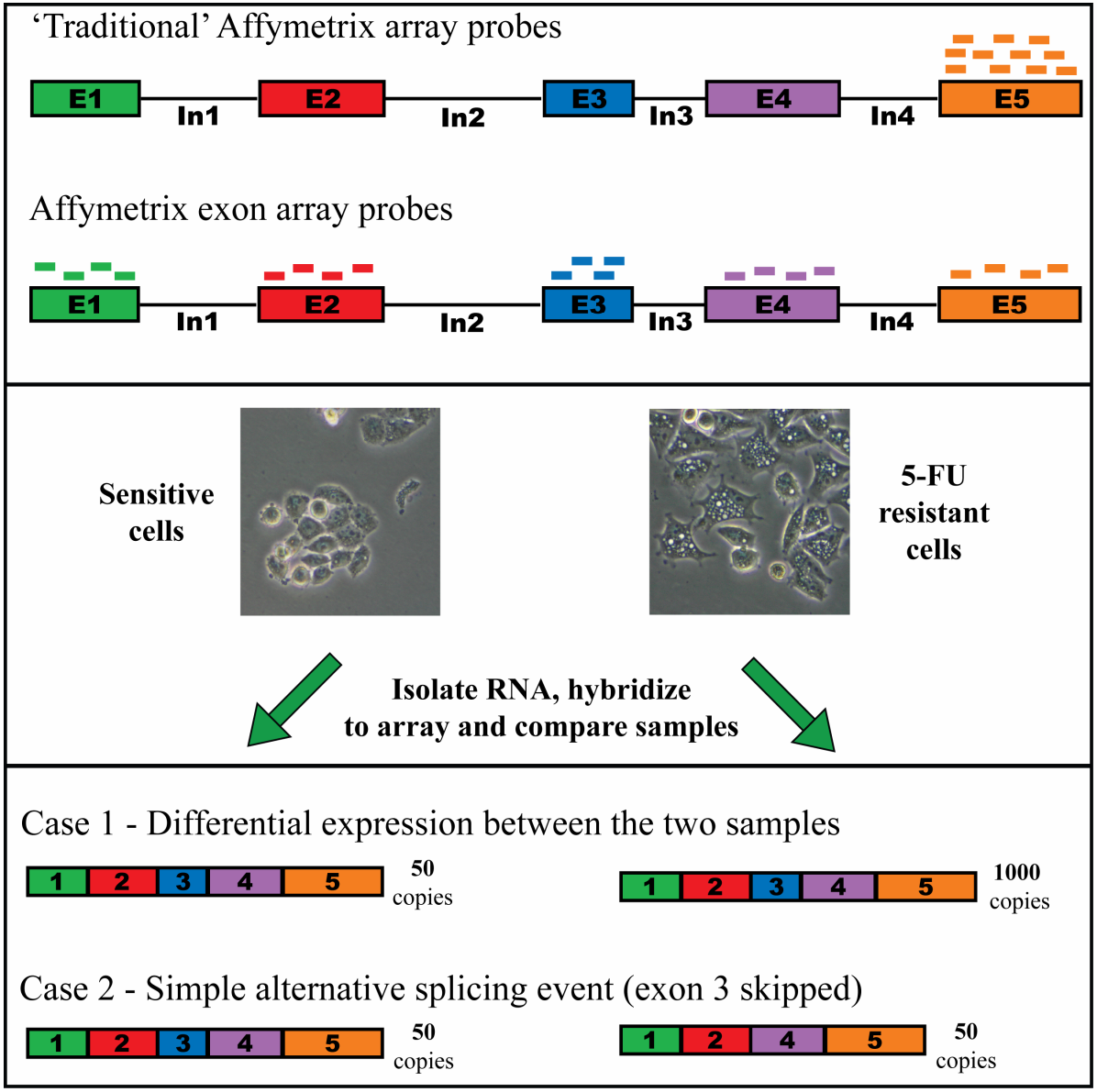


Therapeutic antibody specific to CD44 alternate exon 6 (Venables, 2006)

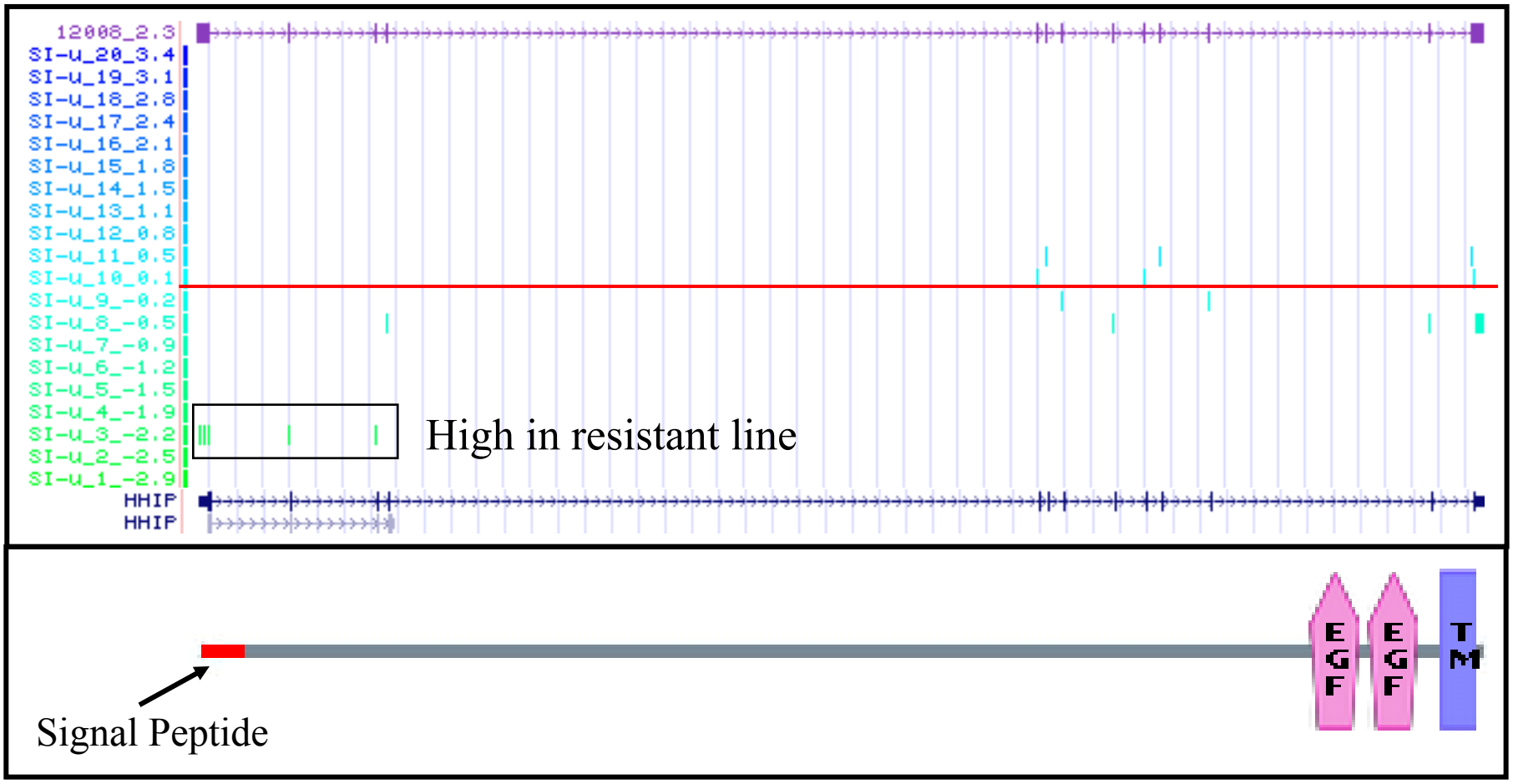
A) Description of Affymetrix exon arrays

- Array design (Human exon 1.0 ST)
  - ~150,000 loci
  - ~5.5 million probes (25-mer oligos)
  - ~1.4 million exons (4 probes per exon).
    - ~60% of these exons are 'speculative'
- Samples
  - 1-5 μg total RNA input
- Analysis
  - Identify differential transcription events corresponding to one or more exons

Affymetrix exon array strategy



5-FU resistance is associated with over-expression of a short HHIP isoform

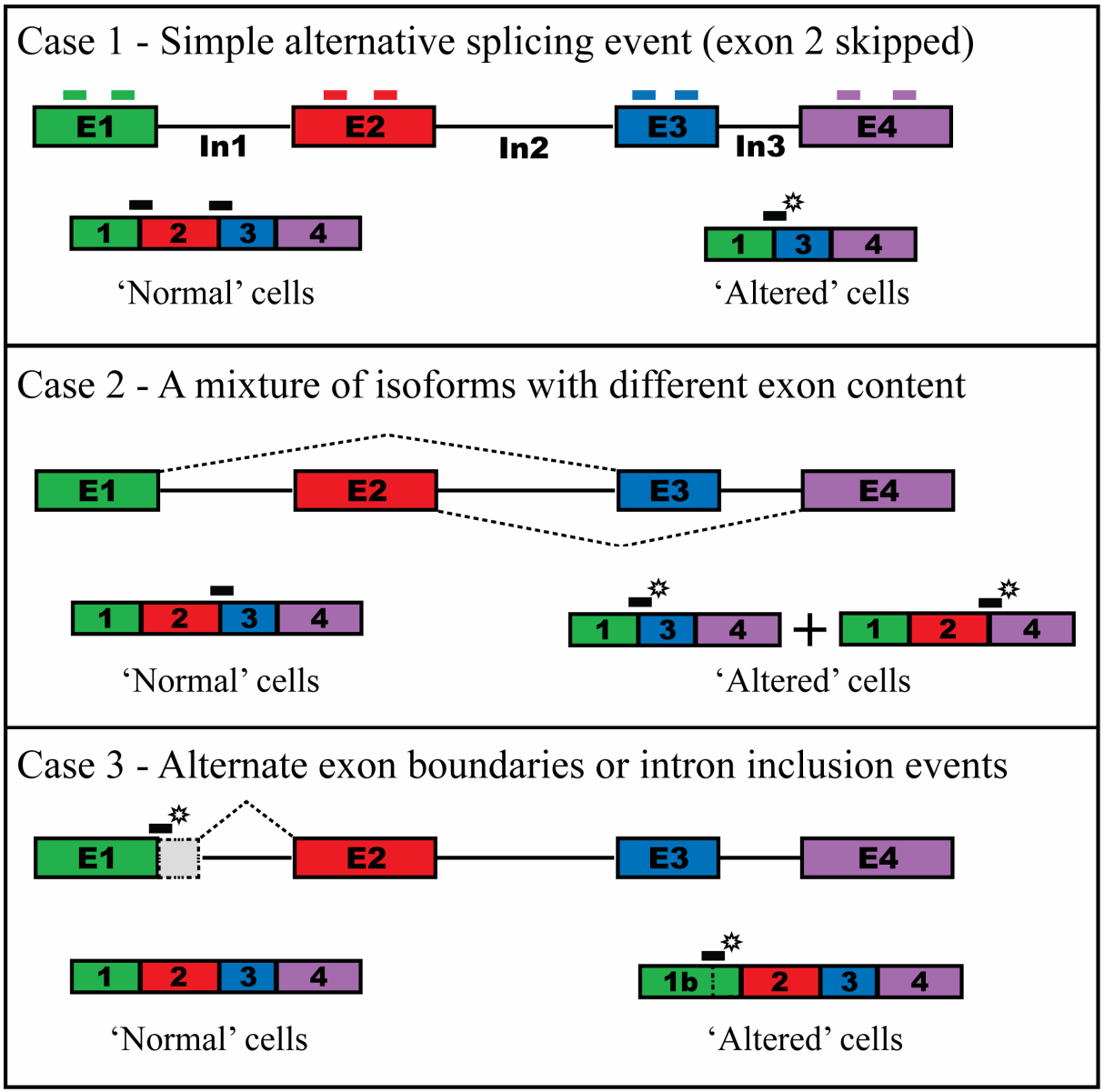


HHIP – Hedgehog interacting protein, a HH antagonist

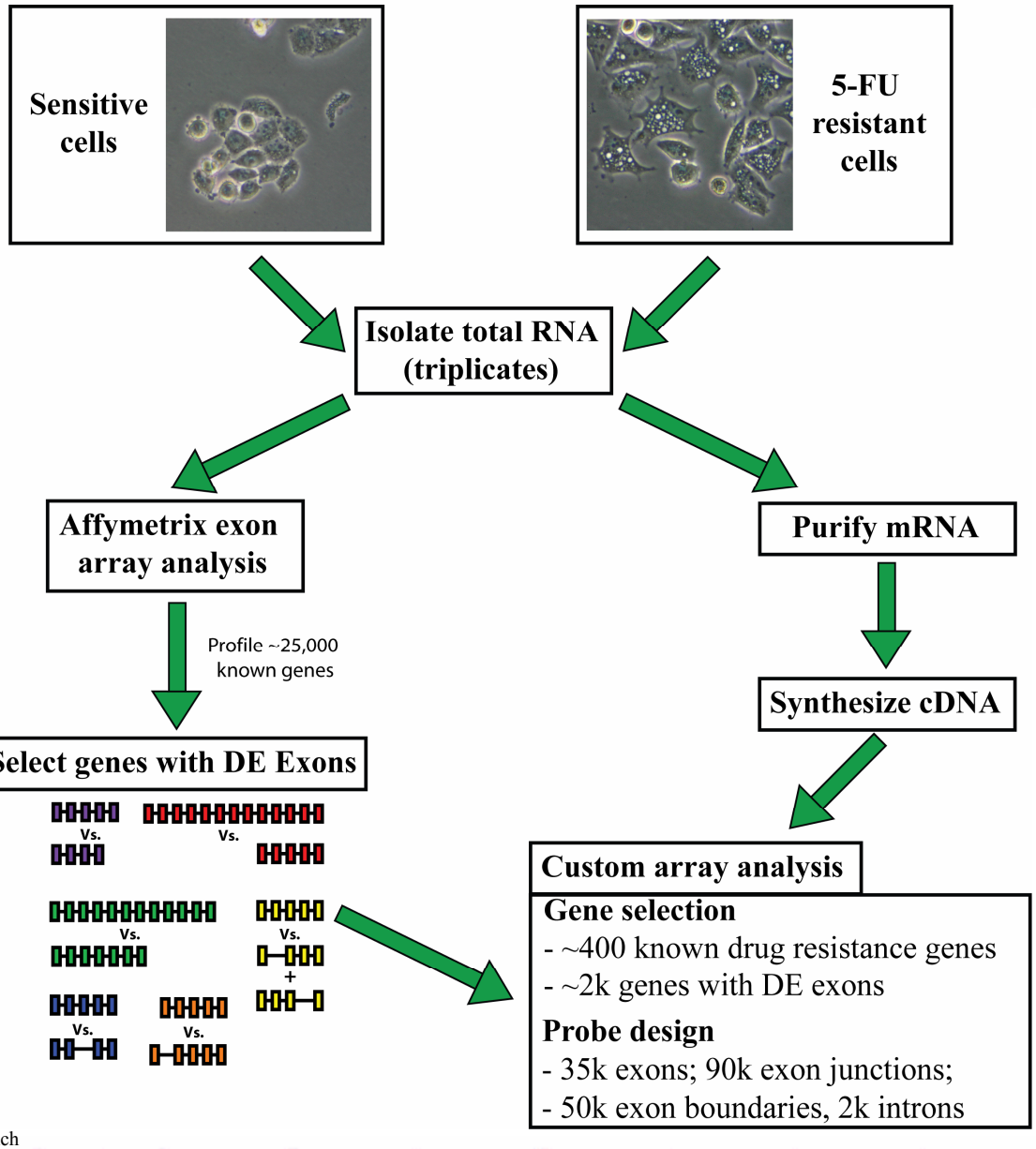
B) Description of ALEXA platform

- Array design
  - ALEXA platform (Perl, R, mySQL)
    - Works on any EnsEMBL genome
  - Extract probes corresponding to all exons, exon-exon junctions, exon boundaries and introns
    - Vary probe length to achieve target Tm (e.g. 36 bp +/- 10 bp)
  - Filter probes for specificity and thermodynamic properties
  - Select ~400,000 probes and submit to NimbleGen
- Samples
  - 4 µg mRNA (polyA+) input
- Analysis
  - In addition to identifying differential exons, the connections and boundaries of exons are interrogated

ALEXA splicing array strategy



Affymetrix versus custom ALEXA arrays



Conclusions

- Preliminary experiments with Affymetrix exon arrays seem promising
  - Identify differential expression of novel and known isoforms. 35 events with 4-fold change or greater
    - 25 affect ORF
    - 9 in 5′ UTR and
    - 1 in 3′ UTR
  - Low cost, low sample requirements, fast turn-over
- Proof-of-principle custom ALEXA experiments with NimbleGen arrays are also encouraging
  - Moderate cost, high sample requirements, moderate turn-over, customizable, open source, species generic
- Experiments to directly compare platforms underway

Acknowledgements

Marco Marra
Joseph Connors
Stephane Flibotte
Steve Jones
Gregg Morin

Prostate Cancer Group
- Marianne Sadar
- Tammy Romanuik

Bioinformatics
- Obi Griffith, Anca Petrescu

MGC Group
- Martin Hirst, Thomas Zeng

GI Cancer Group
- Isabella Tai, Michelle Tang

Affymetrix Group
- Agnes Baross, Susanna Chan, Jennifer Asano

Proteomics Group
- Gregg Morin, Grace Cheng

Michael Smith Foundation for Health Research

GenomeBritishColumbia

NSERC CRSNG