RGASP workshop

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Analysis pipeline

- Filter input
- Classify pairs
- Spliced alignment
- Group alignments
- Generate output

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Filter input

- Low quality nucleotides -> N
- Trim Ns on both ends
- Limit multiplicative effect
- Detect and eliminate repeats

Classify pairs (1)

- Use fetchGWI
- Find unique exact matches
- Verify paired reads distance and orientation

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Classify pairs (2)

	Pairs 2 matched	Pairs 1 matched	Pairs 0 matched	Pairs non-canonical	Singlet Mapped	Singlet No match	Total
GMr1	7.4 M	19.0 M	58.3 M	1.7 M	0.06 M	0.2 M	86.6 M
GMr2	5.9 M	12.2 M	28.7 M	1.7 M	0.03 M	0.3 M	48.8 M
Kr1	5.8 M	18.5 M	45.5 M	1.4 M	0.04 M	0.1 M	71.4 M

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Classify pairs (3)

- Use megablast on unmapped pairs
- Verify distance and orientation

	GMr1	GMr2	Kr1
Pairs 0 matched	58250606	28657924	45522710
megablast	12412160	5801018	11162500

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Spliced alignment (1)

- Use paired information to restrict search area
- Use SIBsim4

Spliced alignment (2)

```
>chr|NC_000001|NC_000001.10 Chromosome 1; LEN=160430423
>tag|4_8_1524_762.1|- clone 4_8_1524_762; LEN=76

160295414-160295442  (1-29)   100% <- (GT/AG) 24
160302238-160302284  (30-76)   100%

        0     .    :    .    :    .    :    .    :    .    :
160295414 ACCAGGGGACAGGTTTTTTTTCCTCAGACCTT...CACCAGAAATATCCC
          |||||||||||||||||||||||||||||<<<...<<<||||||||||||
        1 ACCAGGGGACAGGTTTTTTTTCCTCAGAC         CAGAAATATCCC

       50     .    :    .    :    .    :    .
160302250 AAACGCGCACAGTCTGGTCCAGGCTGGCTGATACT
          |||||||||||||||||||||||||||||||||||
       42 AAACGCGCACAGTCTGGTCCAGGCTGGCTGATACT
```

Group alignments (1)

- Use 'tromer' to combine alignments
- Use paired reads information
- Predict transcripts

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Group alignments (2)

```
>map|NC_000001_37|NC_000001.10|+ Chromosome 1 541739..567794
H0 [541739] 0,1
 =E0=> D0 1,7
D0 [541752] 1,1
 H0 1,7 =E0=>
 -I0-> A0 1,7
A0 [567506] 1,1
 D0 1,7 -I0->
 =E1=> T0 2,7
T0 [567794] 1,0
 A0 2,7 =E1=>

E0 541739..541752 0,1
 t:4_57_813_1318.2 6..19 (541739..541752) 85%
E1 567506..567794 0,2
 L:4_50_1286_288 1..253 (567542..567794) 100%
 t:4_57_813_1318.2 20..76 (567506..567561) 98%
I0 541753..567505 1
 t:4_57_813_1318.2 19..20 GT/AG 7
```

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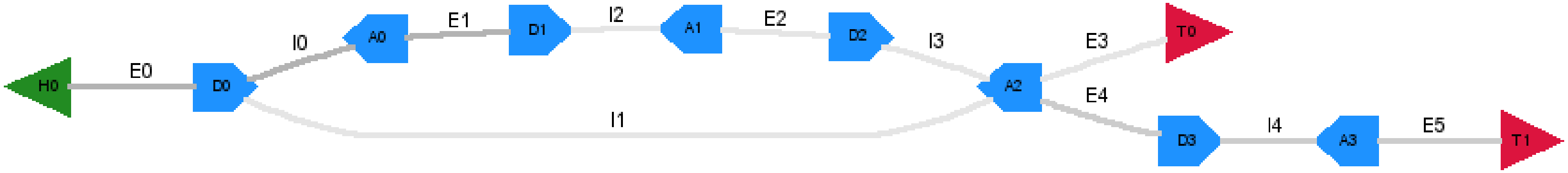
Group alignments (3)

	Reads	Pairs	Graphs
GM12878 r1	35,210,742	18,189,056	835,017
GM12878 r2	22,208,026	11,479,864	497,331
GM12878	57,418,768	29,501,799	895,252
K562 r1	32,250,482	16,661,568	660,902

Generate output (1)



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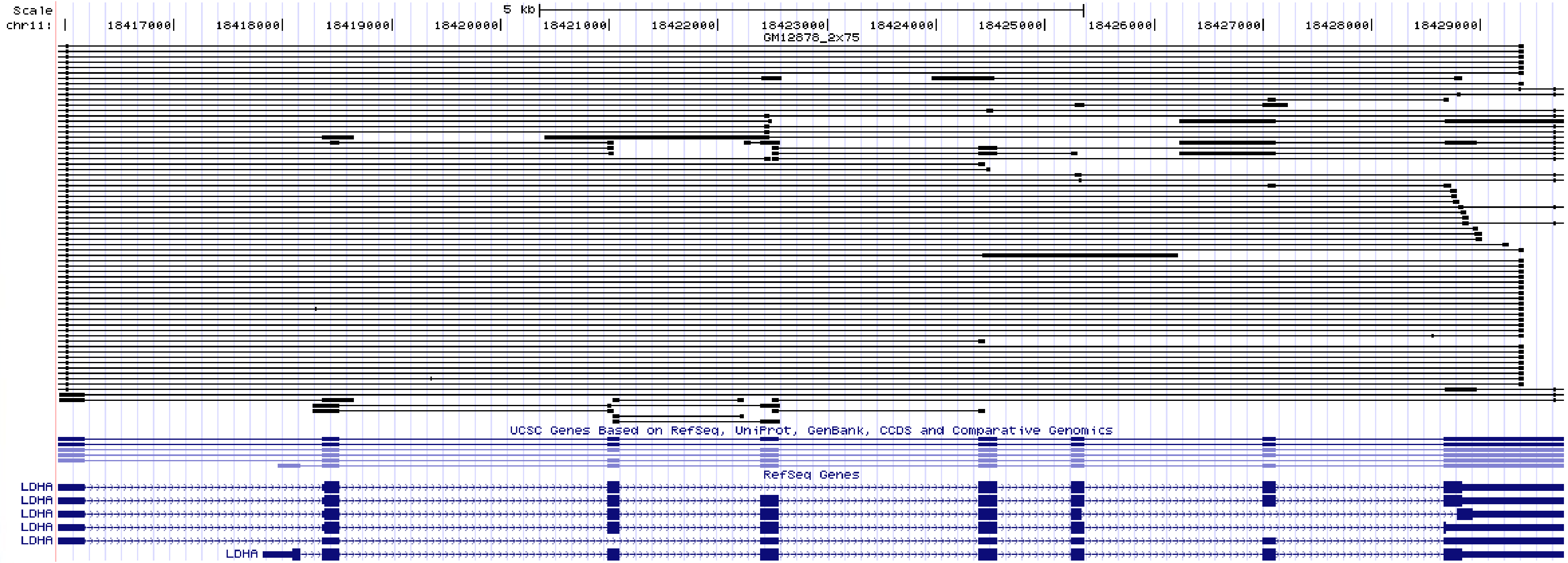
Generate output (2)



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