3. Genomic variation

- 3.1 Genomic variations
- 3.2 SNP calling
- 3.3 Detect structural variations
- 3.4 Application of genomic variations

3.1 Genomic variations

• Types of genomic variations

• Genotyping approaches

Genomic DNA Variation

= variations between the genome of different individuals

Single Nucleotide Polymorphisms (SNPs) = substitutions, short indels (one to a few nucleotides)

Micro- and mini-satellite expansion and contraction (typically less than 100 bp variation)

Transposable Elements insertion/excision (ranging from ~100 bp to less than 10 kb)

Segmental Duplications = Low copy repeats (LCRs) (>1 kb- 3 Mb with similarity >90%) -- include copy number variants (CNVs)

Large chromosomal rearrangements: Mb-range duplication, insertion, deletion, inversion, translocation (microscopic structural variation)

Changes in chromosome numbers = aneuploidy (typically deleterious) (microscopic structural variation)

Types of genomic variation

- **SNP**
- Indel: insertion/deletion
- Structural variation (SV)
- CNV: copy number variation; PAV
- Inversion
- Translocation: intra-/inter-chromosomal
- Duplication
- Rearrangement
- Methylation

Two main types

- SNP (also small indel)
- Structural variation
 - It consists of many kinds of variation in the genome of one species, and usually includes microscopic and submicroscopic types, such as deletions, duplications, copy-number variants, insertions, inversions and translocations.
 Typically a structure variation affects a sequence length about 1Kb to 3Mb, which is larger than SNPs.

Genotyping approaches

- Genome re-sequencing
- Targeted sequence capture: Exome-SEQ

Sequencing

- RAD-SEQ
- RNA-SEQ
- Environmental (mixed) samples

Cytogenetic detection

Cytogenetic detection of structural genomic variation

• FISH

• Array-based Comparative Genome Hybridization (CGH)

CNVs detected by fiber FISH



Array-CGH technology



• Using this method, copy number changes at a level of 5-10 kilobases of DNA sequences can be detected. Today even high-resolution CGH (HR-CGH) arrays are accurate to detect structural variations (SV) at resolution of 200 bp (Urban et al. 2006).

3.2 SNP calling

• About SNP

• SNP calling

Single Nucleotide Polymorphism

• A Single Nucleotide Polymorphisms (SNP), is a genetic variation when a single nucleotide (i.e., A, T, C, or G) is altered and kept through heredity.

- SNP: Tag SNP
- Mutation
- Haplotype: haplotype block
- Genotype

SNPs are very common

- SNPs are very common in the human population.
- Between any two people, there is an average of one SNP every 1000 bases.
- Most of these have no phenotypic effect
 - only <1% of all human SNPs impact protein function (non-coding regions)
 - Selection against mis-sense mutations

SNP标记的优势

基因组上数量多密度大

- Maize: 1 per 48 bp (non-coding); 1 per 131 bp (coding)
- Soybean: 1 per 294 bp
- Arabidopsis: 1 per 3,300 bp
- Cotton: 1 per 100 bp
- Human: 1 per 1000bp

 可以通过简单但高通量方法检测,如测 序和芯片检测等,效率高

SNP检测技术已被大量开发:

- MALTI-TOF MS: Sequenom, San Diego, CA,
- USATaqMan: Applied Biosystems, Foster City, CA,
- USAInvader: Third Wave Technologies, Madison, WI, USA
- SNPStream: Beckman-Coulter, Fullerton , CA, USA
- Pyroequencing: Uppsala, Sweden
- Illumina: La Jolla, CA, USA
- Biotrove OpenArray: Woburn, MA, USA
- Array Tape System: Alexandria, MN, USA

- Alleles of function related genes: Genetic markers that are linked to every gene
- Population diversity & history
- Genetic Association studies in populations
- Molecular assistant breeding ...

SNP and mutation

SNP: Single DNA base variation found >1%

Mutation: Single DNA base variation found <1%



Mutations and SNPs



Major SNP

- A SNP is usually assumed to be a binary variable.
 - The probability of repeat mutation at the same SNP locus is quite small.
 - The tri-allele cases are usually considered to be the effect of genotyping errors.
- The nucleotide on a SNP locus is called
 - a major allele (if allele frequency > 50%), or
 - a minor allele (if allele frequency < 50%).

94% \rightarrow ACTTAGCTT \leftarrow T: Major allele 6% \rightarrow ACTTAGCTC \leftarrow C: Minor allele

Haplotypes

A haplotype stands for a set of linked SNPs on the same chromosome.

Genotypes

- In large sequencing projects, **genotypes** instead of haplotypes are collected due to cost consideration.
- Heterogenous genomic sites

Problems of Genotypes

- Genotypes only tell us the alleles at each SNP locus.
 - But we don't know the connection of alleles at different SNP loci.
 - There could be several possible haplotypes for the same genotype.

SNP calling

- With reference
 - Genome
 - transcripts
- Without reference

The reliability of short read alignment

- Repeats and sequencing errors
- Limited mismatch numbers within a region
- Always report a single alignment
- Fully utilize the mate-pair information of paired reads
- Produce a consensus genotype sequence from the alignment inferred from a statistical model

Finding higher quality SNPs

• Look at the number of reads covering the position with the SNP and discard those covered by three or fewer reads.

• Consensus quality is important, but SNP quality is more important. Discard a SNP with a quality score lower than 20.

Challenges of mapping-based approaches

- Reference genome is not available
- Hemi-SNP
- Large size of genomic variation

Genotyping SNP when reference genome is not available

SNP genotyping a genetic population/germplasm population...
RAD-SEQ; RNA-SEQ; ect.

A complex case for SNP

	Called bases	
Diploid species		
Cultivar 1 locus	а G С Т А <mark>G</mark> С Т	A G C T A <mark>G</mark> C T
Cultivar 2 locus	А G С Т А С С Т	AGCTA C CT
	simple SNP	
Allotetraploid		
Cultivar 1 locus Cultivar 1 homoeologue	А G С Т А G С Т А G Т Т А С С Т	AGYTA <mark>S</mark> CT
Cultivar 2 locus Cultivar 2 homoeologue	А G С Т А С С Т А G Т Т А С С Т	AGYTA C CT
inter-hor	noeologue polymorphism	hemi-SNP

3.3 Detect structural variations

• PE read-based

• Deletion, insertion, inversion, translocation

• *de novo* assembly

Read number-based

• CNV

deletion

(Wang *et al.* 2012)

Insertion: T-DNA/ Tos17

Dot position porientation porientation porientation 25 FCC020TYACXX:11:100:1563:0874#ACAGTGAT Chr8 24761748 + 8109 + An insection 17 FCC020TYACXX:11:206:6416:32126#ACAGTGAT Chr8 24761788 + 8006 + 23 FCC020TYACXX:11:2106:6416:32126#ACAGTGAT Chr8 24761786 + 8005 + 24 FCC020TYACXX:11:2106:6416:32126#ACAGTGAT Chr8 24761860 + 8002 + 49 FCC020TYACXX:11:201:3161:4829292#ACAGTGAT Chr8 24761860 + 8031 + 28 FCC020TYACXX:11:301:3183:29292#ACAGTGAT Chr8 24761865 7998 + 29 FCC020TYACXX:11:301:14364939831#ACAGTGAT Chr8 24761895 7950 + 29 FCC020TYACXX:11:301:14364998381#ACAGTGAT Chr8 24761993 7960 + 21 FCC020TYACXX:11:301:14364998981#ACAGTGAT Chr8 24761993 7986 + 22 FCC020TYACXX:11:101:158180:1682#ACAGTGAT	No	read TD		referenc	e	T-	DNA	results
25 FCC02UTACXX:1:1301:1605:36574#CAGTCAT Chr8 24761768 → 8109 → An insertion 23 FCC02UTACXX:1:100:15454:6427#ACAGTCAT Chr8 24761768 → 8096 → 47 FCC02UTACXX:1:200:546:312880#ACAGTCAT Chr8 24761865 → 8093 → 47 FCC02UTACXX:1:200:546:31287#ACAGTCAT Chr8 24761865 → 8029 → 43 FCC02UTACXX:1:204:3521:418972#ACAGTCAT Chr8 24761865 → 8020 → 44 FCC02UTACXX:1:104:304:3183:29929#ACAGTCAT Chr8 24761866 → 8020 → 28 FCC02UTACXX:1:1030:14182:14051#ACAGTCAT Chr8 24761806 → 8020 → 28 FCC02UTACXX:1:1030:14175:14510#ACAGTCAT Chr8 24761807 → 7995 → 29 FCC02UTACXX:1:100:11800:197175#ACAGTCAT Chr8 24761935 → 7960 → → → → → → → → → → → → → → → → → → → <	NO.	1640 12	pos:	ition	orientation	position	orientation	
117 FCC02UTACXX:1:106:15643:6427#ACAGTCAT Chr.8 24761768 → 8107 → 23 FCC02UTACXX:1:1207:19712:375684ACAGTCAT Chr.8 24761866 → 8096 → 24 FCC02UTACXX:1:12106:5416:32126#ACAGTCAT Chr.8 24761866 → 8029 → 27 FCC02UTACXX:1:1204:3514:18072#ACAGTCAT Chr.8 24761866 → 8020 → 49 FCC02UTACXX:1:1304:13882:399:66248#ACAGTCAT Chr.8 24761866 → 7998 → 28 FCC02UTACXX:1:1304:13832:399:66248#ACAGTCAT Chr.8 24761875 → 7998 → 29 FCC02UTACXX:1:1303:14175:145106#ACAGTCAT Chr.8 24761895 → 7995 → 29 FCC02UTACXX:1:1303:14175:145106#ACAGTCAT Chr.8 24761936 → 7996 → 20 FCC02UTACXX:1:101:1505197175:84CAGTCAT Chr.8 24761936 → 7950 →	25	FCC02UYACXX:1:1301:16055:36574#ACAGTGAT	Chr8	24761748	+	8109	+	An insertion
23 FCC02UTACXX:1:1207:19712:37586#ACA6TGAT Chr8 247619788 → 8096 47 FCC02UTACXX:1:1306:516:32126#ACA6TGAT Chr8 24761866 → 8003 → 43 FCC02UTACXX:1:1206:5161.32126#ACA6TGAT Chr8 24761860 → 8020 → 43 FCC02UTACXX:1:220414422:140561#ACA6TGAT Chr8 24761860 → 8031 → 24 FCC02UTACXX:1:220414422:140561#ACA6TGAT Chr8 24761866 → 7998 → 28 FCC02UTACXX:1:2107:118149991#ACA6TGAT Chr8 24761875 → 7996 → 29 FCC02UTACXX:1:103:11475:14510@#ACA6TGAT Chr8 24761919 → 7950 → 20 FCC02UTACXX:1:103:1818#ACA6TGAT Chr8 24761936 → 7960 → 10 FCC02UTACXX:1:102:5318:10572#ACA6TGAT Chr8 24761936 → 7960 → 21 FCC02UTACXX:1:102:5318:10572#ACA6TGAT Chr8 24761936 → 7960 → 26 FCC02UTACXX:1:102:538:10572#ACA6TGAT Chr8 24761936 → 7817	17	FCC02UYACXX:1:1106:15543:6427#ACAGTGAT	Chr8	24761768	+	8107	→	
417 PCC02UTAXXX:1:2106:5616:32126#ACAGTGAT Chr.8 24761808 → 8063 → 27 PCC02UTAXXX:1:2104:3514:18972#ACAGTGAT Chr.8 24761860 → 8020 → 43 PCC02UTAXX:1:2104:3514:18972#ACAGTGAT Chr.8 24761860 → 8020 → 49 PCC02UTAXX:1:1304:1318:2920#ACAGTGAT Chr.8 24761866 → 8031 → 28 PCC02UTAXX:1:1304:1318:2920#ACAGTGAT Chr.8 24761875 → 7998 → 28 PCC02UTAXX:1:1201:18141*9991#ACAGTGAT Chr.8 24761919 → 79950 → 29 PCC02UTAXX:1:1303:1475:145106#ACAGTGAT Chr.8 24761936 → 7967 → 20 PCC02UTAXX:1:101:19509:197756#ACAGTGAT Chr.8 24761943 → 7936 → 21 PCC02UTAXX:1:101:197756#ACAGTGAT Chr.8 24761943 → 7936 → 26 PCC02UTAXX:1:101:1972:14246#ACAGTGAT Chr.8 24761943 → 7936 → 21 PCC02UTAXX:1:101:1973:1426#ACAGTGAT Chr.8 24761945 →	23	FCC02UYACXX:1:1207:19712:37586#ACAGTGAT	Chr8	24761798	+	8096	+	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	47	FCC02UYACXX:1:2106:5416:32126#ACAGTGAT	Chr8	24761809	*	8063	+	
43 FCC02UTACXX:1:2104.35141:18972#ACAGTGAT Chr8 24761860 → 8020 → 49 FCC02UTACXX:1:1304:13188:2992#ACAGTGAT Chr8 24761860 → 8031 → 22 FCC02UTACXX:1:1304:13188:2992#ACAGTGAT Chr8 24761866 → 7998 → 28 FCC02UTACXX:1:1304:13188:2992#ACAGTGAT Chr8 24761897 → 7995 → 29 FCC02UTACXX:1:1207:11814/9931#ACAGTGAT Chr8 24761931 → 7950 → 20 FCC02UTACXX:1:1010:1500919775#ACAGTGAT Chr8 24761936 → 7950 → 210 FCC02UTACXX:1:1101:15019919775#ACAGTGAT Chr8 24761936 → 7950 → 26 FCC02UTACXX:1:1102:19175#ACAGTGAT Chr8 24761936 → 7956 → 14 FCC02UTACXX:1:1102:151866589#ACAGTGAT Chr8 24761936 → 7936 → 14 FCC02UTACXX:1:1102:1518672#ACAGTGAT Chr8 24761946 → 7871 → 16 FCC02UTACXX:1:1102:154672*1182046ACAGTGAT Chr8 24762016 →	27	FCC02UYACXX:1:1301:13037:138787#ACAGTGAT	Chr8	24761856	*	8029	+	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	43	FCC02UYACXX:1:2104:3514:18972#ACAGTGAT	Chr8	24761860	*	8020	+	
32 FCC02UTACXX:1:1304:13188:12992#ACAGTGAT Chr8 24761866 → 7998 → 28 FCC02UTACXX:1:1302:8239:66248#ACAGTGAT Chr8 24761876 → 7995 → 29 FCC02UTACXX:1:1300:11475:145106#ACAGTGAT Chr8 24761897 → 7995 → 22 FCC02UTACXX:1:1206:449931#ACAGTGAT Chr8 24761936 → 7960 → 21 FCC02UTACXX:1:100:451818#ACAGTGAT Chr8 24761936 → 7960 → 26 FCC02UTACXX:1:100:306:56369#ACAGTGAT Chr8 24761967 → 7960 → 14 FCC02UTACXX:1:100:5318:105782#ACAGTGAT Chr8 24761967 → 7922 → 11 FCC02UTACXX:1:102:5318:105782#ACAGTGAT Chr8 24762016 → 7830 → 46 FCC02UTACXX:1:2101:5782:11829#ACAGTGAT Chr8 24762032 → 7836 → 14 FCC02UTACXX:1:2101:5782:11829#ACAGTGAT Chr8 24762035 → 7836 → 42 FCC02UTACXX:1:2101:5782:11829#ACAGTGAT Chr8 24762035 → <t< td=""><td>49</td><td>FCC02UYACXX:1:2204:14422:140561#ACAGTGAT</td><td>Chr8</td><td>24761860</td><td>+</td><td>8031</td><td>+</td><td></td></t<>	49	FCC02UYACXX:1:2204:14422:140561#ACAGTGAT	Chr8	24761860	+	8031	+	
28 FCC02UTACXX:1:102:8239:66248#ACAGTGAT Chr8 24761875 7998 48 FCC02UTACXX:1:2107:11814:99931#ACAGTGAT Chr8 24761879 7995 29 FCC02UTACXX:1:1203:11475:145106#ACAGTGAT Chr8 24761919 7950 20 FCC02UTACXX:1:1201:19509:181758#ACAGTGAT Chr8 24761936 7960 26 FCC02UTACXX:1:1101:300:156569#ACAGTGAT Chr8 24761943 7936 14 FCC02UTACXX:1:1102:5318:105782#ACAGTGAT Chr8 24761965 7871 16 FCC02UTACXX:1:2105:1828C2:131262#ACAGTGAT Chr8 24762014 7847 46 FCC02UTACXX:1:2105:15782:131262#ACAGTGAT Chr8 24762014 7847 47 FCC02UTACXX:1:2105:138:163332#ACAGTGAT Chr8 24762027 7856 40 FCC02UTACXX:1:105:18400:15332#ACAGTGAT Chr8 24762032 7832 - 41 FCC02UTACXX:1:105:18400:15332#ACAGTGAT Chr8 24762032 7836 - 42 FCC02UTACXX:1:105:18400:15332#ACAGTGAT Chr8 24762032 7832 - 41 FCC02UTACXX:1:1000:18400:15332#ACAGTGAT Chr8	32	FCC02UYACXX:1:1304:13188:29929#ACAGTGAT	Chr8	24761866	→	7998	+	
48 FCC02UTACXX:1:2107:11814:99931#ACAGTGAT Chr.8 24761991 → 7995 → 29 FCC02UTACXX:1:1204:8499:81818#ACAGTGAT Chr.8 24761931 → 7957 → 10 FCC02UTACXX:1:1101:19509:19775#ACAGTGAT Chr.8 24761931 → 7957 → 11 FCC02UTACXX:1:1101:8306:56369#ACAGTGAT Chr.8 24761936 → 7960 → 28 FCC02UTACXX:1:1103:8427:29496#ACAGTGAT Chr.8 24761943 → 7936 → 14 FCC02UTACXX:1:1103:8427:29496#ACAGTGAT Chr.8 24761947 → 7922 → 11 FCC02UTACXX:1:12001:1802:1429292#ACAGTGAT Chr.8 24762014 → 7847 → 46 FCC02UTACXX:1:2101:15782#ACAGTGAT Chr.8 24762032 → 7830 → → 47 FCC02UTACXX:1:1105:1840:15332#ACAGTGAT Chr.8 24762032 → 7830 → → + 4762032 → 7836 → + 42762032 → 7836 → + 42762032 → 7836 → + <td>28</td> <td>FCC02UYACXX:1:1302:8239:66248#ACAGTGAT</td> <td>Chr8</td> <td>24761875</td> <td>+</td> <td>7998</td> <td>+</td> <td></td>	28	FCC02UYACXX:1:1302:8239:66248#ACAGTGAT	Chr8	24761875	+	7998	+	
29 FCC02UTACXX:1:1303:11475:145106#ACAGTCAT Chr8 24761931 → 7950 22 FCC02UTACXX:1:100:15609:197758#ACAGTCAT Chr8 24761936 → 7967 → 26 FCC02UTACXX:1:100:15609:197758#ACAGTCAT Chr8 24761936 → 7960 → 26 FCC02UTACXX:1:100:530:165782#ACAGTCAT Chr8 24761947 → 7922 → 11 FCC02UTACXX:1:100:513:105782#ACAGTCAT Chr8 24761967 → 7922 → 50 FCC02UTACXX:1:200:15782:105782#ACAGTCAT Chr8 24762016 → 7831 → 46 FCC02UTACXX:1:200:15782:1689#ACAGTCAT Chr8 24762027 → 7856 → 16 FCC02UTACXX:1:1201:15782:1689#ACAGTCAT Chr8 24762032 → 7832 → 42 FCC02UTACXX:1:1201:1370:22111#ACAGTGAT Chr8 2476205 → 7836 → 18 FCC02UTACXX:1:1201:1370:2211#ACAGTGAT Chr8 2476205 → 7836 → 19 FCC02UTACXX:1:1300:18482:45896#ACAGTGAT Chr8 2476214 ← 227	48	FCC02UYACXX:1:2107:11814:99931#ACAGTGAT	Chr8	24761897	→	7995	+	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	29	FCC02UYACXX:1:1303:11475:145106#ACAGTGAT	Chr8	24761919	→	7950	+	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	22	FCC02UYACXX:1:1204:8499:81818#ACAGTGAT	Chr8	24761931	+	7957	+	
26 PCC02UYACXX:1:1301:8306:56369#ACAGTGAT Chr8 24761943 → 7936 14 PCC02UYACXX:1:1103:8427:22496#ACAGTGAT Chr8 24761997 7922 11 FCC02UYACXX:1:102:6318:105782#ACAGTGAT Chr8 24762014 7847 50 FCC02UYACXX:1:2105:2952:131262#ACAGTGAT Chr8 24762014 7830 46 FCC02UYACXX:1:2105:1848:16589#ACAGTGAT Chr8 24762027 7856 40 FCC02UYACXX:1:2101:15782:16589#ACAGTGAT Chr8 24762032 42 FCC02UYACXX:1:101:51848:15332#ACAGTGAT Chr8 24762059 41 FCC02UYACXX:1:201:370:2211#ACAGTGAT Chr8 24762154 - 292 33 FCC02UYACXX:1:1301:15479:158826#ACAGTGAT Chr8 24762162 272 9 FCC02UYACXX:1:1301:1370:1850#ACAGTGAT Chr8 24762106 252 18 FCC02UYACXX:1:1301:13736:176419#ACAGTGAT Chr8 24762200 252	10	FCC02UYACXX:1:1101:19509:197758#ACAGTGAT	Chr8	24761936	+	7960	+	
14 FCC02UYACXX:1:1103:8427:29496#ACAGTGAT Chr8 24761967 7922 + 11 FCC02UYACXX:1:1102:5318:105782#ACAGTGAT Chr8 24762014 7811 + 50 FCC02UYACXX:1:2004:19802:142992#ACAGTGAT Chr8 24762016 + 7830 + 46 FCC02UYACXX:1:101:15782:16589#ACAGTGAT Chr8 24762016 + 7830 + 40 FCC02UYACXX:1:101:15782:16589#ACAGTGAT Chr8 24762032 + 7836 + 41 FCC02UYACXX:1:1201:5977:29193#ACAGTGAT Chr8 24762035 + 7836 + 41 FCC02UYACXX:1:1201:15479:15882#ACAGTGAT Chr8 24762164 + 292 + 33 FCC02UYACXX:1:1304:1811:36152#ACAGTGAT Chr8 24762162 + 272 + 9 FCC02UYACXX:1:1304:18790:58656#ACAGTGAT Chr8 24762162 + 227 + 9 FCC02UYACXX:1:1304:18790:58656#ACAGTGAT Chr8 24762204 + 236 + 13 FCC02UYACXX:1:1307:1830##ACAGTGAT Chr8 24762214 + 222 +	26	FCC02UYACXX:1:1301:8306:56369#ACAGTGAT	Chr8	24761943	+	7936	+	
11 FCC02UYACXX:1:1102:5318:105782#ACAGTGAT Chr8 24761995 7871 7871 50 FCC02UYACXX:1:2204:19802:142992#ACAGTGAT Chr8 24762014 7847 46 FCC02UYACXX:1:2105:2952:131262#ACAGTGAT Chr8 24762016 7830 40 FCC02UYACXX:1:2101:15782:16589#ACAGTGAT Chr8 24762027 7856 16 FCC02UYACXX:1:2102:5977:29193#ACAGTGAT Chr8 24762032 7836 42 FCC02UYACXX:1:2101:5179:193#ACAGTGAT Chr8 24762059 7831 19 FCC02UYACXX:1:101:15479:158826#ACAGTGAT Chr8 24762154 292 33 FCC02UYACXX:1:1304:1811:36152#ACAGTGAT Chr8 24762195 227 34 FCC02UYACXX:1:107:18498#ACAGTGAT Chr8 24762204 227 35 FCC02UYACXX:1:100:18489:15806#ACAGTGAT Chr8 24762204 226 45 FCC02UYACXX:1:100:18488:18304#ACAGTGAT Chr8 24762204 226 35 FCC02UYACXX:1:1010:18409:16ACAGTGAT Chr8	14	FCC02UYACXX:1:1103:8427:29496#ACAGTGAT	Chr8	24761967	→	7922	→]
50 FCC02UYACXX:1:2204:19802:142992#ACAGTGAT Chr8 24762014 \rightarrow 7847 \rightarrow 46 FCC02UYACXX:1:2105:2952:131262#ACAGTGAT Chr8 24762016 \rightarrow 7856 \rightarrow 40 FCC02UYACXX:1:2101:15782:16589#ACAGTGAT Chr8 24762032 \rightarrow 7836 \rightarrow 42 FCC02UYACXX:1:105:18480:153332#ACAGTGAT Chr8 24762032 \rightarrow 7836 \rightarrow 41 FCC02UYACXX:1:1201:5370:22111#ACAGTGAT Chr8 24762059 \rightarrow 7831 \rightarrow 19 FCC02UYACXX:1:104:1811:36152#ACAGTGAT Chr8 24762162 $=$ 272 \leftarrow 33 FCC02UYACXX:1:1101:18411:36152#ACAGTGAT Chr8 24762162 $=$ 272 \leftarrow 38 FCC02UYACXX:1:1101:1842:25950#ACAGTGAT Chr8 24762204 \leftarrow 226 \leftarrow $=$	11	FCC02UYACXX:1:1102:5318:105782#ACAGTGAT	Chr8	24761995	→	7871	→]
46 FCC02UYACXX:1:2105:2952:131262#ACAGTGAT Chr8 24762016 → 7830 → 40 FCC02UYACXX:1:2101:15782:16589#ACAGTGAT Chr8 24762027 → 7856 → 16 FCC02UYACXX:1:2101:577:29193#ACAGTGAT Chr8 24762032 → 7836 → 42 FCC02UYACXX:1:2101:3370:22111#ACAGTGAT Chr8 24762059 → 7836 → 19 FCC02UYACXX:1:1201:3370:22111#ACAGTGAT Chr8 24762154 ← 292 ← 33 FCC02UYACXX:1:1201:15479:158826#ACAGTGAT Chr8 24762162 ← 272 ← 34 FCC02UYACXX:1:1304:1811:36152#ACAGTGAT Chr8 24762162 ← 272 ← 35 FCC02UYACXX:1:101:8482:25950#ACAGTGAT Chr8 24762204 ← 225 ← 35 FCC02UYACXX:1:101:8482:25950#ACAGTGAT Chr8 24762204 ← 226 ← 36 FCC02UYACXX:1:101:818482484CAGTGAT Chr8 24762214 ← 226 ← 37 FCC02UYACXX:1:1038:184CAGTGAT Chr8 24762231 ← 215	50	FCC02UYACXX:1:2204:19802:142992#ACAGTGAT	Chr8	24762014	→	7847	→]
40 FCC02UYACXX:1:2101:15782:16589#ACAGTGAT Chr8 24762027 → 7856 → 16 FCC02UYACXX:1:105:18480:153332#ACAGTGAT Chr8 24762032 → 7832 → 42 FCC02UYACXX:1:2102:5977:29193#ACAGTGAT Chr8 24762055 → 7836 → 41 FCC02UYACXX:1:2101:15479:158826#ACAGTGAT Chr8 24762055 → 7831 → 19 FCC02UYACXX:1:101:15479:158826#ACAGTGAT Chr8 24762162 ← 272 ← 33 FCC02UYACXX:1:100:15479:158826#ACAGTGAT Chr8 24762195 ← 227 ← 34 FCC02UYACXX:1:100:18482:25950#ACAGTGAT Chr8 24762200 ← 252 ← 9 FCC02UYACXX:1:101:370:18790:5866#ACAGTGAT Chr8 24762201 ← 236 ← 18 FCC02UYACXX:1:101:13842:25950#ACAGTGAT Chr8 24762231 ← 215 ← 13 FCC02UYACXX:1:101:21276:118304#ACAGTGAT Chr8 24762241 ← 197 ← 13 FCC02UYACXX:1:102:19788:183949#ACAGTGAT Chr8 24762241 ← </td <td>46</td> <td>FCC02UYACXX:1:2105:2952:131262#ACAGTGAT</td> <td>Chr8</td> <td>24762016</td> <td>+</td> <td>7830</td> <td>→</td> <td>1</td>	46	FCC02UYACXX:1:2105:2952:131262#ACAGTGAT	Chr8	24762016	+	7830	→	1
16 FCC02UYACXX:1:1105:18480:153332#ACAGTGAT Chr8 24762032 \rightarrow 7832 \rightarrow 42 FCC02UYACXX:1:210:3370:22111#ACAGTGAT Chr8 24762035 \rightarrow 7836 \rightarrow 41 FCC02UYACXX:1:2101:3370:22111#ACAGTGAT Chr8 24762059 \rightarrow 7831 \rightarrow 19 FCC02UYACXX:1:1201:15479:158826#ACAGTGAT Chr8 24762164 \leftarrow 292 \leftarrow 33 FCC02UYACXX:1:1301:15479:158826#ACAGTGAT Chr8 24762162 \leftarrow 272 \leftarrow 38 FCC02UYACXX:1:1301:18479:158826#ACAGTGAT Chr8 24762195 \leftarrow 227 \leftarrow 9 FCC02UYACXX:1:1304:13736:176499#ACAGTGAT Chr8 24762200 \leftarrow 252 \leftarrow 35 FCC02UYACXX:1:1010:18482:25950#ACAGTGAT Chr8 24762214 \leftarrow 226 \leftarrow 45 FCC02UYACXX:1:100:19788:183949#ACAGTGAT Chr8 24762231 \leftarrow 215 \leftarrow 39 FCC02UYACXX:1:102:19788:183949#ACAGTGAT Chr8 24762241 \leftarrow 197 \leftarrow 36 FCC02UYACXX:1:100:15682#ACAGTGAT Chr8	40	FCC02UYACXX:1:2101:15782:16589#ACAGTGAT	Chr8	24762027	+	7856	→	1
42 FCC02UYACXX:1:2102:5977:29193#ACAGTGAT Chr.8 24762035 \rightarrow 7836 \rightarrow 41 FCC02UYACXX:1:2101:3370:22111#ACAGTGAT Chr.8 24762059 \rightarrow 7831 \rightarrow 19 FCC02UYACXX:1:1201:15479:158826#ACAGTGAT Chr.8 24762154 \leftarrow 292 \leftarrow 33 FCC02UYACXX:1:1304:1811:36152#ACAGTGAT Chr.8 24762162 \leftarrow 272 \leftarrow 38 FCC02UYACXX:1:1307:18790:58656#ACAGTGAT Chr.8 24762105 \leftarrow 227 \leftarrow 9 FCC02UYACXX:1:1304:13736:176499#ACAGTGAT Chr.8 24762200 \leftarrow 252 \leftarrow 35 FCC02UYACXX:1:107:2176:118304#ACAGTGAT Chr.8 24762204 \leftarrow 236 \leftarrow 18 FCC02UYACXX:1:10107:2176:118304#ACAGTGAT Chr.8 24762214 \leftarrow 215 \leftarrow 13 FCC02UYACXX:1:1010:19788:18394#ACAGTGAT Chr.8 24762241 \leftarrow 193 \leftarrow 21 FCC02UYACXX:1:1308:18679:38205#ACAGTGAT Chr.8 24762262 \leftarrow 175 \leftarrow 33 FCC02UYACXX:1:1308:18309:440ACAGTGAT Chr.	16	FCC02UYACXX:1:1105:18480:153332#ACAGTGAT	Chr8	24762032	+	7832	→	1
41 FCC02UYACXX:1:2101:3370:22111#ACAGTGAT Chr8 24762059 → 7831 → 19 FCC02UYACXX:1:1201:15479:158826#ACAGTGAT Chr8 24762154 ← 292 ← 33 FCC02UYACXX:1:1304:1811:36152#ACAGTGAT Chr8 24762162 ← 272 ← 38 FCC02UYACXX:1:1307:18790:58656#ACAGTGAT Chr8 24762195 ← 227 ← 9 FCC02UYACXX:1:101:8482:25950#ACAGTGAT Chr8 24762200 ← 252 ← 35 FCC02UYACXX:1:107:21276:118304#ACAGTGAT Chr8 24762204 ← 226 ← 45 FCC02UYACXX:1:2104:2002:113801#ACAGTGAT Chr8 24762231 ← 215 ← 39 FCC02UYACXX:1:107:21276:118304#ACAGTGAT Chr8 24762241 ← 197 ← 39 FCC02UYACXX:1:1038:1679:38205#ACAGTGAT Chr8 24762241 ← 193 ← 21 FCC02UYACXX:1:103:18103:28232#ACAGTGAT Chr8 24762262 ← 175 ← 36 FCC02UYACXX:1:103:18103:28232#ACAGTGAT Chr8 24762262 ←	42	FCC02UYACXX:1:2102:5977:29193#ACAGTGAT	Chr8	24762035	+	7836	→	1
19 FCC02UYACXX:1:1201:15479:158826#ACAGTGAT Chr8 24762154 \leftarrow 292 \leftarrow 33 FCC02UYACXX:1:1304:1811:36152#ACAGTGAT Chr8 24762162 \leftarrow 272 \leftarrow 38 FCC02UYACXX:1:1307:18790:58656#ACAGTGAT Chr8 24762195 \leftarrow 227 \leftarrow 9 FCC02UYACXX:1:1101:8482:25950#ACAGTGAT Chr8 24762200 \leftarrow 252 \leftarrow 35 FCC02UYACXX:1:1304:13736:176499#ACAGTGAT Chr8 24762204 \leftarrow 236 \leftarrow 18 FCC02UYACXX:1:107:21276:118304#ACAGTGAT Chr8 24762214 \leftarrow 222 \leftarrow 45 FCC02UYACXX:1:102:19788:183949#ACAGTGAT Chr8 24762211 \leftarrow 197 \leftarrow 39 FCC02UYACXX:1:102:19788:183949#ACAGTGAT Chr8 24762241 \leftarrow 193 \leftarrow 36 FCC02UYACXX:1:1030:28232#ACAGTGAT Chr8 24762273 \leftarrow 189 \leftarrow 36 FCC02UYACXX:1:1305:18309:8419#ACAGTGAT Chr8 24762273 \leftarrow 189 \leftarrow 36 FCC02UYACXX:1:1304:19011:56582#ACAGTGAT Chr	41	FCC02UYACXX:1:2101:3370:22111#ACAGTGAT	Chr8	24762059	+	7831	→	1
33FCC02UYACXX:1:1304:1811:36152#ACAGTGATChr824762162 \leftarrow 272 \leftarrow 38FCC02UYACXX:1:1307:18790:58656#ACAGTGATChr824762195 \leftarrow 227 \leftarrow 9FCC02UYACXX:1:1101:8482:25950#ACAGTGATChr824762200 \leftarrow 252 \leftarrow 35FCC02UYACXX:1:1304:13736:176499#ACAGTGATChr824762204 \leftarrow 236 \leftarrow 18FCC02UYACXX:1:107:21276:118304#ACAGTGATChr824762214 \leftarrow 222 \leftarrow 45FCC02UYACXX:1:2104:2002:113881#ACAGTGATChr824762231 \leftarrow 215 \leftarrow 13FCC02UYACXX:1:1002:19788:183949#ACAGTGATChr824762241 \leftarrow 197 \leftarrow 39FCC02UYACXX:1:1003:18103:2823#ACAGTGATChr824762241 \leftarrow 193 \leftarrow 21FCC02UYACXX:1:1305:18309:8419#ACAGTGATChr824762262 \leftarrow 175 \leftarrow 34FCC02UYACXX:1:1304:19011:56582#ACAGTGATChr824762285 \leftarrow 159 \leftarrow 34FCC02UYACXX:1:1304:19011:56582#ACAGTGATChr824762318 \leftarrow 124 \leftarrow 37FCC02UYACXX:1:1308:163344#ACAGTGATChr824762318 \leftarrow 124 \leftarrow 37FCC02UYACXX:1:1306:20471:51487#ACAGTGATChr824762341 \leftarrow 94 \leftarrow 38FCC02UYACXX:1:1306:20471:51487#ACAGTGATChr824762318 \leftarrow 124 \leftarrow 39FCC02UYACXX:1:1306:20471:51487#ACAGTGATChr824762364 \leftarrow 75 \leftarrow 30FCC02UYACXX:1:1009	19	FCC02UYACXX:1:1201:15479:158826#ACAGTGAT	Chr8	24762154	*	292	+	1
$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	33	FCC02UYACXX:1:1304:1811:36152#ACAGTGAT	Chr8	24762162	*	272	+	
9 FCC02UYACXX:1:1101:8482:25950#ACAGTGAT Chr8 24762200 \leftarrow 252 \leftarrow 35 FCC02UYACXX:1:1304:13736:176499#ACAGTGAT Chr8 24762204 \leftarrow 236 \leftarrow 18 FCC02UYACXX:1:1107:21276:118304#ACAGTGAT Chr8 24762214 \leftarrow 222 \leftarrow 45 FCC02UYACXX:1:2104:2002:113881#ACAGTGAT Chr8 24762231 \leftarrow 215 \leftarrow 13 FCC02UYACXX:1:1102:19788:183949#ACAGTGAT Chr8 24762241 \leftarrow 197 \leftarrow 39 FCC02UYACXX:1:1308:18679:38205#ACAGTGAT Chr8 24762262 \leftarrow 175 \leftarrow 36 FCC02UYACXX:1:1305:18309:8419#ACAGTGAT Chr8 24762273 \leftarrow 189 \leftarrow 34 FCC02UYACXX:1:1304:19011:56582#ACAGTGAT Chr8 24762285 \leftarrow 159 \leftarrow 124 \leftarrow 37 FCC02UYACXX:1:1306:20471:51487#ACAGTGAT Chr8 24762318 \leftarrow 124 \leftarrow \leftarrow 37 FCC02UYACXX:1:1306:20471:51487#ACAGTGAT Chr8 24762364 \leftarrow 94 \leftarrow \leftarrow 44 FCC02UYACXX:1:1306:6195:15420	38	FCC02UYACXX:1:1307:18790:58656#ACAGTGAT	Chr8	24762195	+	227	+	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	9	FCC02UYACXX:1:1101:8482:25950#ACAGTGAT	Chr8	24762200	+	252	+	
18 FCC02UYACXX:1:1107:21276:118304#ACAGTGAT Chr8 24762214 \leftarrow 222 \leftarrow 45 FCC02UYACXX:1:2104:2002:113881#ACAGTGAT Chr8 24762231 \leftarrow 215 \leftarrow 13 FCC02UYACXX:1:1102:19788:183949#ACAGTGAT Chr8 24762241 \leftarrow 197 \leftarrow 39 FCC02UYACXX:1:108:18679:38205#ACAGTGAT Chr8 24762241 \leftarrow 193 \leftarrow 21 FCC02UYACXX:1:103:18103:28232#ACAGTGAT Chr8 24762262 \leftarrow 175 \leftarrow 36 FCC02UYACXX:1:1305:18309:8419#ACAGTGAT Chr8 24762285 \leftarrow 159 \leftarrow 34 FCC02UYACXX:1:1304:19011:56582#ACAGTGAT Chr8 24762318 \leftarrow 124 \leftarrow 37 FCC02UYACXX:1:1306:20471:51487#ACAGTGAT Chr8 24762341 \leftarrow 94 \leftarrow 44 FCC02UYACXX:1:2104:20778:95349#ACAGTGAT Chr8 24762359 \leftarrow 83 \leftarrow $=$ 30 FCC02UYACXX:1:1303:6195:154204#ACAGTGAT Chr8 24762364 \leftarrow 75 \leftarrow $=$ 30 FCC02UYACXX:1:1303:6195:154204	35	FCC02UYACXX:1:1304:13736:176499#ACAGTGAT	Chr8	24762204	+	236	+	
45 FCC02UYACXX:1:2104:2002:113881#ACAGTGAT Chr8 24762231 \leftarrow 215 \leftarrow 13 FCC02UYACXX:1:1102:19788:183949#ACAGTGAT Chr8 24762241 \leftarrow 197 \leftarrow 39 FCC02UYACXX:1:1308:18679:38205#ACAGTGAT Chr8 24762241 \leftarrow 193 \leftarrow 21 FCC02UYACXX:1:103:18103:28232#ACAGTGAT Chr8 24762262 \leftarrow 175 \leftarrow 36 FCC02UYACXX:1:1305:18309:8419#ACAGTGAT Chr8 24762285 \leftarrow 159 \leftarrow 34 FCC02UYACXX:1:1304:19011:56582#ACAGTGAT Chr8 24762318 \leftarrow 124 \leftarrow 37 FCC02UYACXX:1:1306:20471:51487#ACAGTGAT Chr8 24762341 \leftarrow 94 \leftarrow 44 FCC02UYACXX:1:2104:20778:95349#ACAGTGAT Chr8 24762359 \leftarrow 83 \leftarrow 30 FCC02UYACXX:1:1303:6195:154204#ACAGTGAT Chr8 24762364 \leftarrow 71 \leftarrow	18	FCC02UYACXX:1:1107:21276:118304#ACAGTGAT	Chr8	24762214	+	222	+	
13 FCC02UYACXX:1:1102:19788:183949#ACAGTGAT Chr8 24762241 \leftarrow 197 \leftarrow 39 FCC02UYACXX:1:1308:18679:38205#ACAGTGAT Chr8 24762241 \leftarrow 193 \leftarrow 21 FCC02UYACXX:1:1203:18103:28232#ACAGTGAT Chr8 24762262 \leftarrow 175 \leftarrow 36 FCC02UYACXX:1:1305:18309:8419#ACAGTGAT Chr8 24762273 \leftarrow 189 \leftarrow 34 FCC02UYACXX:1:1304:19011:56582#ACAGTGAT Chr8 24762285 \leftarrow 159 \leftarrow 12 FCC02UYACXX:1:102:10398:163344#ACAGTGAT Chr8 24762318 \leftarrow 124 \leftarrow 37 FCC02UYACXX:1:204:20778:95349#ACAGTGAT Chr8 24762341 \leftarrow 94 \leftarrow 44 FCC02UYACXX:1:2104:20778:95349#ACAGTGAT Chr8 24762359 \leftarrow 83 \leftarrow 30 FCC02UYACXX:1:1303:6195:154204#ACAGTGAT Chr8 24762364 \leftarrow 75 \leftarrow $=$ 30 FCC02UYACXX:1:1303:6195:154204#ACAGTGAT Chr8 24762364 \leftarrow 75 \leftarrow $=$ $=$ $=$ $=$ $=$	45	FCC02UYACXX:1:2104:2002:113881#ACAGTGAT	Chr8	24762231	+	215	*	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	13	FCC02UYACXX:1:1102:19788:183949#ACAGTGAT	Chr8	24762241	+	197	*	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	39	FCC02UYACXX:1:1308:18679:38205#ACAGTGAT	Chr8	24762241	+	193	*	
36 FCC02UYACXX:1:1305:18309:8419#ACAGTGAT Chr8 24762273 \leftarrow 189 \leftarrow 34 FCC02UYACXX:1:1304:19011:56582#ACAGTGAT Chr8 24762285 \leftarrow 159 \leftarrow 12 FCC02UYACXX:1:1102:10398:163344#ACAGTGAT Chr8 24762318 \leftarrow 124 \leftarrow 37 FCC02UYACXX:1:1306:20471:51487#ACAGTGAT Chr8 24762341 \leftarrow 94 \leftarrow 44 FCC02UYACXX:1:2104:20778:95349#ACAGTGAT Chr8 24762359 \leftarrow 83 \leftarrow 30 FCC02UYACXX:1:1303:6195:154204#ACAGTGAT Chr8 24762364 \leftarrow 75 \leftarrow 15 FCC02UYACXX:1:100:12089:782954##ACAGTGAT Chr8 24762370 \leftarrow 71 \leftarrow	21	FCC02UYACXX:1:1203:18103:28232#ACAGTGAT	Chr8	24762262	+	175	*	
34 FCC02UYACXX:1:1304:19011:56582#ACAGTGAT Chr8 24762285 ← 159 ← 12 FCC02UYACXX:1:1102:10398:163344#ACAGTGAT Chr8 24762318 ← 124 ← 37 FCC02UYACXX:1:1306:20471:51487#ACAGTGAT Chr8 24762341 ← 94 ← 44 FCC02UYACXX:1:2104:20778:95349#ACAGTGAT Chr8 24762359 ← 83 ← 30 FCC02UYACXX:1:1303:6195:154204#ACAGTGAT Chr8 24762364 ← 75 ← 15 FCC02UYACXX:1:100:12089:1054204#ACAGTGAT Chr8 24762364 ← 71 ←	36	FCC02UYACXX:1:1305:18309:8419#ACAGTGAT	Chr8	24762273	+	189	*	
12 FCC02UYACXX:1:1102:10398:163344#ACAGTGAT Chr8 24762318 ← 124 ← 37 FCC02UYACXX:1:1306:20471:51487#ACAGTGAT Chr8 24762341 ← 94 ← 44 FCC02UYACXX:1:2104:20778:95349#ACAGTGAT Chr8 24762359 ← 83 ← 30 FCC02UYACXX:1:1303:6195:154204#ACAGTGAT Chr8 24762364 ← 75 ← 15 FCC02UYACXX:1:100:2087#ACAGTGAT Chr8 24762370 ← 71 ←	34	FCC02UYACXX:1:1304:19011:56582#ACAGTGAT	Chr8	24762285	+	159	*	
37 FCC02UYACXX:1:1306:20471:51487#ACAGTGAT Chr8 24762341 ← 94 ← 44 FCC02UYACXX:1:2104:20778:95349#ACAGTGAT Chr8 24762359 ← 83 ← 30 FCC02UYACXX:1:1303:6195:154204#ACAGTGAT Chr8 24762364 ← 75 ← 15 FCC02UWACXX:1:1003:6195:154204#ACAGTGAT Chr8 24762370 ← 71	12	FCC02UYACXX:1:1102:10398:163344#ACAGTGAT	Chr8	24762318	+	124	*	
44 FCC02UYACXX:1:2104:20778:95349#ACAGTGAT Chr8 24762359 ← 83 ← 30 FCC02UYACXX:1:1303:6195:154204#ACAGTGAT Chr8 24762364 ← 75 ← 15 FCC02UWACXX:1:1003:6195:154204#ACAGTGAT Chr8 24762364 ← 75 ←	37	FCC02UYACXX:1:1306:20471:51487#ACAGTGAT	Chr8	24762341	+	94	*	
30 FCC02UYACXX:1:1303:6195:154204#ACAGTGAT Chr8 24762364 ← 75 ←	44	FCC02UYACXX:1:2104:20778:95349#ACAGTGAT	Chr8	24762359	+	83	*	
	30	FCC02UYACXX:1:1303:6195:154204#ACAGTGAT	Chr8	24762364	+	75	*	
10 IPUUUZUTAUAA: 1: 1104: 13088: 7258 (#AUAG1GAL UNY8 24 (h2.572 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	15	FCC02UYACXX:1:1104:13088:72587#ACAGTGAT	Chr8	24762372	+	71	*	
31 FCC02IIYACXX:1:1303:19490:191151#ACAGTGAT Cbr8 24762382 ← 44 ←	31	FCC02IIYACXX:1:1303:19490:191151#ACAGTGAT	Chr8	24762382	*	44	*	
24 FCC02UYACXX:1:1207:11152:93193#ACAGTGAT Chr8 24762396 ← 47 ←	24	FCC02UYACXX:1:1207:11152:93193#ACAGTGAT	Chr8	24762396	*	47	*	
20 FCC02UYACXX:1:1202:14593:104111#ACAGTGAT Chr8 24762398 44	20	FCC02UYACXX:1:1202:14593:104111#ACAGTGAT	Chr8	24762398	*	44	*	

Challenge: perfect mapping but failure of experimental validation. CNV?

		L		Q		P		V		A		V		K		A		G		V		S					V		G		L		Q		Ì	
	С	T (G C	A (GC	0 (G	Ţ	T G	C	T G	T (C A	A	GG	G C	C	GG	A	GT	C	TC	C	C	T	G	T	G	GA	С	T (0 0	A	GA	T	C
	10	1		1							U.			1						_	I				,	pe ne		N							51 1	J51
B819BNABXX:3:67:2226:73432#TAGCTTAT	С	T (GC	A (GC	C (G	T	T G	C	G	Τ (CA	A	GG	G C	CI	GG	A	GT	С	TC	C	C	T	G	ΙŢ	G	GA	С	Ţ (0 0	A	GA	T	C
B819BNABXX:3:3:14772:28726#TAGCTTAT	С	T (GC	A (GC	C (G	T	T G	C	T G	T (C A	A	GG	6 C	CI	GG	A	GT	С	TC	C	C	ΓT	G	T T	G	GA	С	Τ (C C	A	GA	T	C
B819BNABXX:3:43:1988:121561#TAGCTTAT	С	T (GC	A (GC	C (G	T	T G	C	T G	T (CA	A	GG) C	CI	GG	A	GT	С	TC	C	C	ΓT	G	T T	G	GA	C	T (CC	A	GA	T	C
A819BBABXX:1:2:10619:139078#CAGATCAT	С	T (GC	A (GC	C (G	T	T G	C	T G	T (C G	A	GG	6 C	C	GG	A	GT	С	TC	C	C	ΓT	G	T T	G	GA	С	T (C C	A	GA	T	C
B819BNABXX:3:25:11391:69040#TAGCTTAT	C	T (GC	A (GC	C (G	T	T G	C	T G	T (C A	A	GG	C C	CI	GG	A	GT	С	TC	C	C	ΓT	G	ΤT	G	GA	C	T (CC	A	GA	T	C
A819BBABXX:1:68:9533:153993#CAGATCAT	С	T (GC	A (G C	C (G	Ţ	T G	C	T G	T (CG	A	GG	C C	CI	GG	A	GT	С	TC	C	C	ΓT	G	T T	G	GA	C	Τ (C C	A	GA	T	C
A819BBABXX:1:66:17996:64414#CAGATCAT	С	T (GC	A (GC	0 (G	Ţ	T G	C	G	T (G	A	GG	6 C	CI	GG	A	GT	С	TC	C	C	ΤT	G	TT	G	GA	C	T (0 0	A	GA	T	C
B819BNABXX:3:68:1367:129909#TAGCTTAT	C	T (GC	A (GC	C (G	T	T G	C	G	T (C A	A	GG	G C	CI	GG	A	GT	C	TC	C	C	TT	G	ĪŢ	G	GA	C	T (0 0	A	GA	T	C

(Li et al., 2015, unpublished)

3.4 Application of genome variations

• Reference genome-based:

- Genetic diversity
- Evolutionary issues
- Artificial selection on crops
- Reference genome-free:
 - Genetic diversity
 - Molecular markers

Recent advances based on genomic variations

- The return of population genetics (Lecture 6)
- Intracultivar genomic heterogeneity was observed
- Intercultivar genomic variation is so big

The return of population genetics

• Population genetics

- Molecular population genetics: PCR-based
 - Molecular population genetics: high throughoutbased
- A bridge between genomics and breeding: artificial selection
 - Breeding theory: genetic diversity, selection targets/strength, etc.
 - A bottom-up approach to find agronomic important genes

Two approaches to find positive/ adaptive genes

- The **top-down** approach:
 - QTL and LD mapping: from phenotype to candidate genes then molecular population genetics for signature of adaptation
- The **bottom-up** approach:
 - From molecular population genetics for signature of adaptation to candidate genes then find its function/phenotype

The effects of demography

The effects of domestication bottleneck on genetic diversity

Domestication bottleneck = domestication selection + demography effect

DNA diversity: π (Tajima 1983) θ (Watterson 1975)

(Whitt and Gaut 2005)

From the bottom up: Molecular population genetics

- Theoretical issues
 - Amount of diversity
 - Reduction of nucleotide diversity

• Frequency distribution of polymorphisms

- Selection skews the population frequency of genetic variants relative neutral equilibrium model (NE) expectations
- An excess of rare variants relative to NE expectations
- Or, with recombination, an excess of high-frequency derived (non-ancestral) mutations
- Degree of association between polymorphisms/linkage disequibrium (LD)
 - Selective sweep increase LD

Intercultivar variation is so big

• A maize pedigree:

• Lai et al. 2010, Genome-wide patterns of genetic variation among elite maize inbred lines. Nature Genetics

Genetic background

Annotation of large-effect SNPs

Numbers of PAVs relative to the B73 reference genome

- 296 high-confidence genes in B73 that were missing from at least one the six inbred lines.
- One large deletion between Mo17 and B73:
 ~2Mb with 24 genes

Intracultivar genomic heterogeneity was observed

- A same phenotype for individuals from a cultivar
- A reference genome of soybean (William 82): Haun et al. Plant Phiso., 2011

The composition and origin of genomic variation among individuals of the soybean reference cultivar Williams 82

- Haun et al. 2011, Plant Physio.
- Williams 82: a Williams × Kingwa BC₆F₃ generation

SNP genotyping (SNP chip) reveals the parental origins of Williams 82 genetic heterogeneity

A Chromosome															Mb						
Wm82 SGC-01			03			06 编制 的复数形式 化化学	07	08		10 10 10 10 10 10 10 10 10 10 10 10 10 1	11 () () () () () () () () () () () () ()	12	13 13 13 14 15 14 15 15 15 15 15 15 15 15 15 15 15 15 15	14	15 99 49 10 10 10 10 10 10 10 10 10 10 10 10 10	16 33555	17			20	-0 -10 -20 -30 -40 -50 -60
Wm82 ISU-01		「「「「「「」」」」を見ていた。 第二語				金融の変形の活動であっていた。	No SN	n-po IPs n	lymo	rphic	SNI	Ps ms	parei	nt	劉耀麗 、劉定克帝二書	ののの方法である		非国际 关系的建立。18-18	新 州 · · · · · · · · · · · · · · · · · · ·	第二十二十二 離離	-0 -10 -20 -30 -40 -50 -60
							SN	IPs n	natch	ning	neith	er pa	arent								

Structural variation (CGH) within regions of heterogeneity between two Williams 82 individuals

Exome resequencing reveals gene content variation between two Williams 82 lines

A model for the origin of genomic heterogeneity in two Williams 82 lines

Implications for the Williams 82 and other plant genome sequences

- Within regions of genetic heterogeneity, the reference sequences consist of a mosaic of the Williams and Kingwa haplotypes.
- Researchers investigating comparative studies of soybean that include Williams 82 as a reference genotype must factor in the inherent differences between each Williams 82 individual and the reference genome sequence.
- Similar considerations will need to be made for a variety of comparative methodologies, such as RNA-SEQ data.
- Similar circumstances may apply to the utility of other plant genome sequences