Towards High-Throughput Transposable Element Markers for Barley

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International Triticeae Mapping Initiative - COST Action Tritigen

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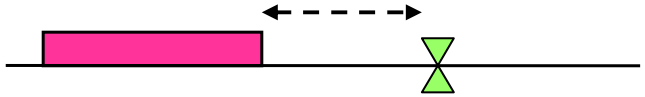
High-Throughput Markers based on TEs insertions

- The main goal is to create **new molecular markers**
- Help span gene-poor segments of the genome where component BAC clones may be fully lacking in genes
- Help to assemble physical maps and their linkage to genetic maps to facilitate the assembly of the genome sequences
- Development of high-throughput marker methods based on **Transposable Elements insertions in barley**

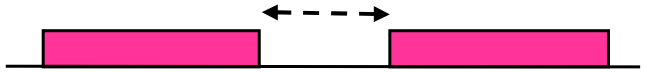
Transposable element-Based Marker Assays

SSAP



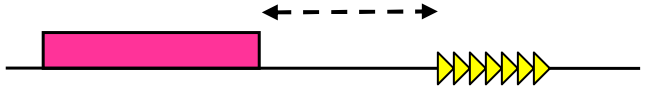
Measure the distance from the transposon to a restriction site

IRAP



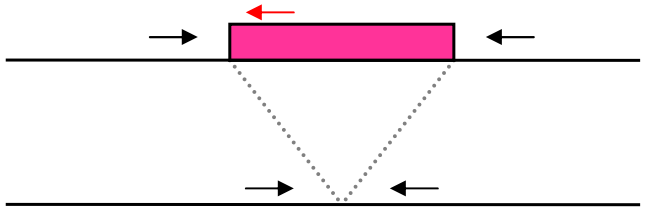
Measure the distance from one transposon to another

REMAP



Measure the distance from the transposon to a microsatellite

RBIP



Detect presence and absence of the transposon by PCR

High-Throughput Markers based on TE insertions

Create **DNA libraries** for different barley varieties

↓

Synthesize **biotinylated oligos** for short TEs

↓

Capture & Sequence

↓

Re-build sequence TE + flanks

↓

Check insertion **polymorphism** by comparing sequence sets

↓

Design PCR primers for **molecular markers** (insertion / empty site)

MITEs

- **Miniature Inverted-repeat Transposable elements**
- **Small TEs** (usually <500 base pairs)
- Containing short **T**erminal **I**nverted **R**epeats (TIRs)
- In high copy numbers
- Evolutionary studies suggest that some MITEs in plant and animal genomes have spread recently

MITEs

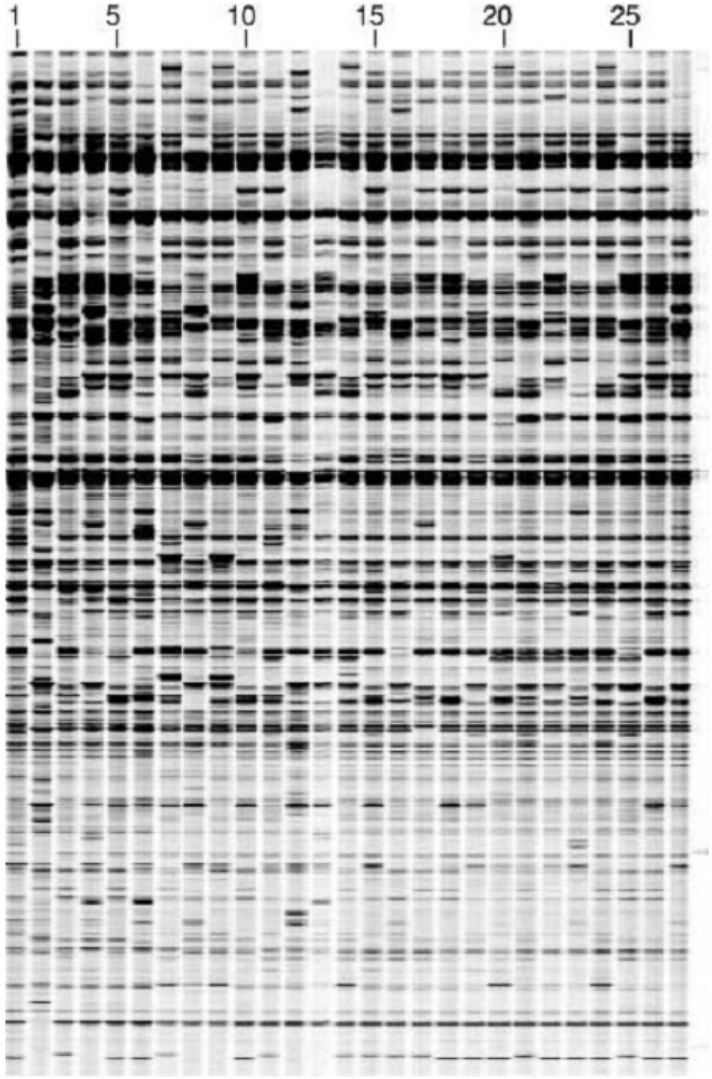


Table 1. Distribution of simple sequence repeat (SSR) and miniature inverted-repeat transposable element (MITE)-amplified fragment length polymorphism (AFLP) loci in the barley genome.

			No. of mapped loci			
Chromosome	Density	cM/locus	SSR	*Stowaway*	*Barfly*	*Pangrangia*
1H	9.6	191.1/20	3	8	4	5
2H	5.5	235.4/43	4	10	12	17
3H	3.4	162.8/48	11	12	12	13
4H	3.5	118.2/34	5	11	11	7
5H	4.3	194.0/45	5	14	13	13
6H	4.2	109.8/26	3	10	4	9
7H	4.0	153.3/38	9	14	12	3
Total	4.6	1164.5/254	40	79	68	67

MITE-AFLP mapping

Takahashi *et al.*, Genome 2006

The MITE-based loci efficiently covered the barley genome

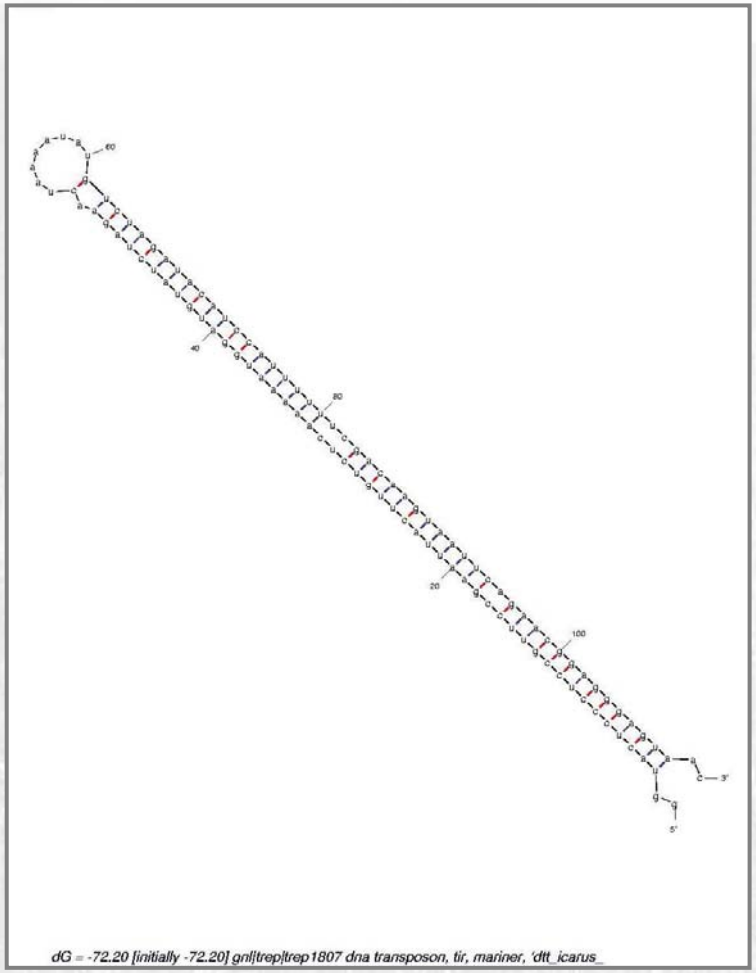
Inter-MITE polymorphism

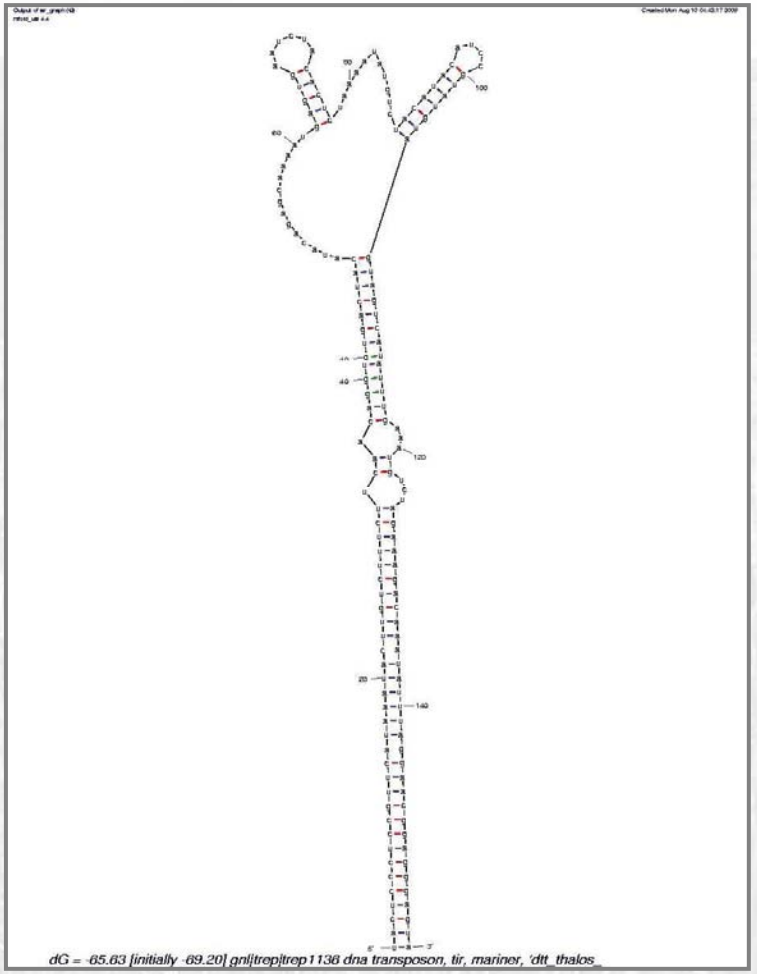
Chang *et al.*, TAG 2001

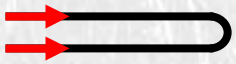
MITEs

- **PCR amplification can span MITE insertion**
- Easier to visualize and score on gel
- **TREP Database** :
  - List of potential short elements
- Particular structure

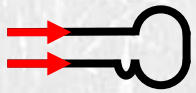
MITEs in Barley







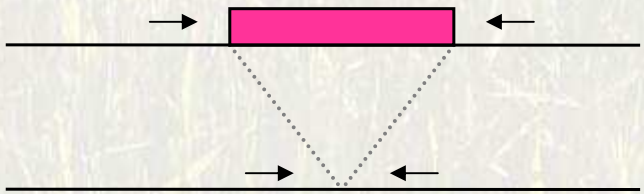
"*perfect*" MITE



"*degenerated*" MITE

Strategy

1. Libraries of single-stranded genomic DNA of barleys
2. Design biotinylated baits for MITE capture
3. Capture on streptavidin beads
4. Design primers for polymorphic insertions



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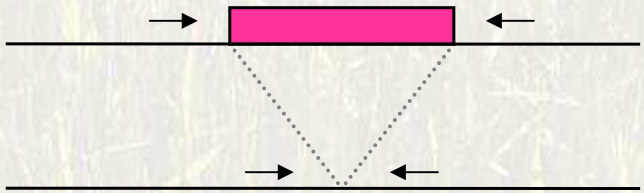
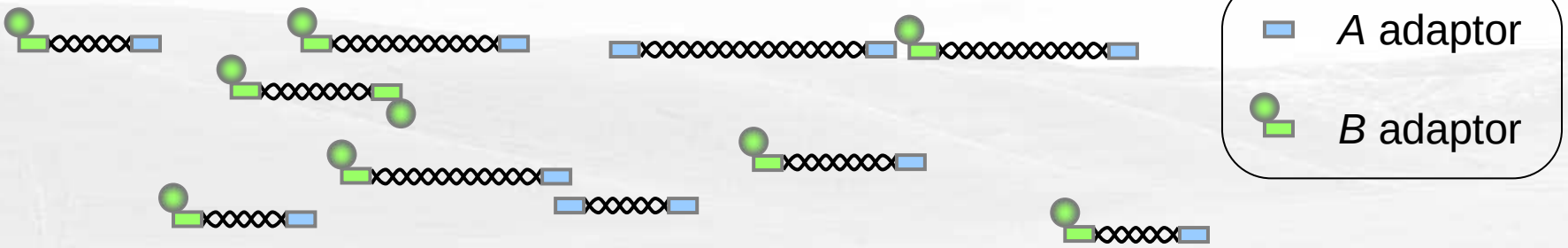


sst DNA Libraries

Random shearing of genomic DNA

sst DNA Libraries

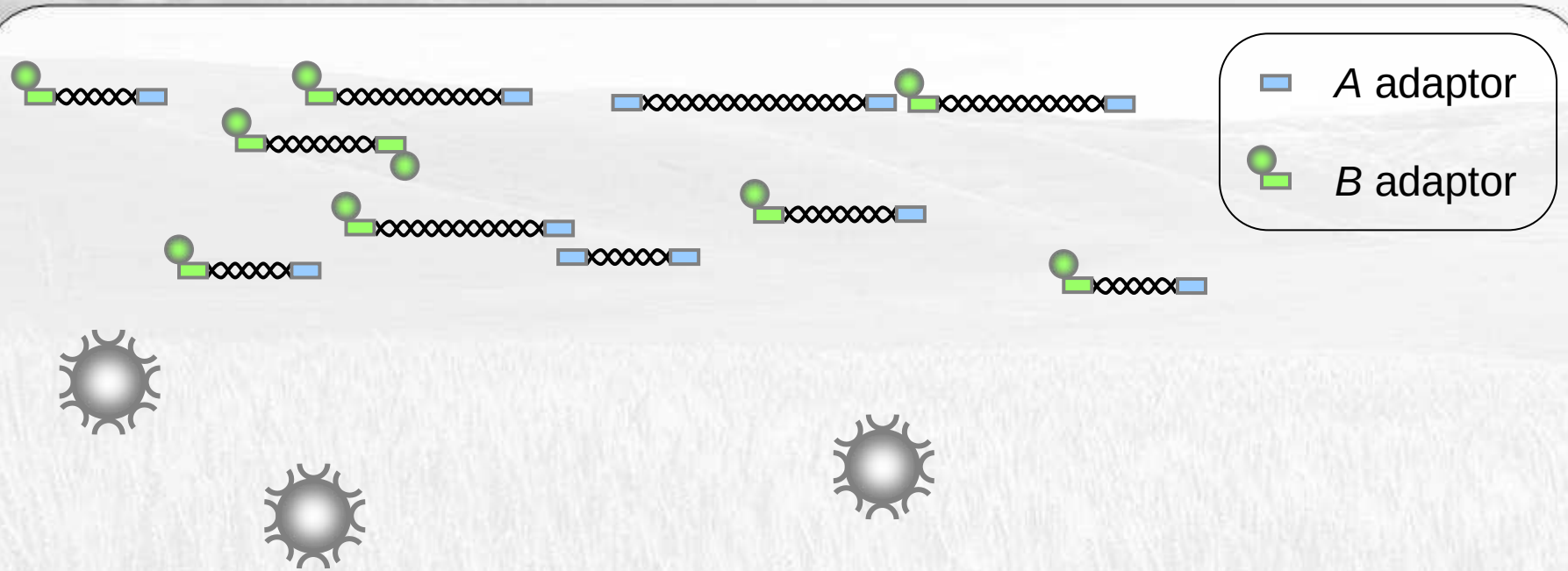


Add adaptors *A* and *B* for 454 sequencer

B adaptor is biotinylated

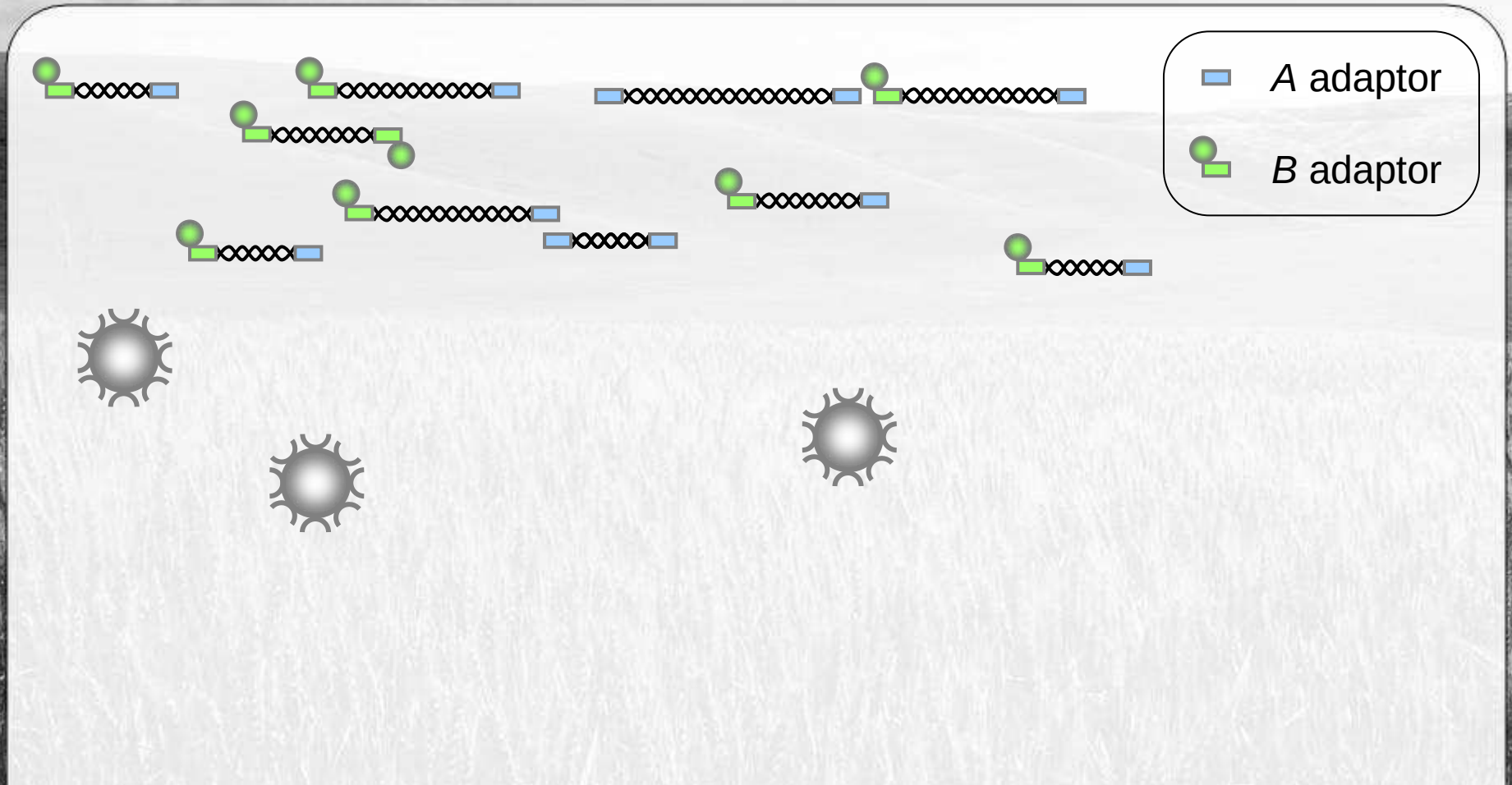
A adaptor is tagged

sst DNA Libraries



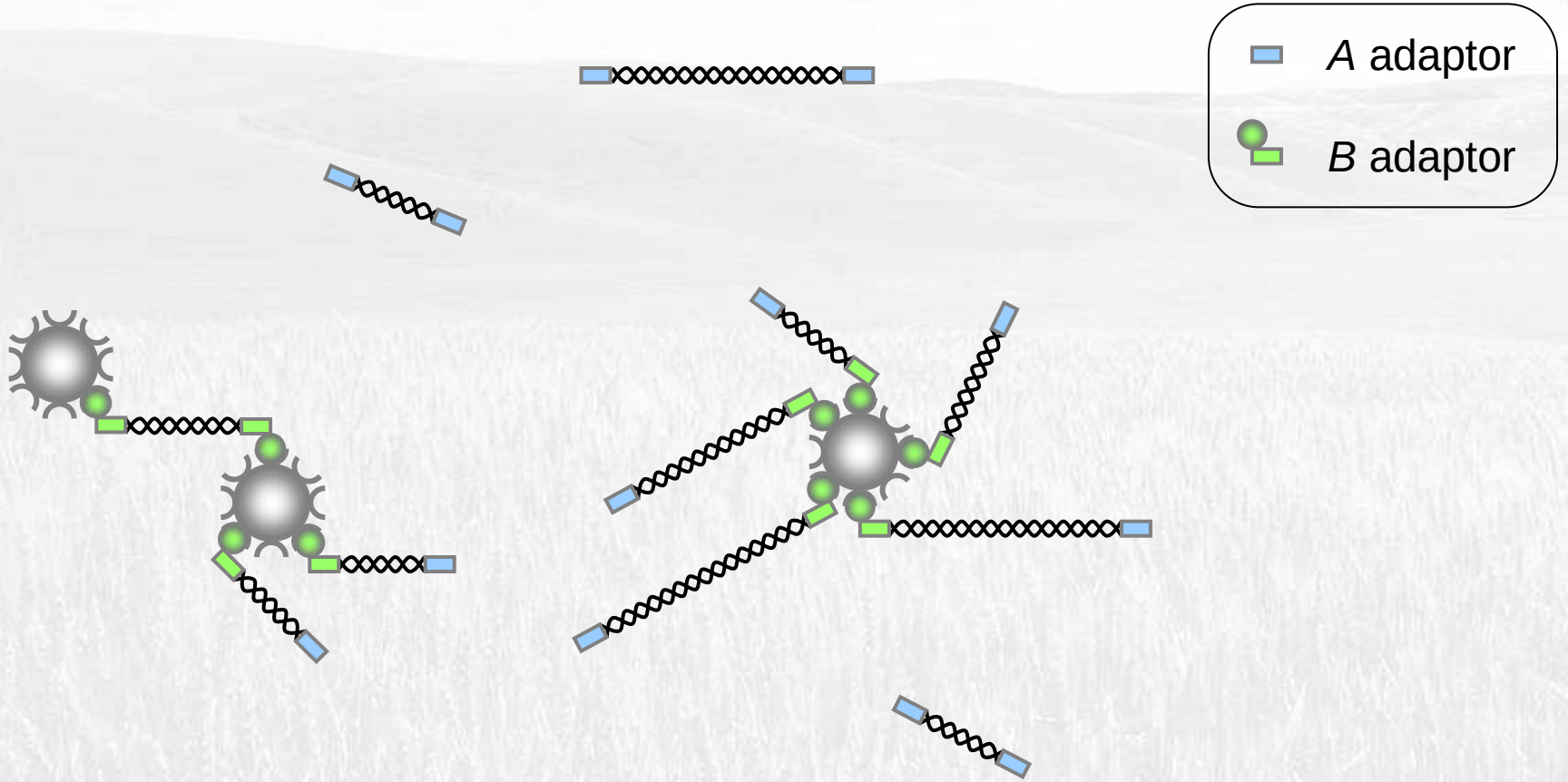
Add Streptavidin beads

sst DNA Libraries



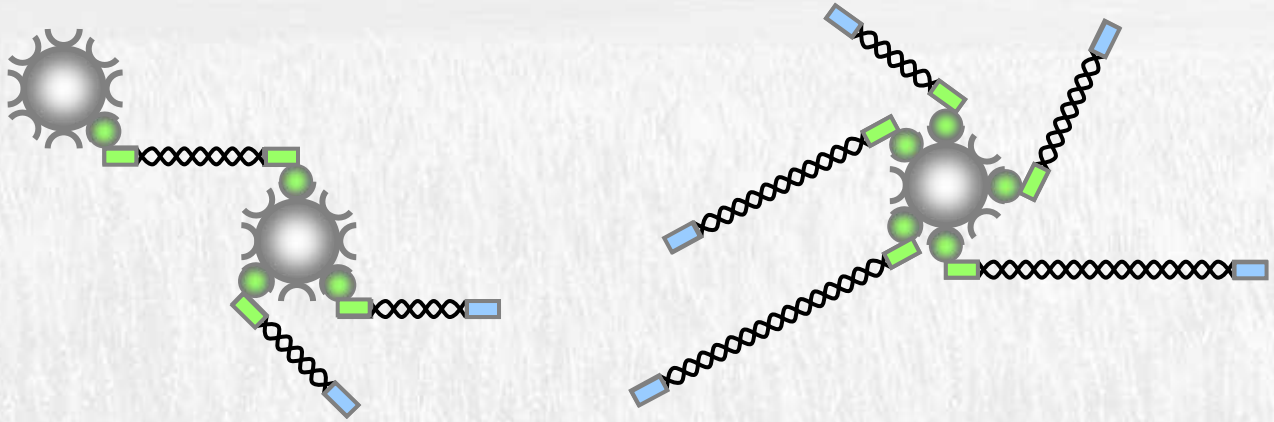
Biotinylated DNA captured

sst DNA Libraries



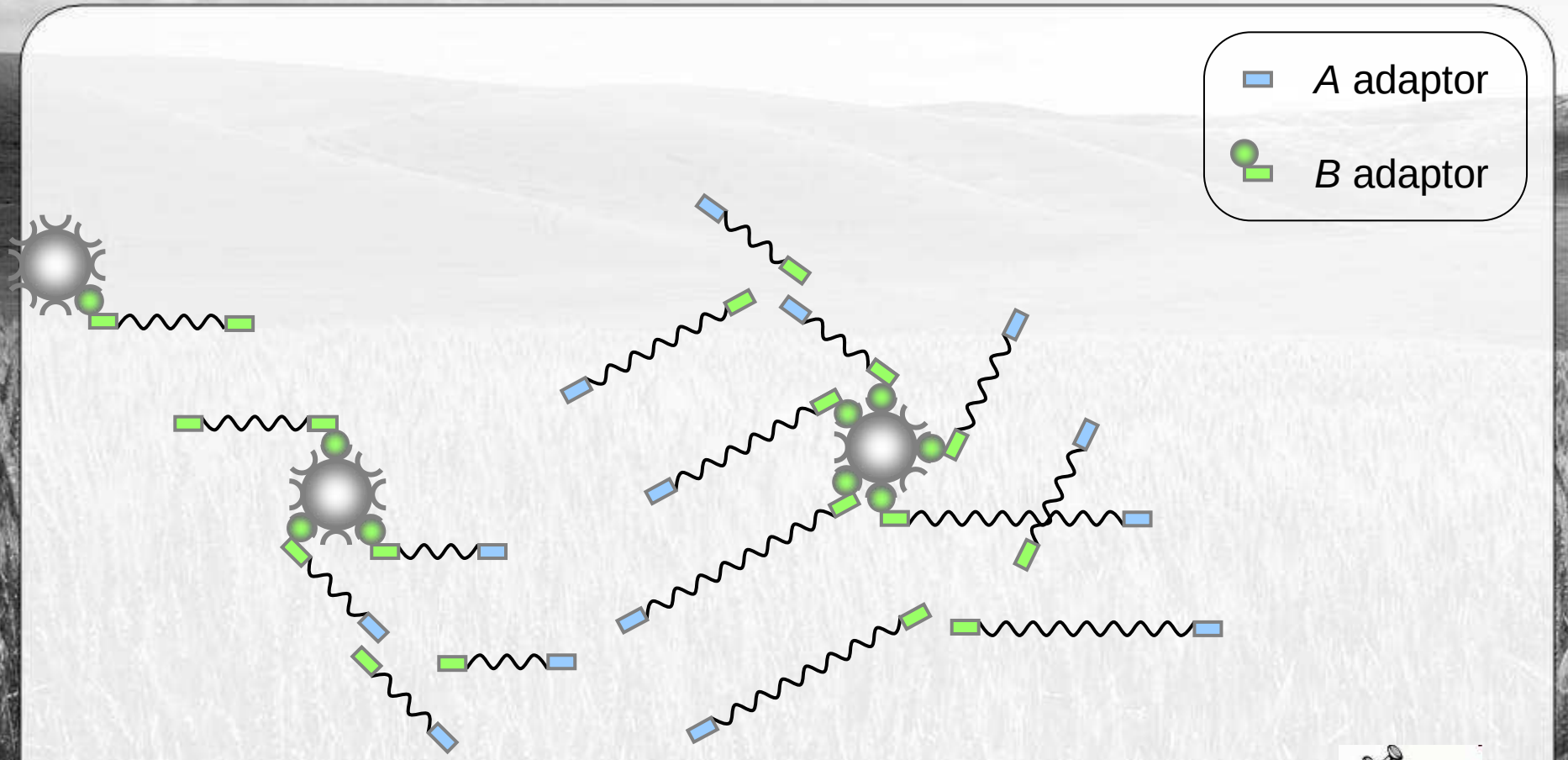
Wash to remove all *A*-*A* molecules

sst DNA Libraries



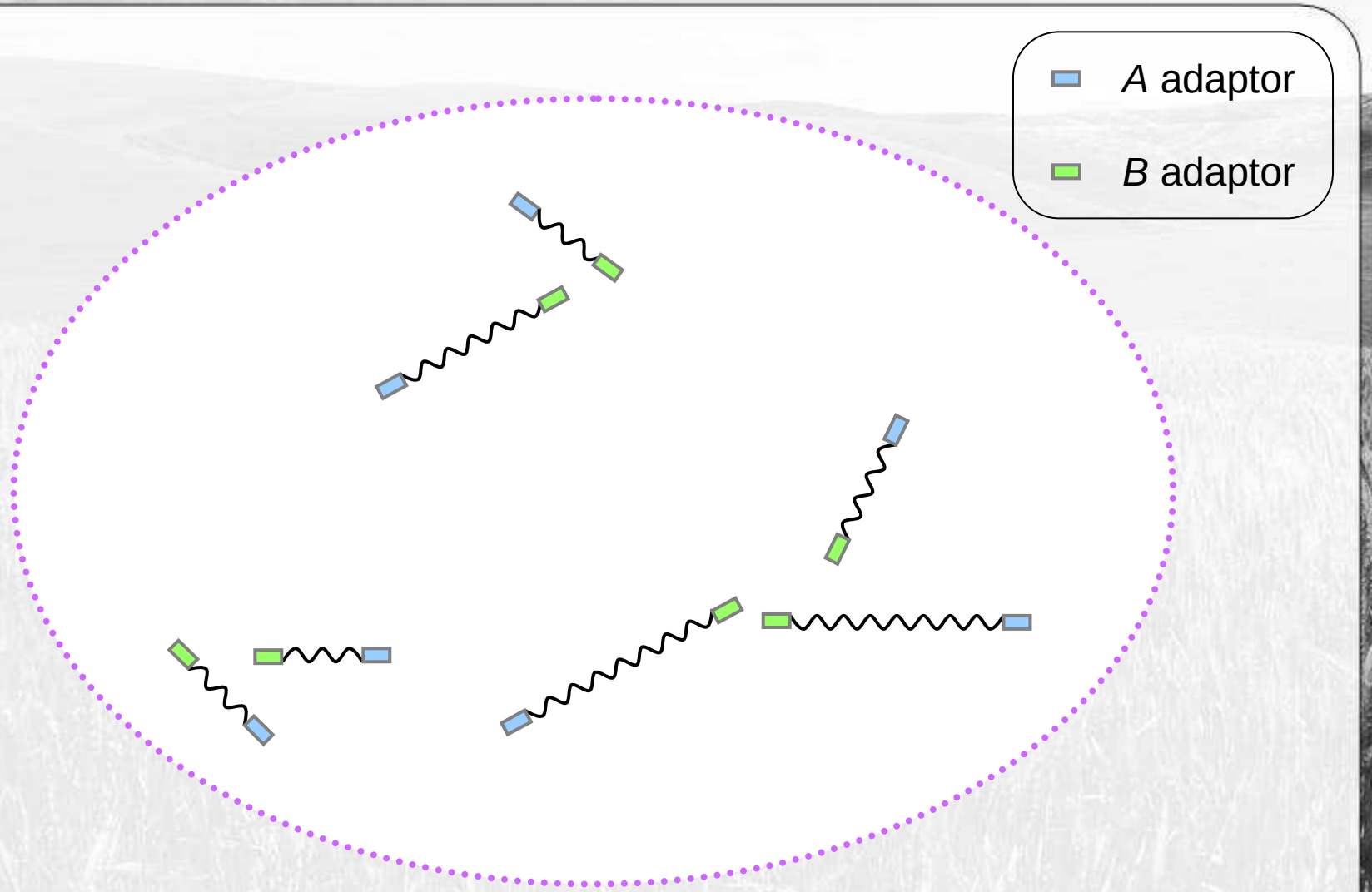
Denaturation

sst DNA Libraries



Remove Streptavidin beads
= remove *B*-*B* and *A*-*B* molecules

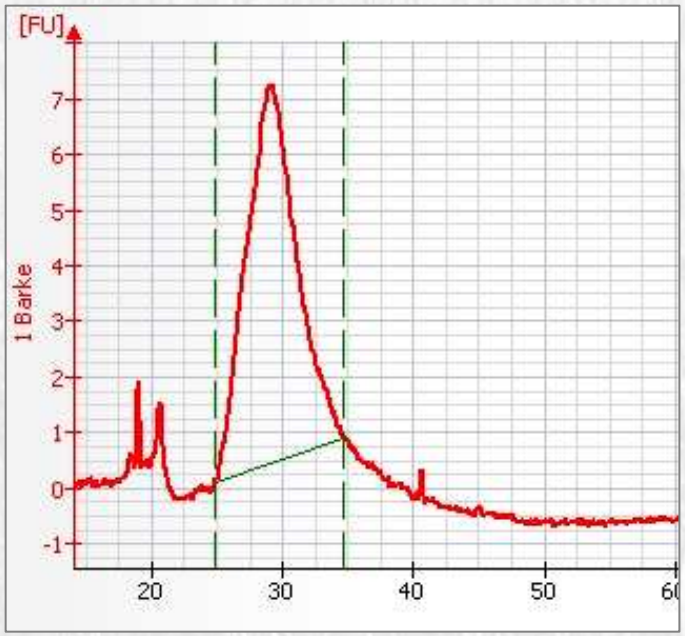
sst DNA Libraries

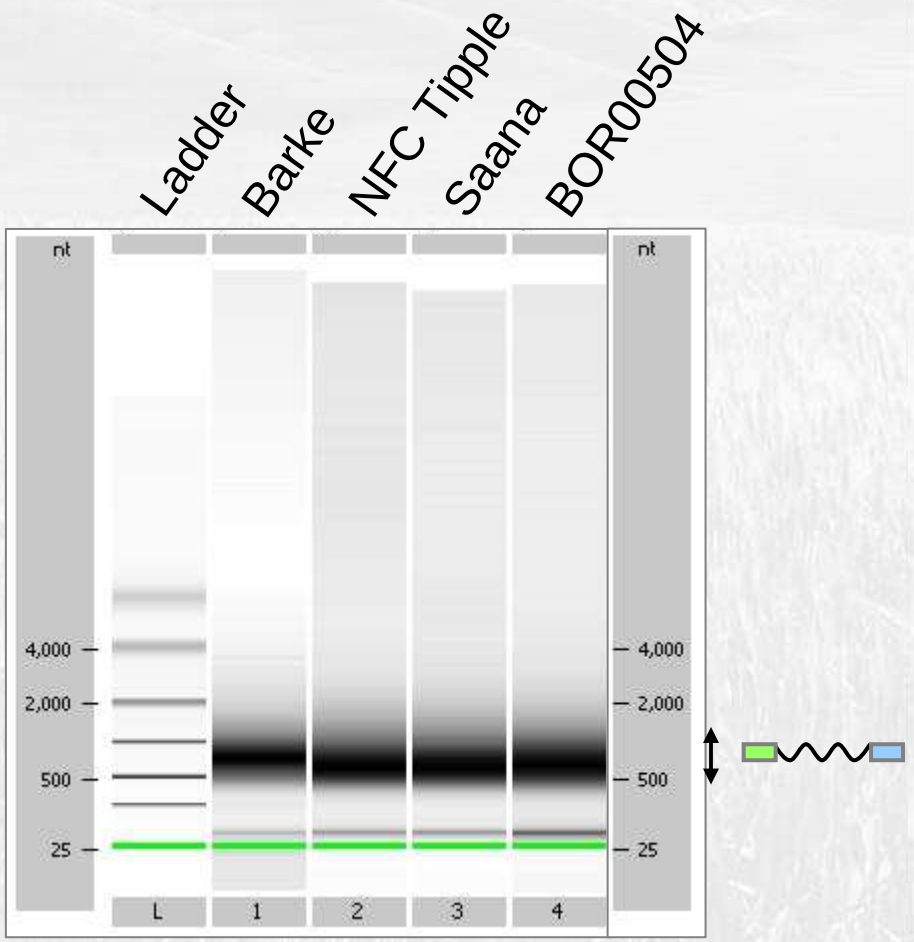


sst DNA libraries of *A*-*B* single-stranded molecules

sst DNA Libraries

Checking quality with Bioanalyzer





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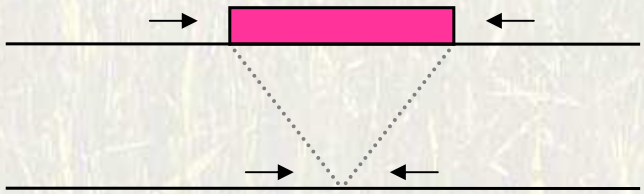


Short TEs in Barley

- **in BAC clones :**
  - NCBI databases : 82 BAC clones = 3.7Mbp
  - Search 49 TE families (size < 1Kbp)

    ⇒ 59 copies full-length with flanks

- **in Bac End Sequences :**
  - Search 25 TEs

	Sequences	bp	MITE hits
BES Arizona	140,907	99,040,801	930
BES Udine_A	44,865	22,946,912	257
BES Udine_B	74,549	52,018,370	292
TOTAL	**260,321**	**174,006,083**	**1,479**

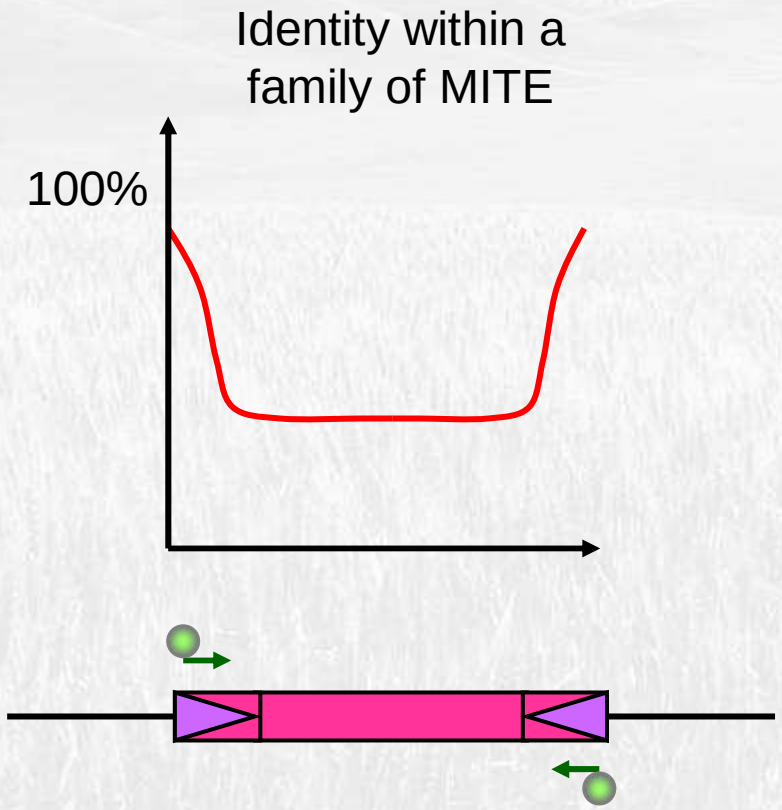
Short TEs in Barley

TEs	Size (bp)	BES Arizona	BES Udine A	BES Udine B	hits in BES	Copies in Barley ?
Belus	177	0	0	0	0	0
Gorgon	66	0	0	0	0	0
Hercules	239	1	0	0	1	50
Polyphemus	241	0	0	1	1	50
Mordred	203	2	0	0	2	100
Jorge	211	2	1	0	3	150
Antonio	108	4	1	0	5	250
Jura	416	2	3	0	5	250
Coeus	273	6	1	1	8	400
Fortuna	316	6	2	2	10	500
Jason	260	6	3	1	10	500
Harbinger	134	11	0	0	11	550
Islay	274	7	1	3	11	550
Phoebus	319	9	2	4	15	750
Orpheus	272	11	5	2	18	900
Xenon	310	13	5	4	22	1,100
Eos/Barfly	354	20	3	8	31	1,550
Stolos	259	22	1	8	31	1,550
Pluto	276	39	8	5	52	2,600
Skiron	93	30	12	16	58	2,900
Kerberos	285	42	10	9	61	3,050
Oleus	150	45	21	18	84	4,200
Xados	116	55	9	23	87	4,350
Icarus	117	97	18	31	146	7,300
Thalos	165	500	151	156	807	40,350
	TOTAL	**930**	**257**	**292**	**1,479**	**73,950**

Baits for MITE capture

- Design oligos

MITE	Size (bp)
Jura	400
Talisker	270
Islay	275
Athos	85-120
Orpheus	272
Eos/Barfly	354
Pluto	276
Kerberos	285
Icarus/Xados	117
Thalos	165



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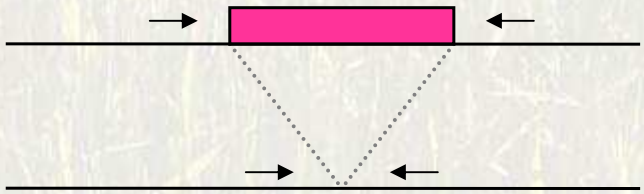
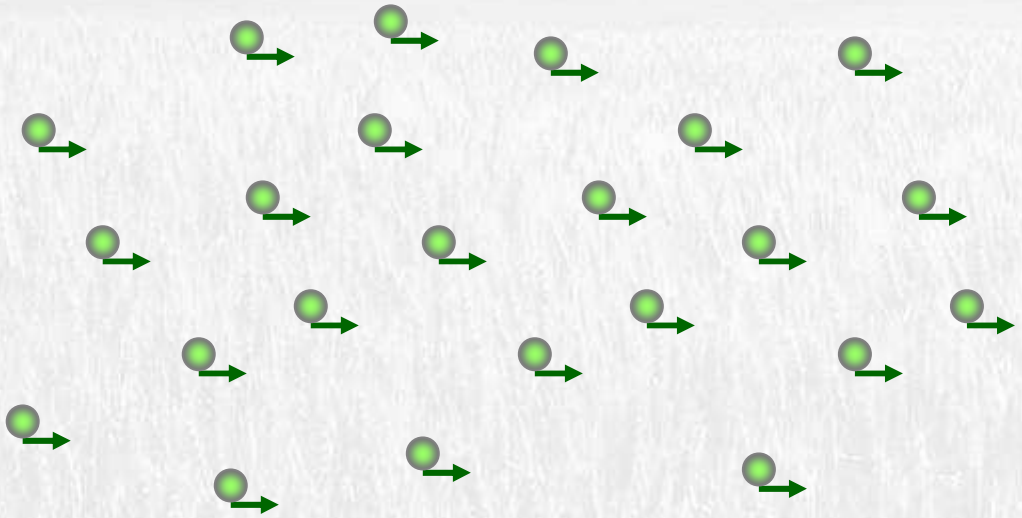
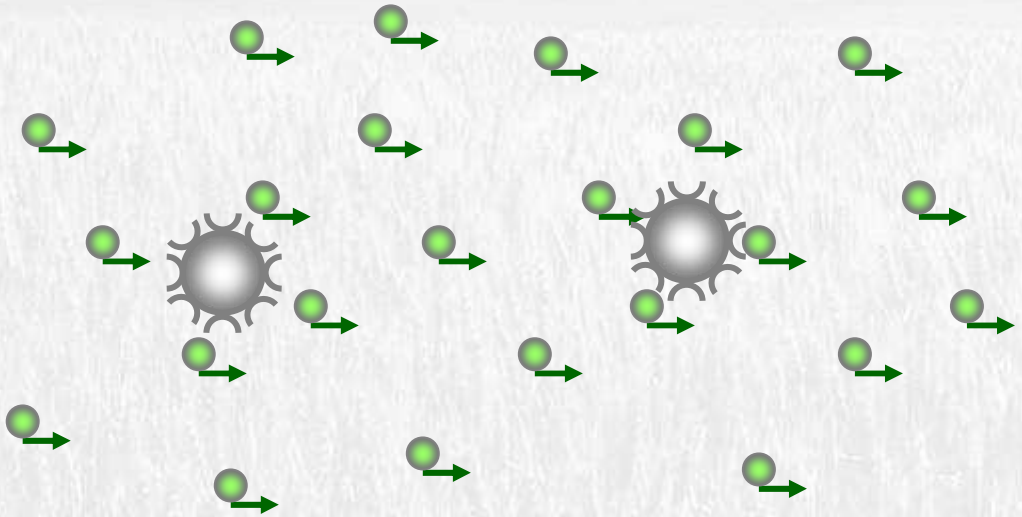


Capture



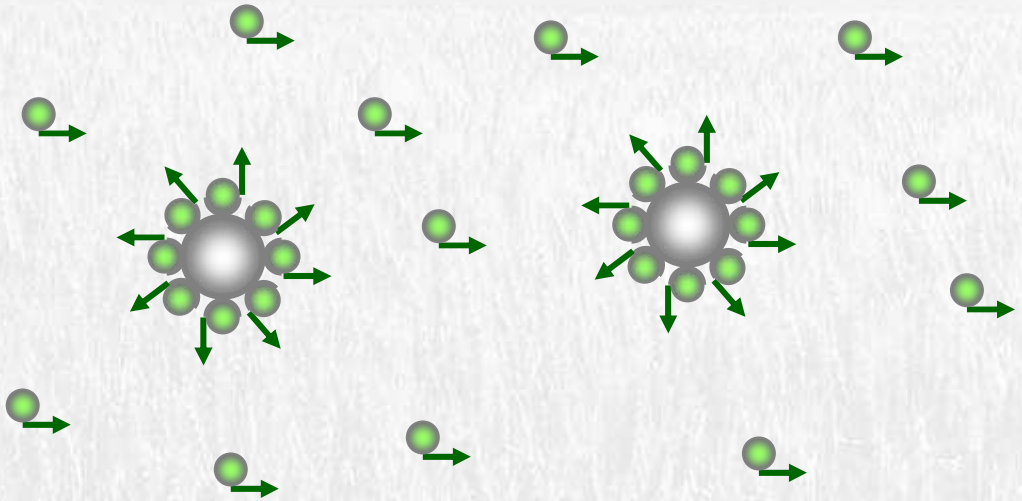
Oligos biotinylated

Capture



Add streptavidin beads

Capture



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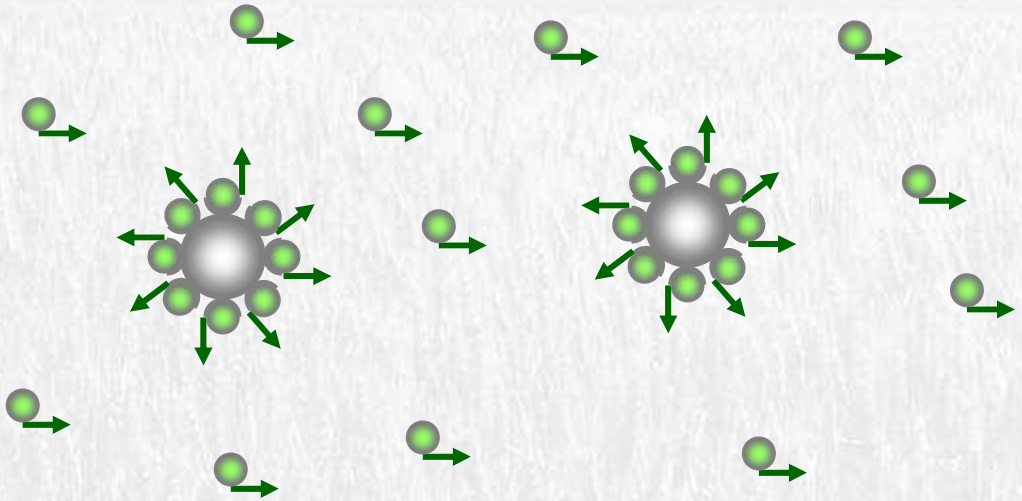
Capture

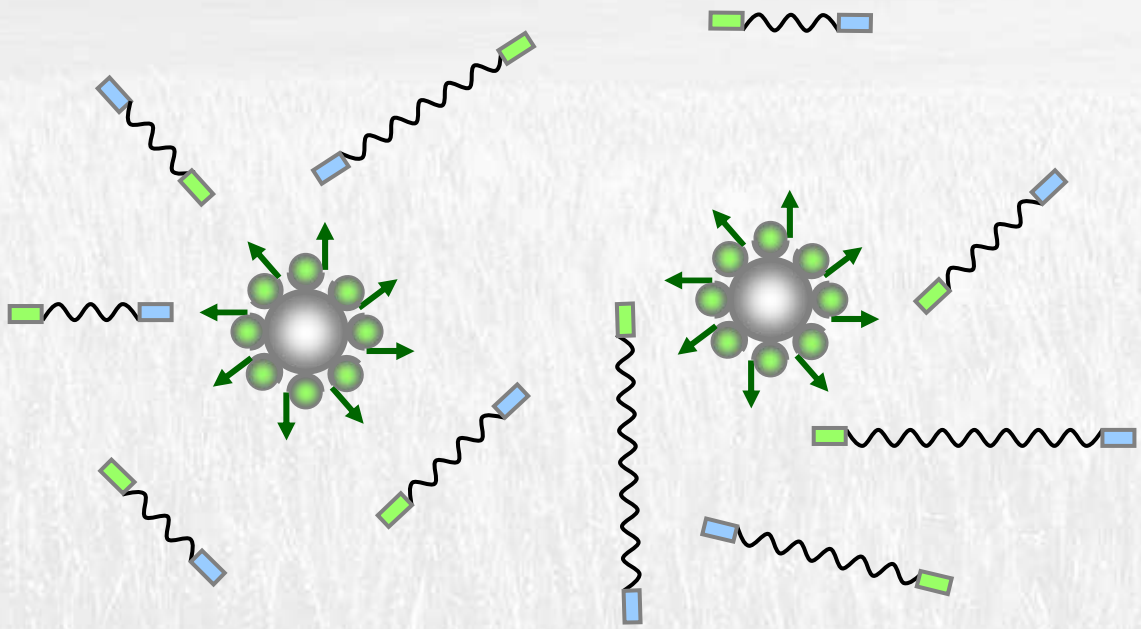


Wash

Capture

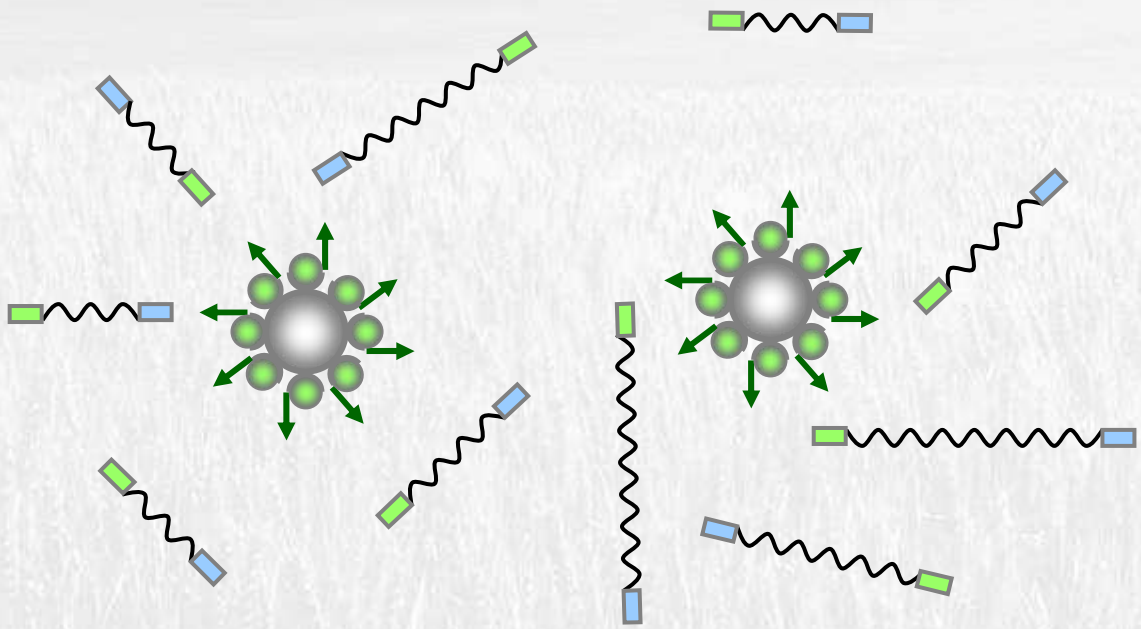
Wash

Capture



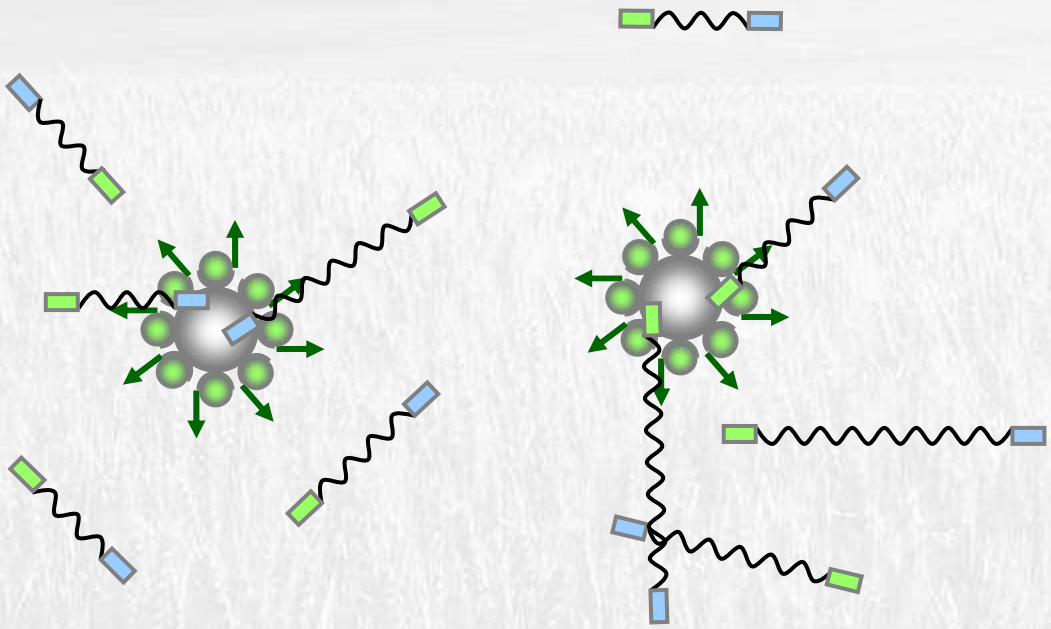
Add sstDNA library

Capture



Hybridization

Capture

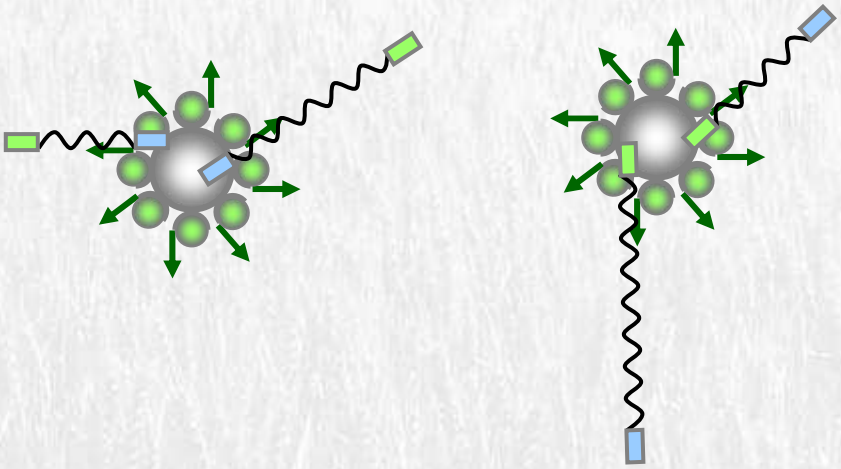


Wash

Capture

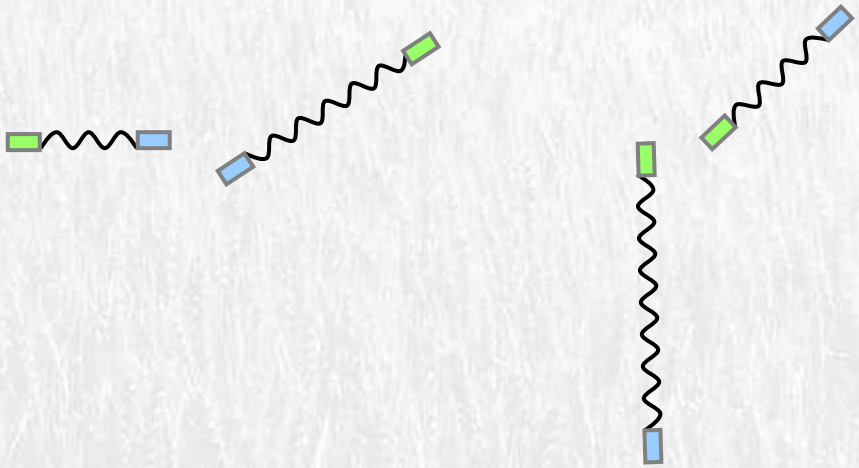
A adaptor

B adaptor



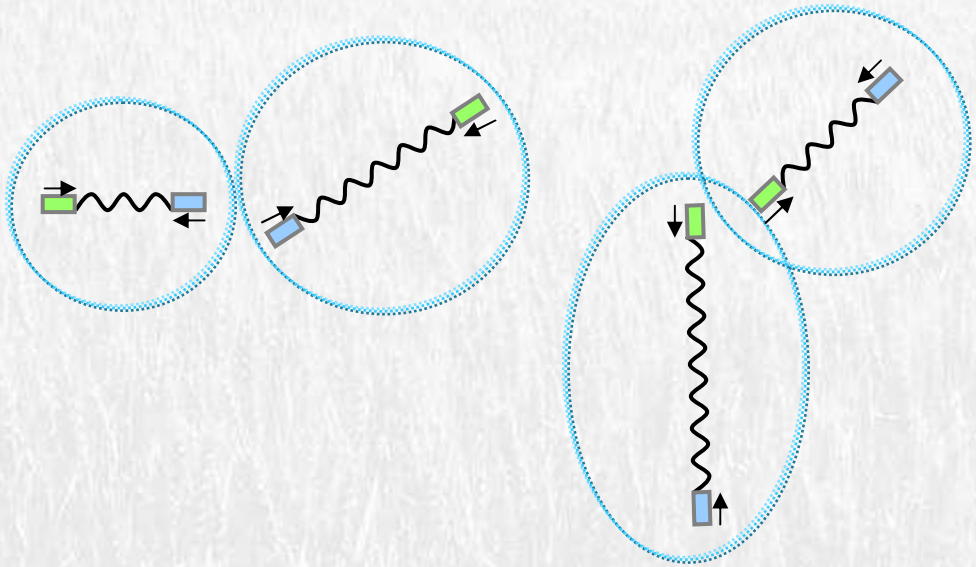
Denaturation & Elution

Capture



Emulsion PCR & 454 Sequencing

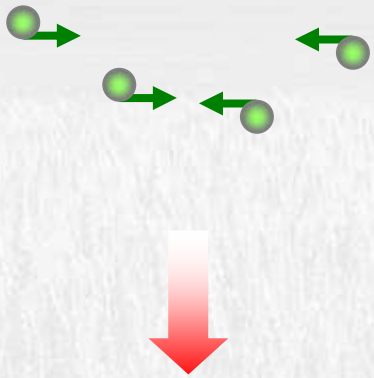
Capture



Emulsion PCR & 454 Sequencing

Capture

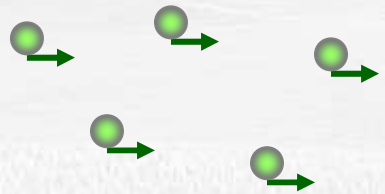
Capture using oligos



consensus oligos (~30nt)

Capture using oligos

- Capture using consensus oligos (~30nt)
  - Get 29,776 sequences (~200bp)



MITE	Size (bp)	Sequences
Jura	400 bp	52
Talisker	270bp	24
Islay	275 bp	10
Athos	85-120 bp	104

Capture using oligos

- Capture using consensus oligos (~30nt)
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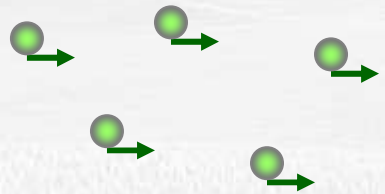

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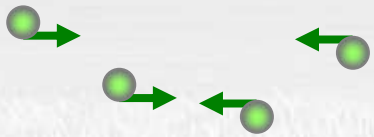
Jura		52
Tali		24
Islay		10
Athos		104

Not specific enough

Capture using oligos

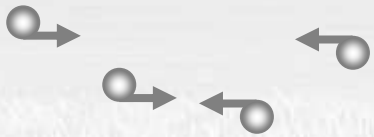
Capture using oligos



Not specific enough

Capture using PCR products

Capture using oligos



Not specific enough

Capture using
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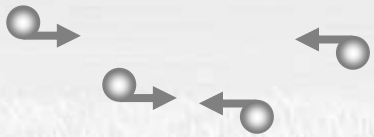
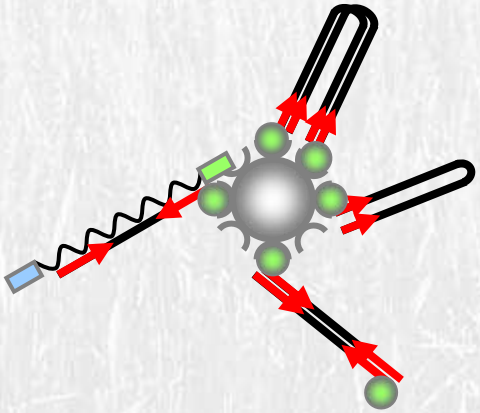
Capture using PCR products

Capture using oligos



Not enough specific

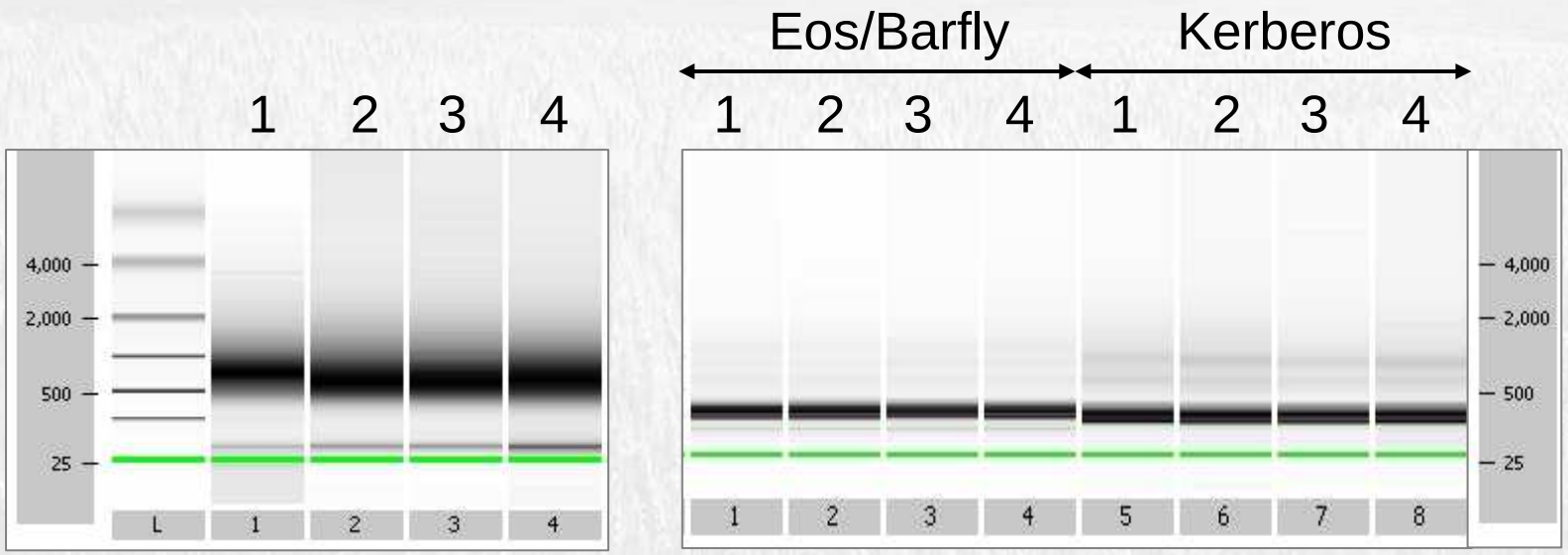
Capture using
PCR products



Capture using PCR products

Capture using
PCR products

1- Barke
2- NFC Tipple
3- Saana
4- BOR00504



sst DNA Libraries

sstDNA captured

Capture using PCR products

Capture using
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1- Barke
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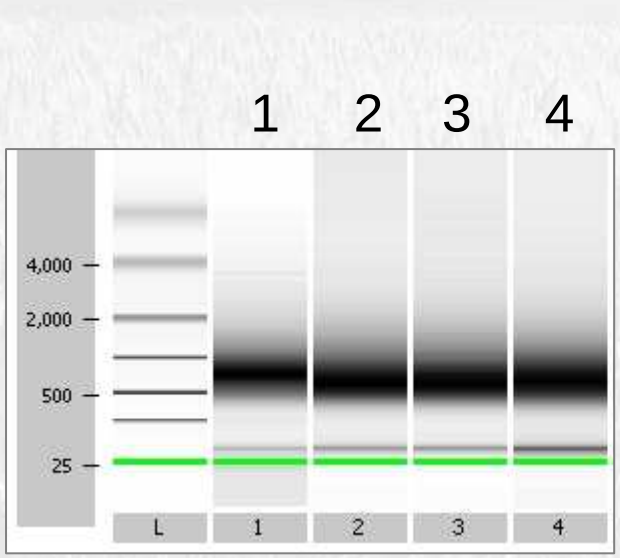
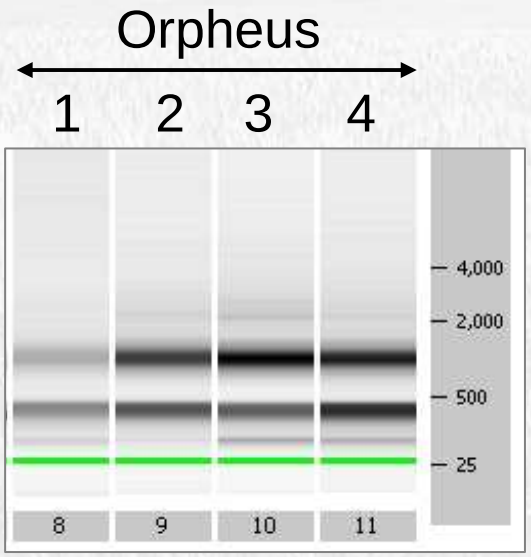


sst DNA Libraries

Orpheus



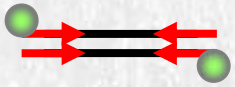
sstDNA captured

Capture using PCR products

Capture using PCR products

Hairpin

Self Hybridization



Prevent capture of targeted sstDNA

PCR product still present in the final elution

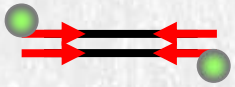
454 sequencer

Capture using PCR products

Capture using
PCR products

Hairpin

Self Hybridization



Prevent capture of
targeted sstDNA

PCR product still
present in the final
elution

PCR product digestion
Exonuclease 3'-5'

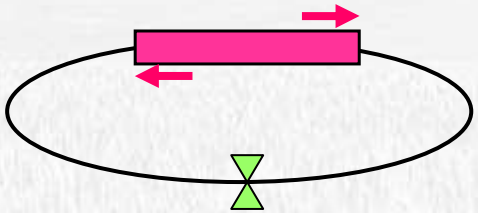
Capture using
RNA/DNA hybridization

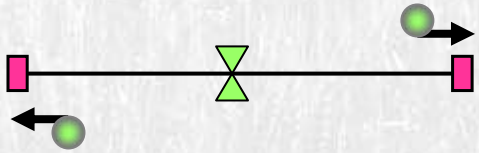
Improvement

Capture using
RNA/DNA hybridization

Should prevent remaining biotinylated PCR product in the final elution

Inverted PCR



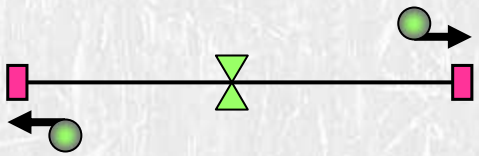


Inverted PCR

>s975_01_a01_001.seq_**Jura**_**SduI**:191

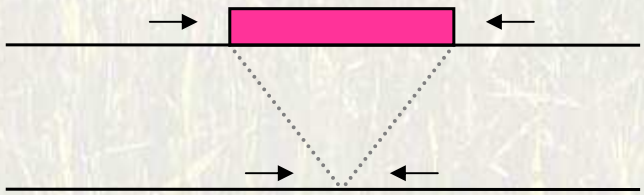
CTTCGAAAATTCTTTGTTATCAAACAGGGCCTaaagccaatagcatgcaacagtcacgccactcagtcaa
ggactccatcaatgcttgaatctctgaatttctcccaagttgtaacttattaccaacttcgaatcgtgaaatagagtttagtaacttggt
aggatatattggaagaggaagccaaagtgct**X**cgttcatctgtatttatgcaaactgaaaccagctcatttcattttggaagcgaa
ctgaacgttagggttgcggtgcgcaagctgaatctagttgccggcatggcccatcatcgacggagtgtcaccgtgggtggtagct
tcagggttttgtttgcttaaacttccaatgttcccagttgccggcatggtccatgcaagtgactaccctaagtttgaccactaatcaaa
ataagtttgcttaaacttccaatgttcccagttcccagttcccagtatcatttttctattaAGGCCCTGTTTGATAACAAA
GAATTTTCGAA

X – restriction site



Strategy

1. Libraries of single-stranded genomic DNA of barleys
2. Design biotinylated baits for MITE capture
3. Capture on streptavidin beads
4. **Design primers for polymorphic insertions**



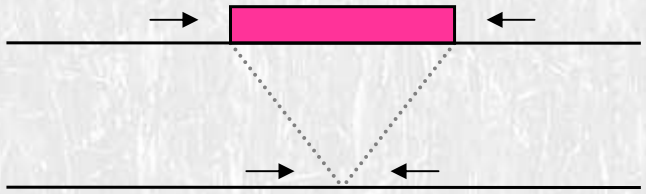
Design primers for polymorphic insertions

BLAST 3 MITEs on 2000 BACs from Morex

Get 1854 sequences MITE + flanks (~490bp) :

Icarus	323
Kerberos	354
Thalos	1154

Design 30 primer pairs to check presence of empty site and polymorphism in 20 barley varieties

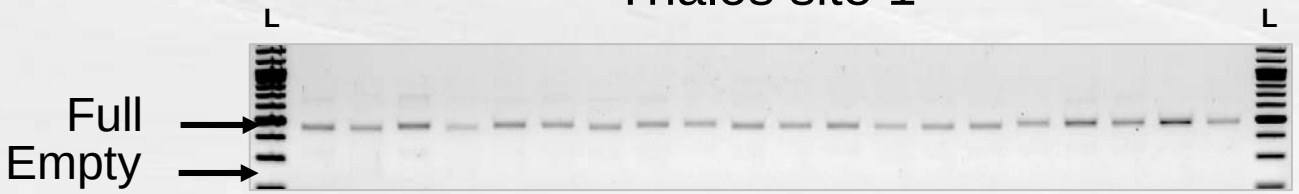


Nils Stein

Burkhard Steuernagel

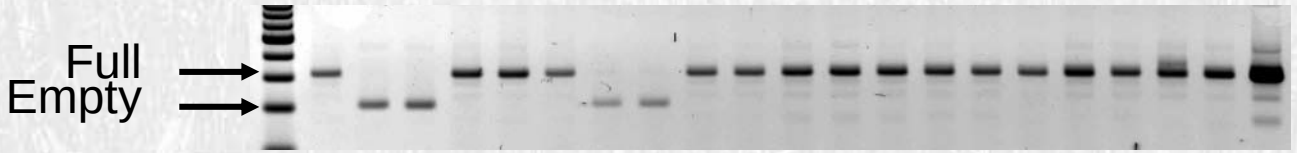
IPK GATERSLEBEN

Thalos site 1



Thalos site 3

Kerberos site 3



1. Arbalet
2. Astoria
3. Barke
4. NFC Tipple
5. Power
6. Prestige
7. Publican
8. Gustav
9. Justina
10. Kinnan
11. Minttu
12. Otira
13. Rekyl
14. Saana
15. Artturi
16. BOR00504
17. BOR00722
18. Botania
19. Edel
20. Jyvä

Conclusions

Conclusions

- Capture with oligos is not specific enough
- Capture with PCR products create troubles when baits remain after elution
- Problems should be solved with RNA/DNA hybridization and inverted PCR strategies
- Sequences from BAC clones to design markers

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