BAC Fingerprinting

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Development of fingerprinting methods

- ***Hin*dIII, stained agarose gels.** **Olson et al. PNAS 83:7826-30, 1986**

- ***Hin*dIII and *Sau*3A, radiolabeled, polyacrylamide gels.**
  Coulson et al. PNAS 83:7821-7825, 1986

- ***Hin*dIII and *Hae*III, radiolabeled, polyacrylamide gels.**
  Klein et al. Genome Res. 10:789-807, 2000

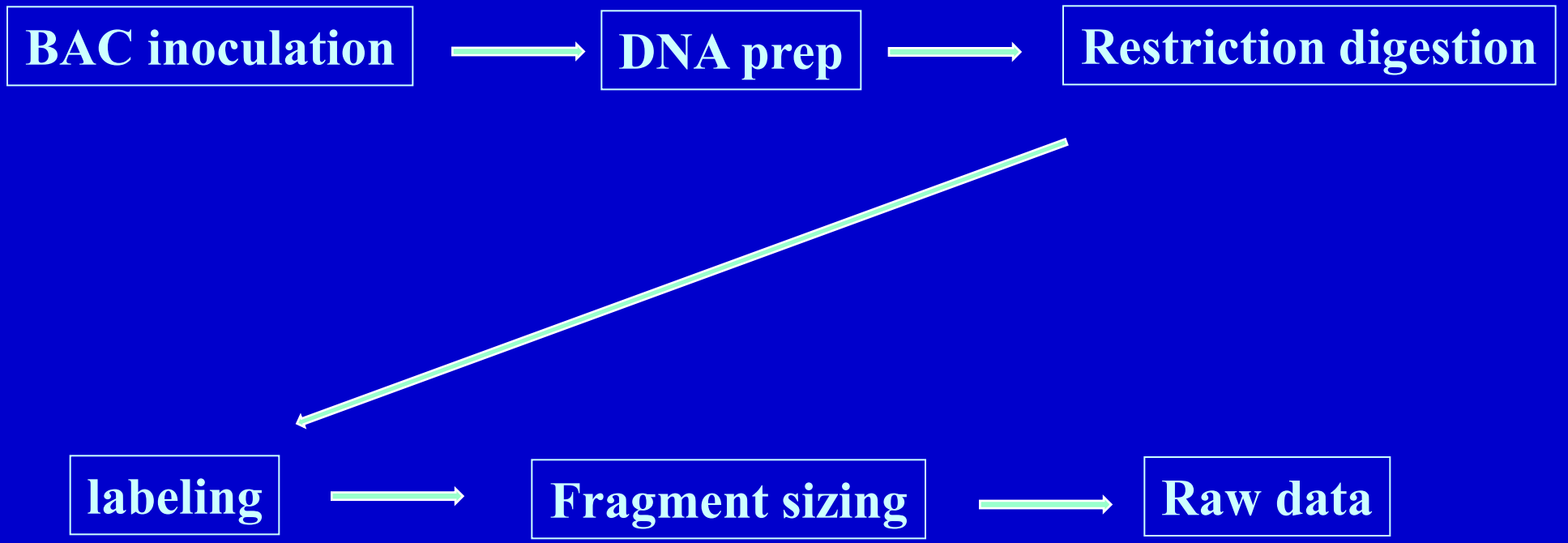
- ***Hin*dIII and *Sau3A,* fluorescence-labeled, polyacrylamide gel based sequencer.**
  Gregory et al. Genome Res. 7:1162-1168, 1997

- **Multiplexing of individul *Hin*dIII-*Hae*III, *Hin*dIII-*Dpn*I, and *Hin*dIII-*Rsa*I, fluorescence-labeled, polyacrylamide gel based sequencer.**
  Ding et al. Genomics 36:237-246, 1999

- **Single type IIS restriction digestion followed by determine of the nucleotide sequence at the cleavage site, fluorescence-labeled, polyacrylamide gel based sequencer.** **Brenner and Livak, PNAS 86:8902-8906, 1989**
  Ding et al. Genomics 74:142-154, 2000

- ***Bam*HI, *Eco*RI, *Xba*I, *Xho*I and *Hae*III, fluorescence-labeled, capillary sequencer.** **Luo et al. Genomics 82:378-389, 2003**

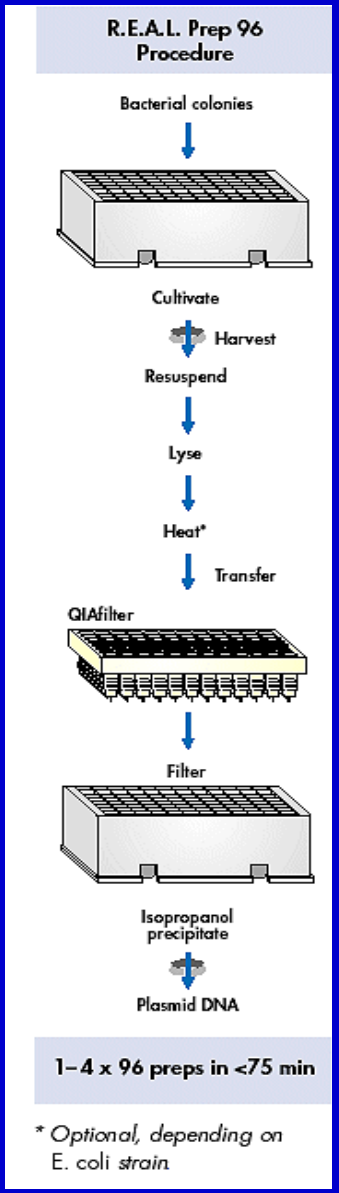
Workflow of BAC fingerprinting



High throughput DNA isolation
(R.E.A.L. Prep kit)

Automatic (4 plates/ 2 hrs)

Qiagen Robot 9600

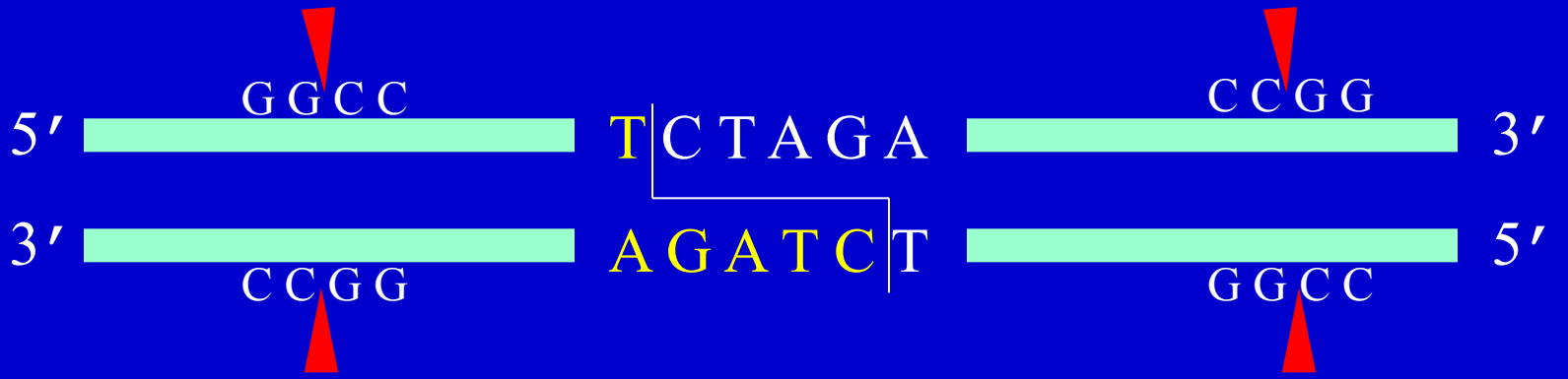


Manual

(4 plates in 70′)

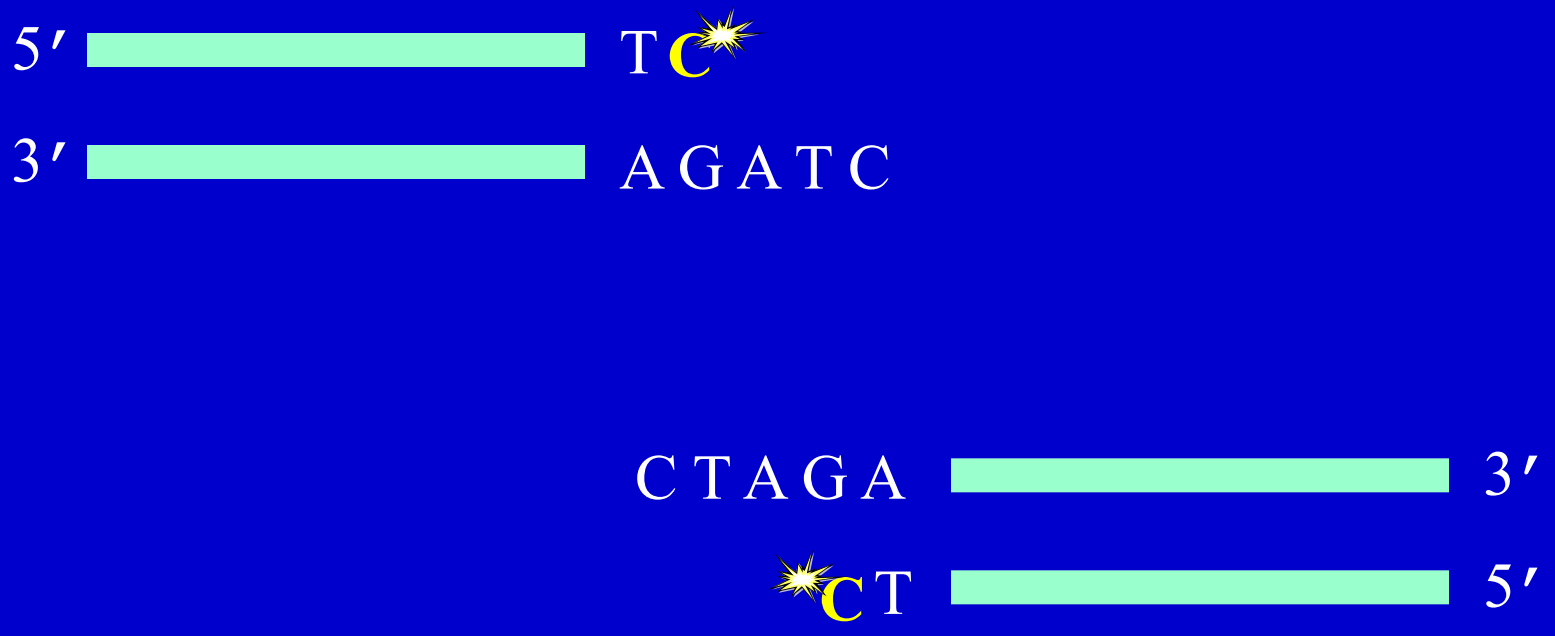
SNaPshot BAC fingerprinting

Restriction cleavage



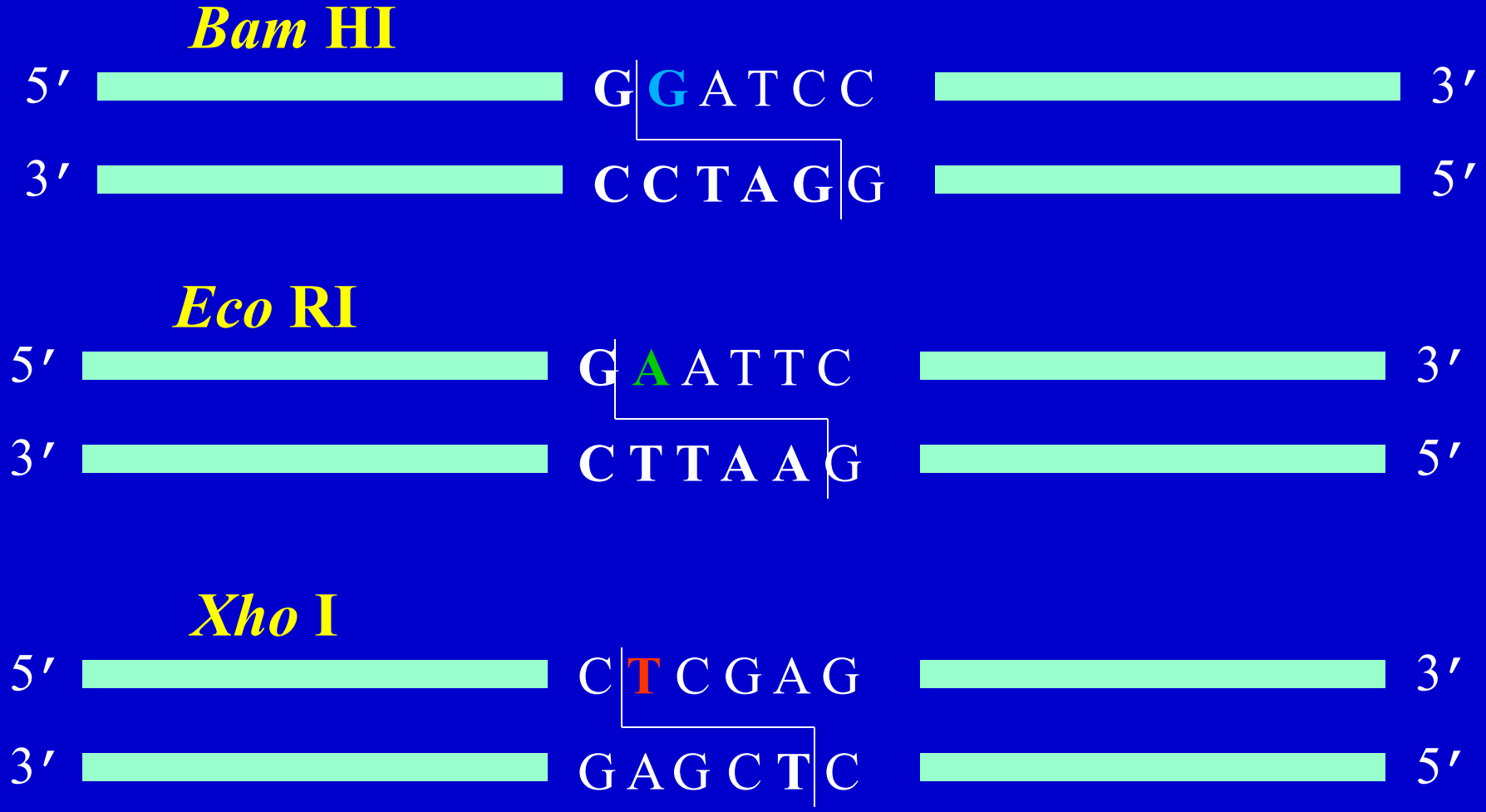
***Xba* I**

Fluorescent labeling



***Xba* I**

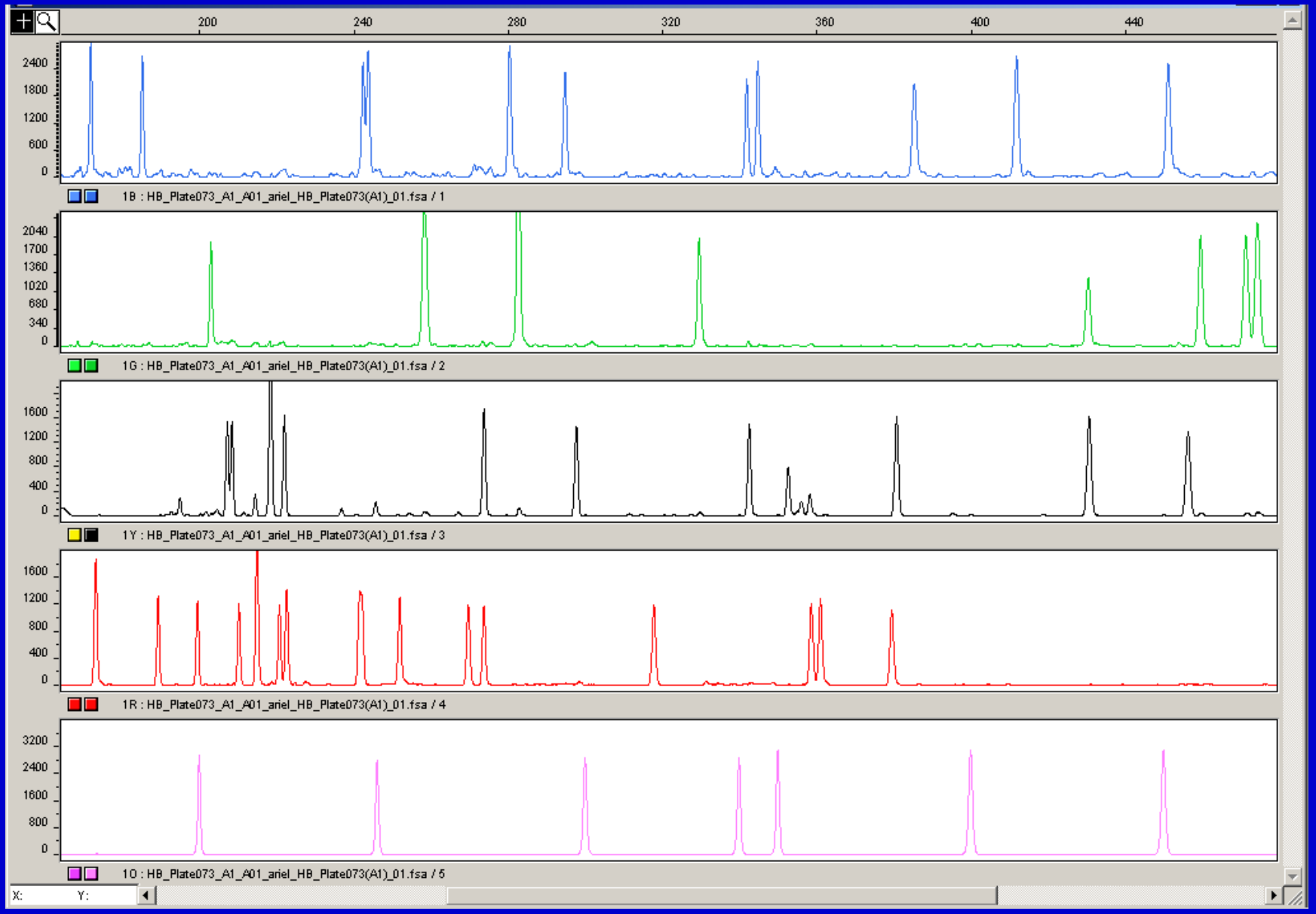
Restriction cleavage and fluorescent labeling



Characteristics of restriction sites and labeling of fragments

Restriction endonuclease	Restriction site	ddNTP	Fluorescent dye	Color of fragment
*Eco*RI	G^AATTC	A	dR6G	Green
*Bam*HI	G^GATTC	G	dR110	Blue
*Xba*I	T^CTAGA	C	dTAMRA	Yellow
*Xho*I	C^TCGAG	T	dROX	Red
*Hae*III	GG^CC	none		

Portion of multi-color fingerprinting profile of a BAC clone



BamHI

***Eco*RI**

***Xba*I**

***Xho*I**

Liz-1200

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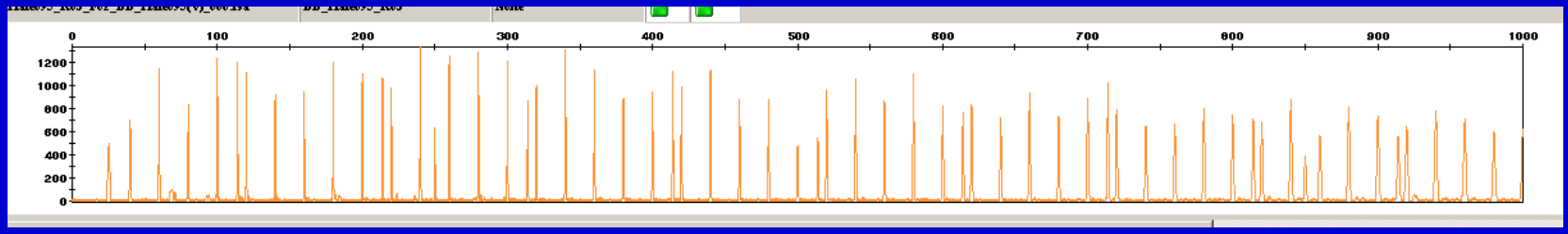
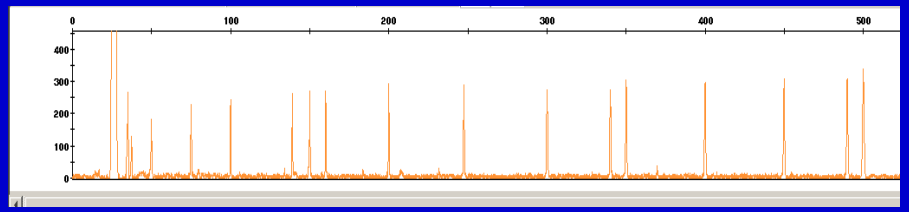
Comparison of GS1200Liz and GS500Liz

Sizing range: 20 –1200 (1000) bp



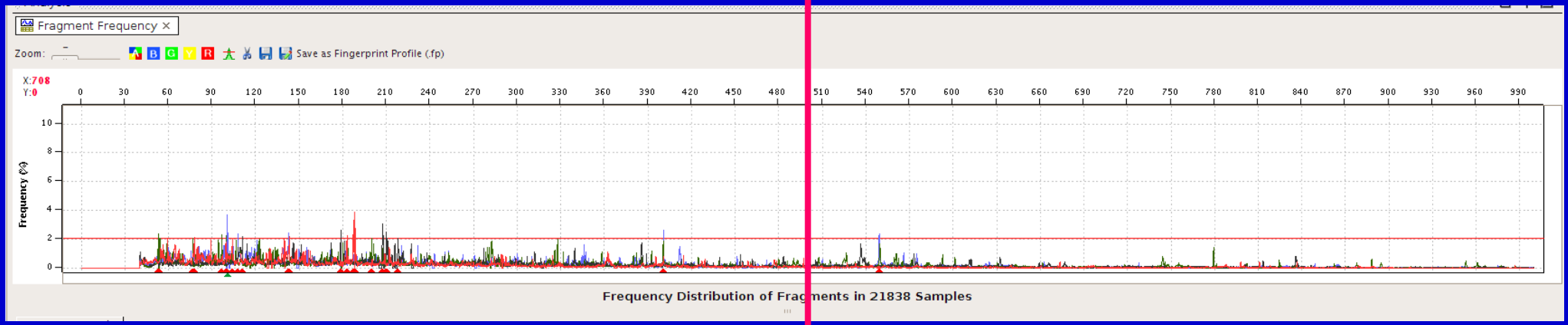
Sizing range: 35 –500 bp



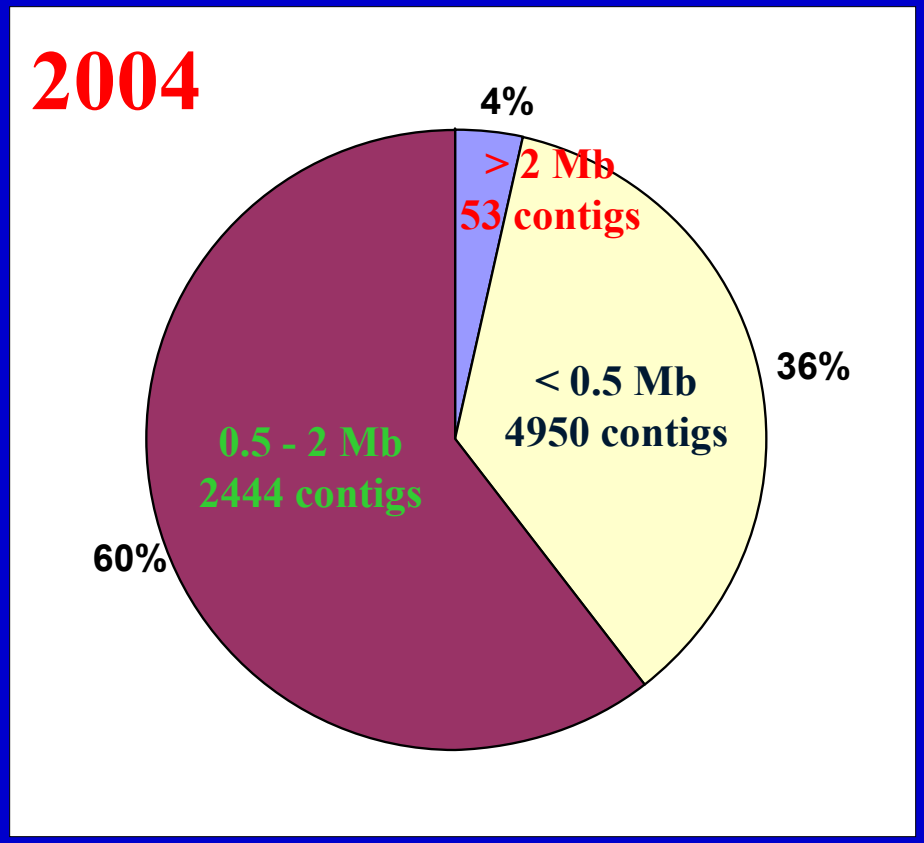
Distribution of fragment frequencies

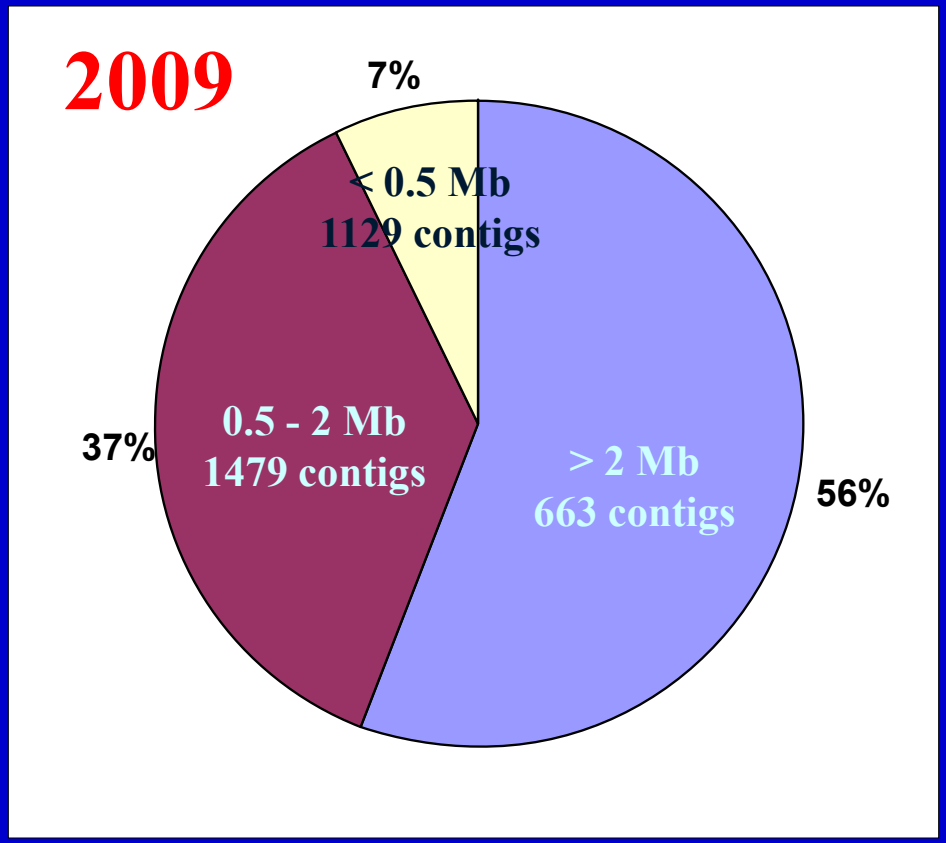
Sizing range: 40 –1200 (1000) bp

500 bp



Comparison of assemblies





Liz500
200K clones

Liz1200
380K clones

DNA isolation

Yields

- Media: 2x YT
- Volume: 1.2 ml
- Cell density (OD ~ 3.5)

Quality

- RNA
- Cell debris
- Salt

Reactions

- Simultaneously multiple restriction digestion
- Fluorescence labeling
- Precipitation

Guideline of enzyme selection

- Compatible restriction site

Guideline of enzyme selection

- Compatible restriction site
- Cost

Affordable?

Required:

- **Generate 5' overhang**
- **AGCT only**
- **6-bp cut ?**

Cheap: 7 More expensive: 40 Don't care: 55

A	G	C	T
*Eco*RI ($212) * *Hin*dIII ($212)	*Bam*HI ($212) *Bgl*II ($1060)	*Xba*I ($840)	*Sal*I ($1120) *Xho*I ($504)

* Prices are based on NEB's list price of 50,000 units at available largest package

Guideline of enzyme selection

- Compatible restriction site
- Cost
- Frequency of restriction site

Predicted numbers of restriction fragments in SNaPshot fingerprints of two *Triticum monococcum* and two *T. turgidum* BACs in the range of 50-500 bp

Enzyme	116F2 (107.3 kb)	115G1 (128.6 kb)	BAC1 (173.4 kb)	BAC2 (147.6 kb)	Total (556.9 kb)
*Eco*RI	**31**	**38**	**32**	**32**	**133**
*Bam*HI	21	36	53	32	141
*Xba*I	31	47	38	41	157
*Xho*I	26	30	46	23	125
Total	108	151	168	128	--
*Hin*dIII	**43**	**51**	**68**	**77**	**239**

Critical factors:

- Optimized reaction conditions

Conditions that contribute to star activity

1. High glycerol concentration [>5% v/v]
2. High units to µg of DNA ratio [Varies with each enzyme, usually >100 units/µg]
3. Low ionic strength [<25 mM]
4. High pH [>pH 8.0]
5. Presence of organic solvents [DMSO, ethanol, ethylene glycol, dimethylacetamide, dimethylformamide, sulphalane]
6. Substitution of Mg^{++} with other divalent cations [Mn^{++}, Cu^{++}, Co^{++}, Zn^{++}]

From: www.neb.com

Inhibiting star activity

1. Use as few units as possible to get a complete digestion. This avoids overdigestion and reduces the final glycerol concentration in the reaction.
2. Make sure the reaction is free of any organic solvents such as alcohols which might be present in the DNA preparation.
3. Raise the ionic strength of the reaction buffer to 100-150 mM (provided the enzyme is not inhibited by high salt).
4. Lower the pH of the reaction buffer to pH 7.0.
5. Use Mg^{++} as the divalent cation.

From: www.neb.com

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- *ß*-mercaptoethanol

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- pH

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- Optimized reaction conditions
- *ß*-mercaptoethanol
- Bovine Serum Albumin (BSA)
- pH
- Optimal temperature for *Taq* FS activity

Updated on 4/22/2007
By M. C. Luo

Protocol of BAC Fingerprinting with SNaPshot™ Kit

1. **Re-suspension:** BAC DNAs isolated by Qiagen R.E.A.L Prep 96 Plasmid kit were re-suspended in 42 µl of ddH_2O in deep well block, vortex gently, and place at 4 °C overnight or longer.
2. **Restriction enzyme digestion:** Add 8.0 µl of the restriction enzyme cocktail (see below); incubate at 37 °C, for 3 hrs.

   Enzyme Cocktail (1x) (NEB enzymes):

Bam HI	2.0 units (0.10 µl)
Eco RI	2.0 units (0.10 µl)
Xba I	2.0 units (0.10 µl)
Xho I	2.0 units (0.10 µl)
Hae III	2.0 units (0.20 µl)
NEBuffer 2	5.0 µl
100X BSA	0.5 µl
RNase A (0.5 µg/µl, DNase free)	1.0 µl
β-Mercaptoethanol (1%)	1.0 µl

3. Transfer 50.0 µl of the digested DNAs into 96-PCR plate.
4. **Labeling:** Add 10.0 µl of SNaPshot labeling cocktail (see below), briefly spin down; incubate at 65 °C for 60′.

   Labeling Cocktail (1x):

SNaPshot Multiplex Ready Reaction Mix (from ABI)	0.3 µl
NEBuffer 2	2.0 µl
100 mM Tris (pH = 9.0)	2.5 µl
ddH_2O	5.2 µl

5. **Precipitation:** Add 5.0 µl of 2.5M Sodium Acetate, 100 µl of pre-chilled ethanol (95%), and place at –80°C for 10-15′. Spin at 4200 rpm for 30′; Wash with 70% ethanol and spin at 3500 rpm for 10′; spin upside down on paper towel at 500 rpm for 2′; air dry for 5′.
6. **Re-suspension:** Re-suspend the pellet with 9.95 µl of Hi-Di formamide, and vortex gently, then 0.05 µl (Liz500) or 0.15 µl (Liz1200) of Liz Size Standard.
7. **ABI 3730XL:** Denature at 95 °C for 5′, and place on ice until ready to load on the ABI 3730XL.

Current working protocol at UC Davis

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Workflow of BAC fingerprinting