

From: [Shujun Luo](#)
To: [John West](#)
Subject: Re: Can you please email me a copy of your slides from this afternoon?
Date: Thursday, November 1, 2012 4:26:21 PM
Attachments: [labupdate121101.pptx](#)

Here it is.

Thanks,

Shujun

On 11/1/12 4:00 PM, "John West" <john.west@personalis.com> wrote:

>Thanks,
>
>John West
>
>Cell (408) 836-5586
>john.west@personalis.com
>



Personalis™

PIONEERING GENOME GUIDED MEDICINE

Exome Plus Lab Update

Shujun Luo

11/01/12



Personalis™

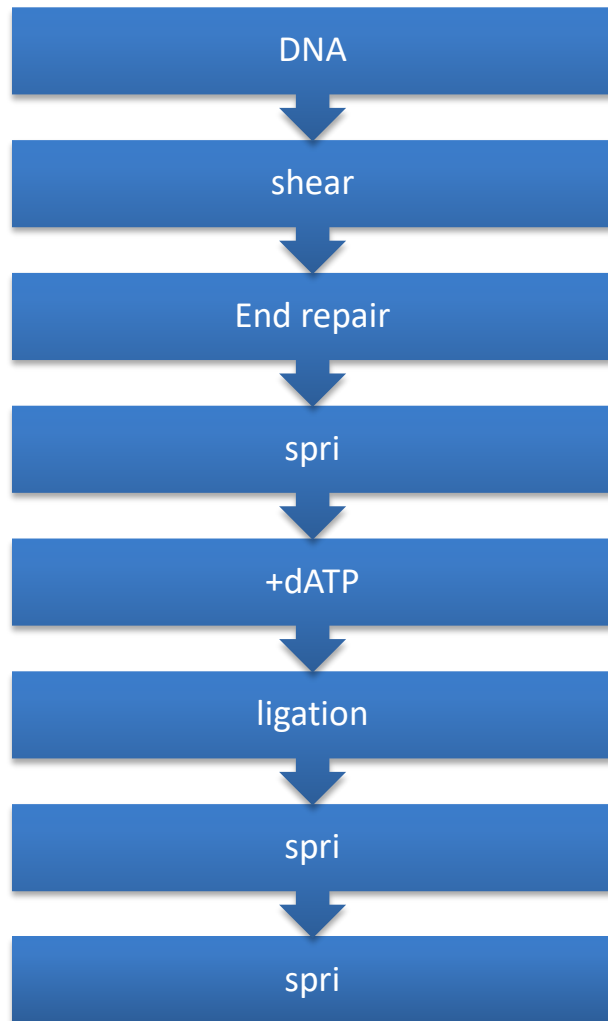
Personalis EX2102

Personalis Exome Plus

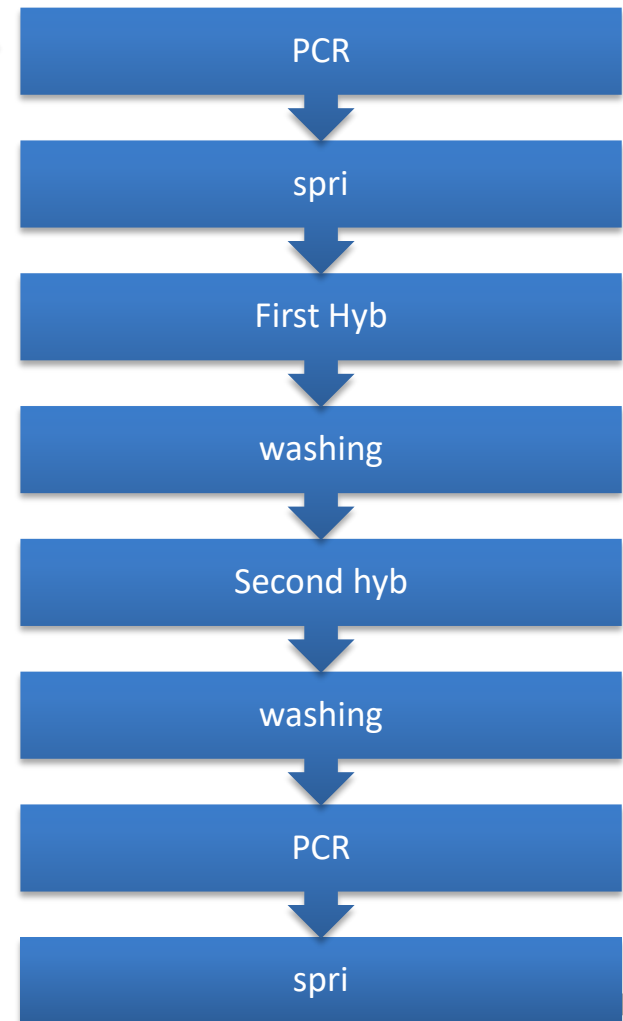
1. Standard Exome
2. ESP
3. LRP
4. HGCP

Workflow

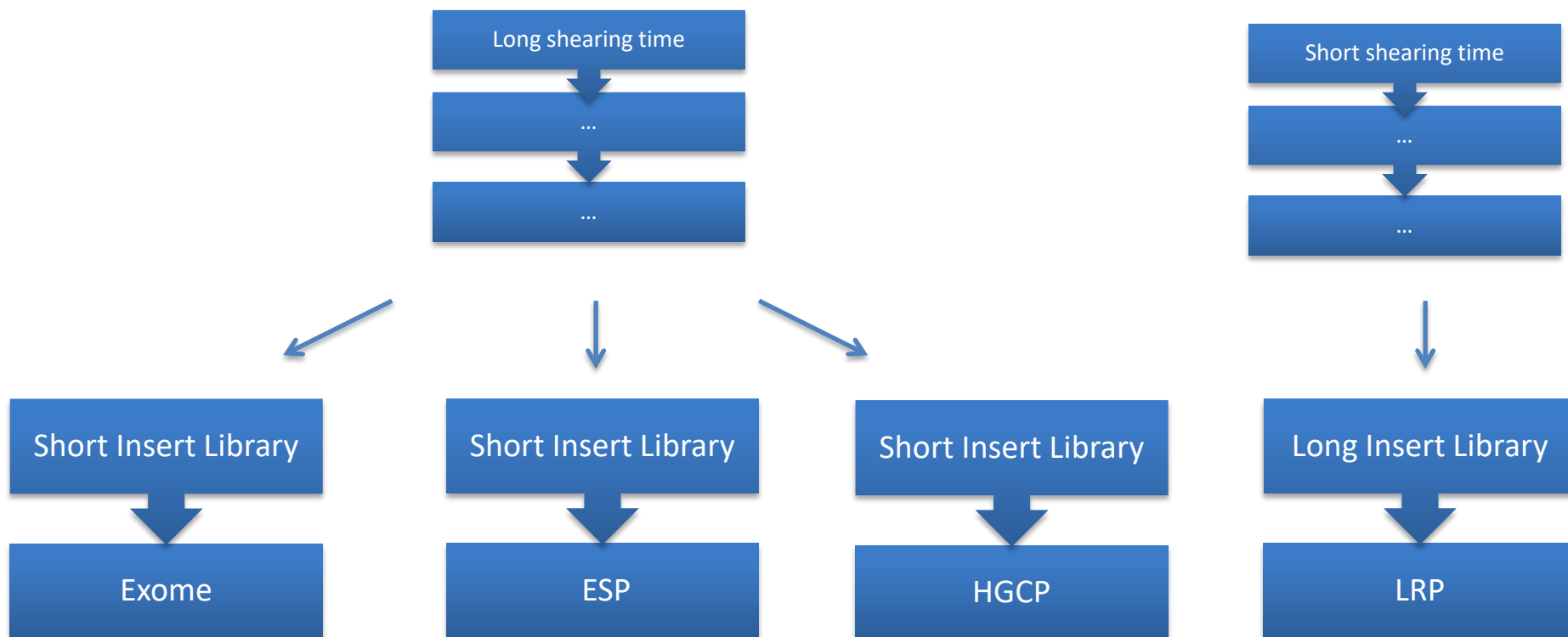
Gel free PCR free genomic prep



Exome pull down prep

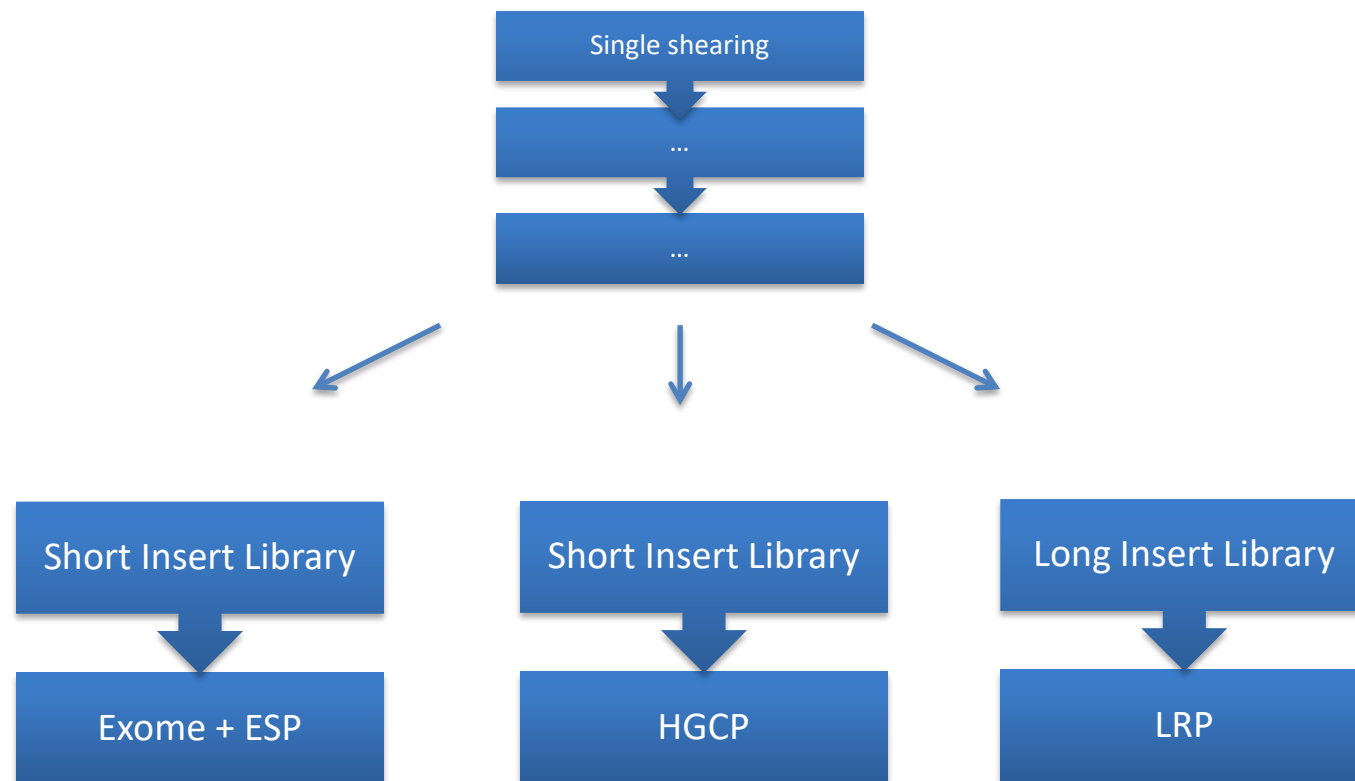


Impacts on Lab Processes

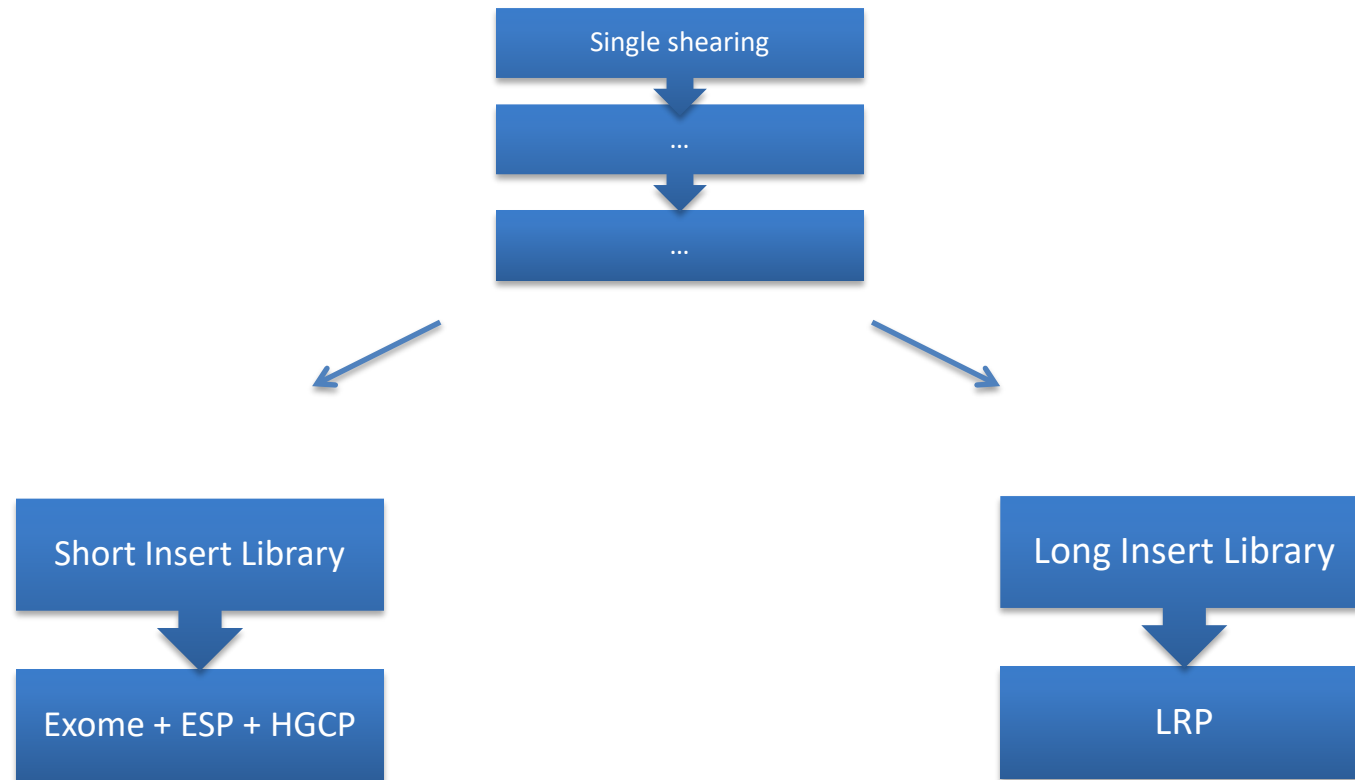


2 library preps
4 hybridizations

Consolidation



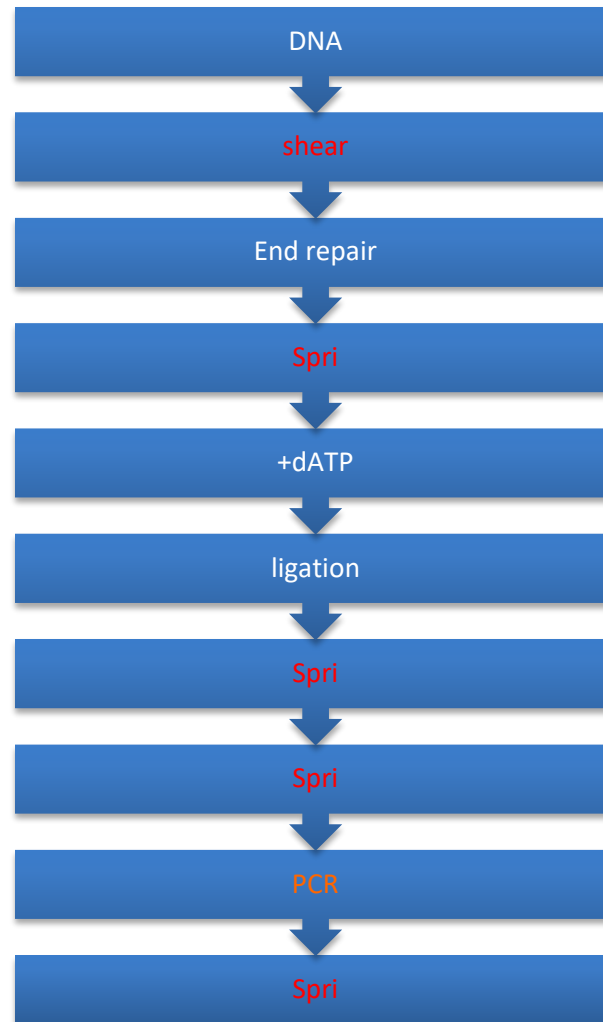
Consolidation



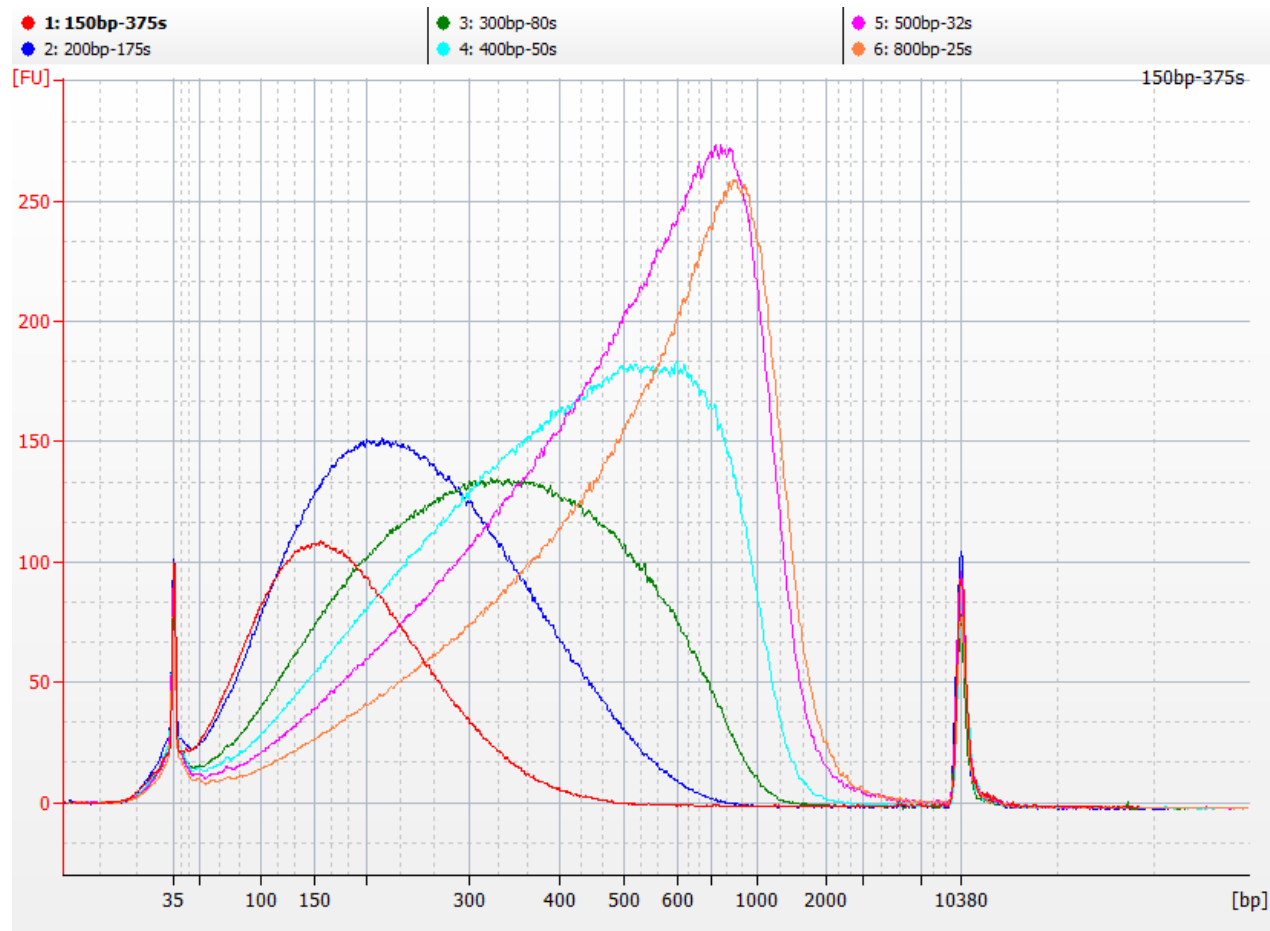
Goals

1. Develop a single protocol to generate two libraries with different insert lengths.
2. Rescue high GC contents and minimize coverage cv for GC%>70%.
3. “Golden protocol” to enable Personalis Exome Plus in one tube.

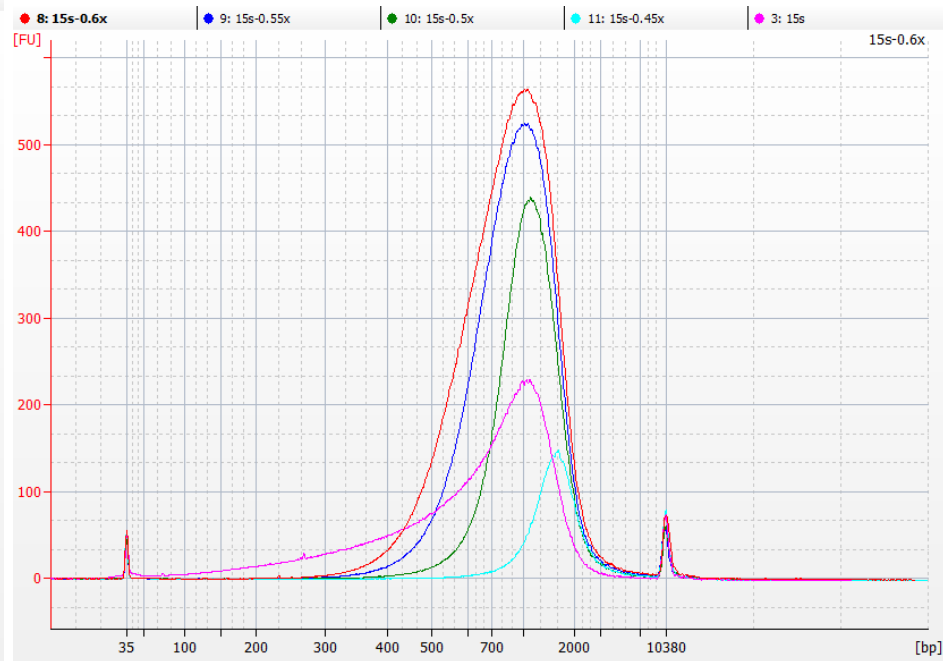
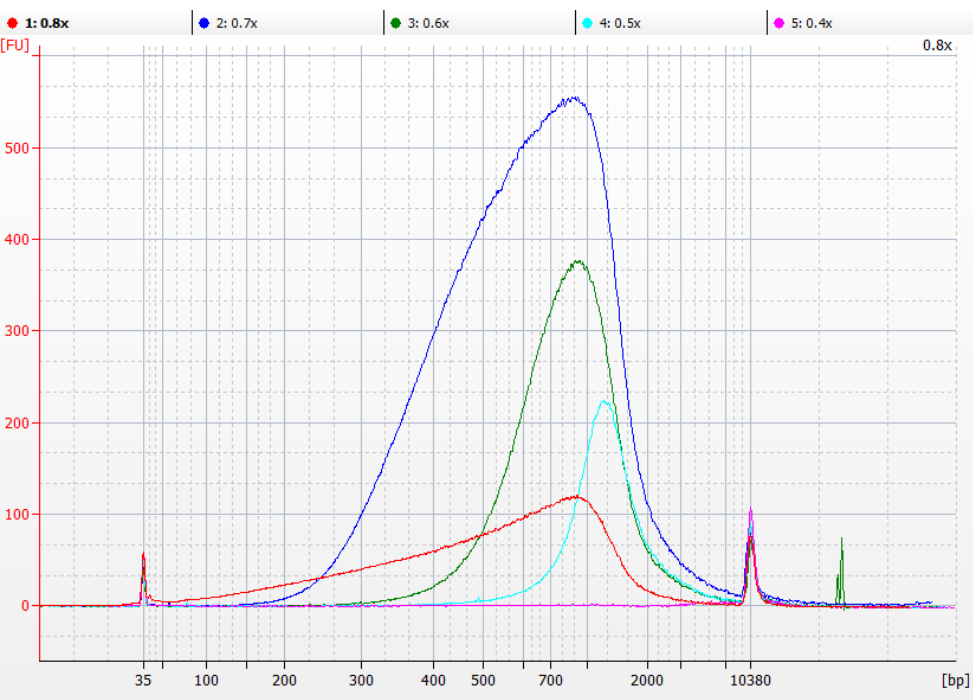
Single protocol two libraries



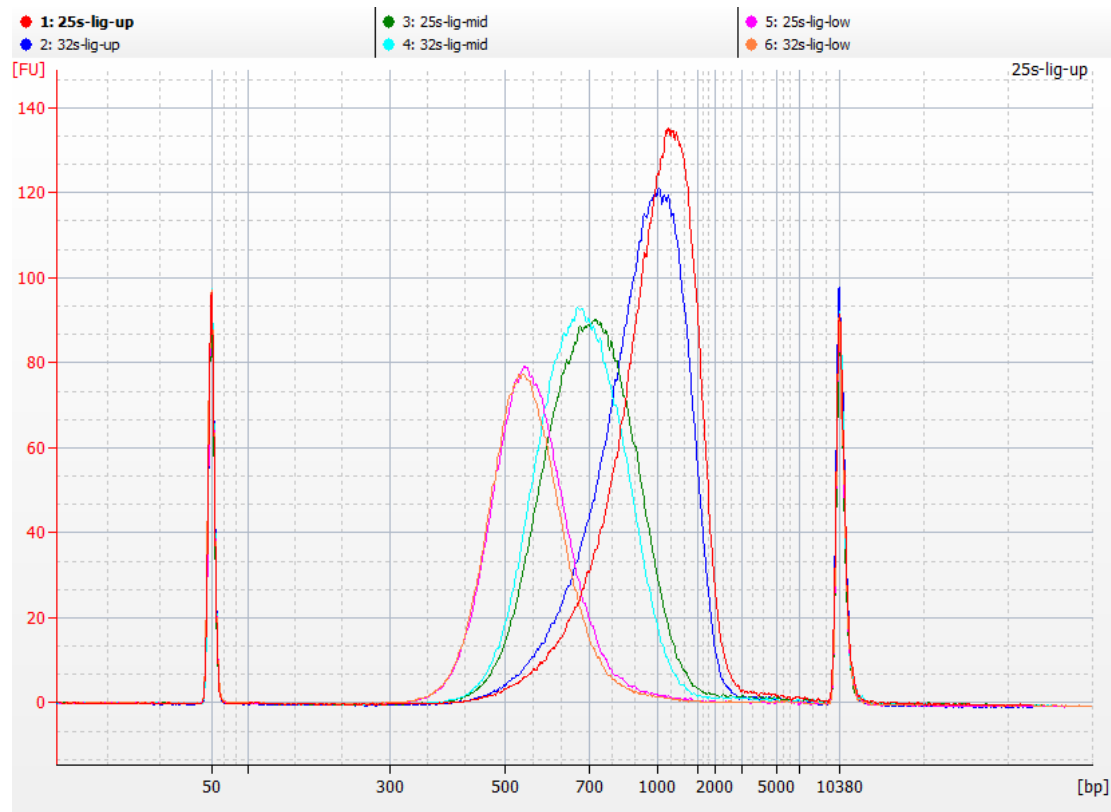
Shear



Spri



Example



GC bias

Model System: Rhodobacter Sphaeroides

The *Rhodobacter sphaeroides* ATCC 17025 genome is 4.56 Million bp long

Browser Chrom/Plasmid Name	Length (bp)	GC Content (%)	Gene Count	NCBI RefSeq Accession	
chr	3217726	68.48	3181	NC_009428	
plasmid_pRSPA01	877879	67.69	849	NC_009429	
plasmid_pRSPA02	289489	67.60	278	NC_009430	
plasmid_pRSPA03	121962	69.36	114	NC_009431	
plasmid_pRSPA04	36198	64.05	32	NC_009432	
plasmid_pRSPA05	13873	58.93	12	NC_009433	

From Gabor:

PST-000190

	mean	third quartile	rhodobacter pcr free			Normalized mean	Delta
			median	first quartile	%_bases_above_15		
GC > 80% (3404 regions):	24.09	29	25	21	96.3	24.4	8.6
GC < 60% (13783 regions):	32.59	38	33	29	99.9	33.1	

PST-000191

			rhodobacter kapa 10 cycle				
GC > 80% (3404 regions):	29.07	35	30	25	98.9	24.0	9.9
GC < 60% (13783 regions):	41.08	47	42	36	100	33.9	

PST-000192

			rhodobacter kapa + betaine 10 cycle				
GC > 80% (3404 regions):	30.1	36	31	27	99	24.1	10.0
GC < 60% (13783 regions):	42.53	49	43	38	100	34.1	

PST-000193

			rhodobacter kapa + DMSO 10 cycle				
GC > 80% (3404 regions):	28.48	34	29	25	99.1	24.1	10.1
GC < 60% (13783 regions):	40.45	46	41	35	100	34.2	

PST-000194

			rhodobacter kapa hifi 10 cycle				
GC > 80% (3404 regions):	25.89	31	27	22	95.1	23.5	10.9
GC < 60% (13783 regions):	37.88	44	38	33	99.9	34.3	

PST-000195

			rhodobacter kapa hifi daza-dGTP 10 cycle				
GC > 80% (3404 regions):	1.03	3	2	1	0	1.4	53.9
GC < 60% (13783 regions):	41.81	55	44	30	93	55.2	

PST-000196

			rhodobacter kapa GC 10 cycle				
GC > 80% (3404 regions):	31.29	37	32	27	99.1	24.1	10.0
GC < 60% (13783 regions):	44.34	51	45	39	100	34.1	

PST-000197

			rhodobacter pmm 10 cycle				
GC > 80% (3404 regions):	25.23	34	28	19	80.8	18.7	17.2
GC < 60% (13783 regions):	48.41	55	48	42	100	35.9	

PST-000198

			rhodobacter pmm long denature 10 cycle				
GC > 80% (3404 regions):	22.86	32	25	16	76	18.2	17.8
GC < 60% (13783 regions):	45.2	52	45	39	100	36.0	

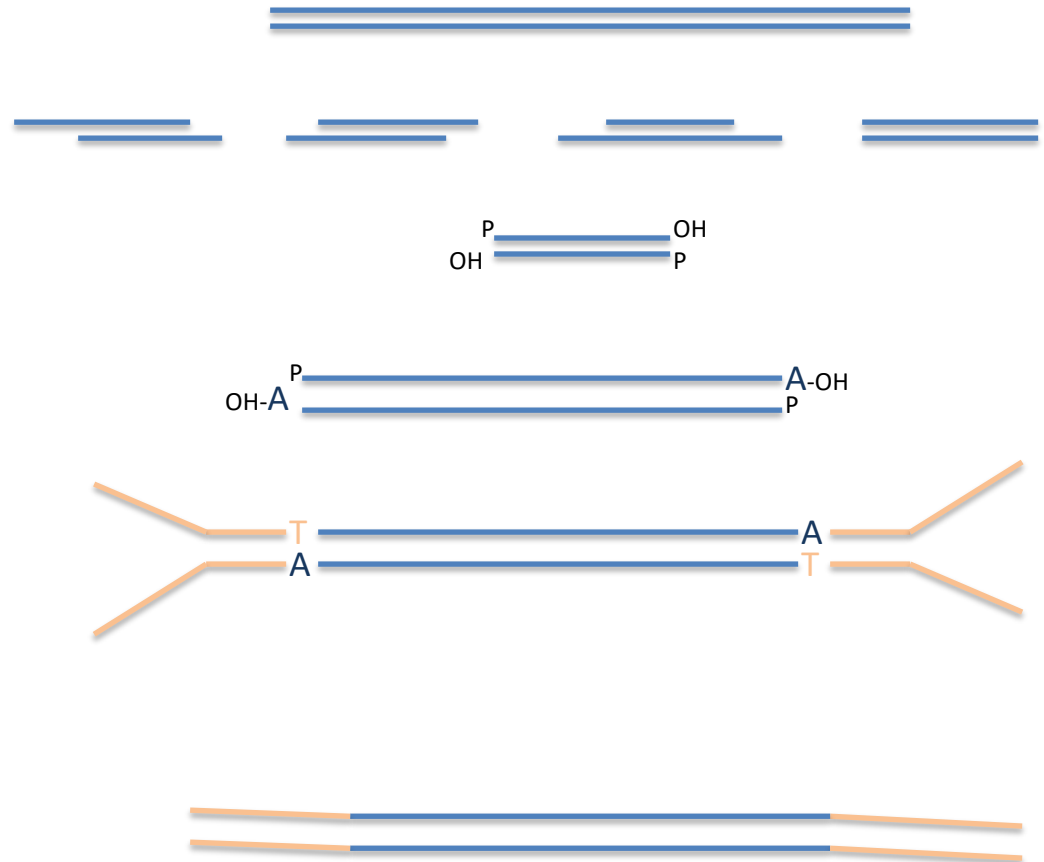
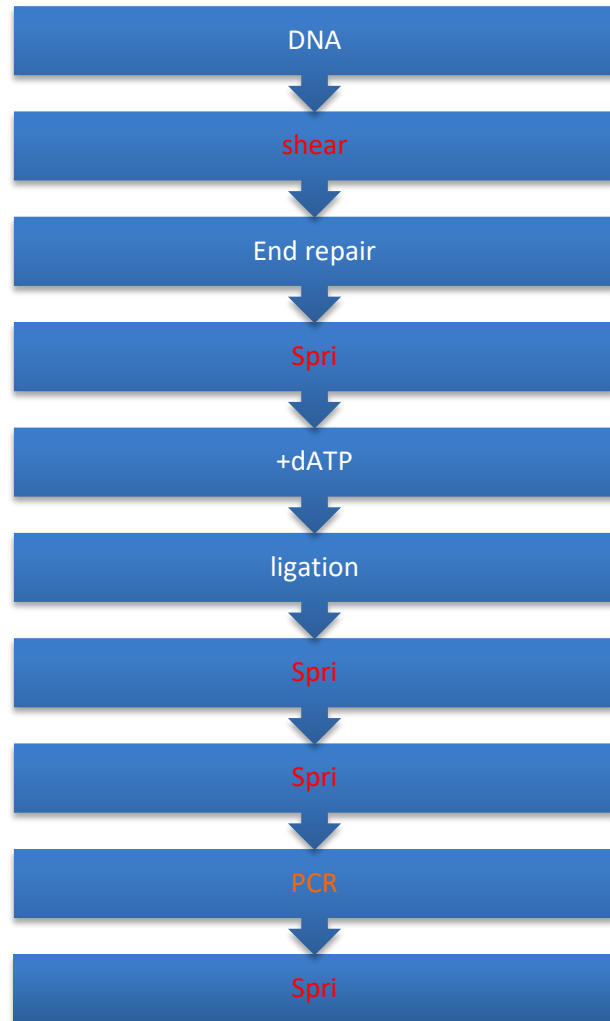
PST-000199

			rhodobacter kapa 8 cycle				
GC > 80% (3404 regions):	23.97	29	25	21	96.5	23.7	10.2
GC < 60% (13783 regions):	34.34	40	35	30	99.9	33.9	



Personalis EX2102

Library Construction



Areas to look into

1. End Repair
2. Shearing