Microbiome Analysis Overview

Josh Granek
May 26, 2016

Microsoft Azure

Thanks!!

Metagenomics

Metagenomics

Amplicon

Shotgun Metagenome

Shotgun Metatranscriptome

Metagenomics

	Information
Amplicon	Who is Present
Shotgun Metagenome	What Genes are Present
Shotgun Metatranscriptome	What is Happening

Metagenomics

	Information	Analogy
Amplicon	Who is Present	Name
Shotgun Metagenome	What Genes are Present	CV
Shotgun Metatranscriptome	What is Happening	Twitter Feed

Metagenomics

	Information	Analogy
Amplicon	Who is Present	Name
Shotgun Metagenome	What Genes are Present	CV
Shotgun Metatranscriptome	What is Happening	Twitter Feed

Metagenomics

	Information	Analogy
Amplicon	Who is Present	Name
Shotgun Metagenome	What Genes are Present	CV
Shotgun Metatranscriptome	What is Happening	Twitter Feed

Metagenomics

	Information	Analogy
Amplicon	Who is Present	Name
Shotgun Metagenome	What Genes are Present	CV
Shotgun Metatranscriptome	What is Happening	Twitter Feed

PubMed.gov
US National Library of Medicine
National Institutes of Health

PubMed ⊙   granek JA   ⊗   Search

Create RSS   Create alert   Advanced   Help

Article types   Summary ⌄   20 per page ⌄   Sort by Most Recent ⌄   Send to: ⌄   Filters: Manage Filters
Clinical Trial
Review   Find related data
Customize ...   **Search results**   Database: Select ⌄

Text availability   **Items: 17**   Find items
Abstract
Free full text
Full text   ☐ Evidence for distinct brain networks in the control of rule-based motor behavior.
                            1. **Granek JA**, Sergio LE.   Search details
PubMed   J Neurophysiol. 2015 Aug;114(2):1298-309. doi: 10.1152/jn.00233.2014. Epub 2015 Jul 1.   granek JA[Author]
Commons   PMID: 26133796
Reader comments   Similar articles
Trending articles
                            ☐ Rapid mapping of insertional mutations to probe cell wall regulation in Cryptococcus neoformans.
Publication dates   2. Esher SK, **Granek JA**, Alspaugh JA.
5 years   Fungal Genet Biol. 2015 Sep;82:9-21. doi: 10.1016/j.fgb.2015.06.003. Epub 2015 Jun 23.   Search   See more...
10 years   PMID: 26112692
Custom range...   Similar articles
                                                                                                    Recent Activity
Species   ☐ Integrating chemical mutagenesis and whole-genome sequencing as a platform for forward and   Turn Off Clear
Humans   3. reverse genetic analysis of Chlamydia.
Other Animals   Kokes M, Dunn JD, **Granek JA**, Nguyen BD, Barker JR, Valdivia RH, Bastidas RJ.   🔍 granek JA (17)
                                Cell Host Microbe. 2015 May 13;17(5):716-25. doi: 10.1016/j.chom.2015.03.014. Epub 2015 Apr 23.   PubMed
Clear all   PMID: 25920978   Free PMC Article
                                Similar articles   🔍 granek J (20)
Show additional filters   PubMed
                            ☐ Antifungal drug resistance evoked via RNAi-dependent epimutations.
                            4. Calo S, Shertz-Wall C, Lee SC, Bastidas RJ, Nicolás FE, **Granek JA**, Mieczkowski P, Torres-Martinez   📄 Global patterns of 16S rRNA diversity at a
                                S, Ruiz-Vázquez RM, Cardenas ME, Heitman J.   depth of millions of sequences per sa. PubMed
                                Nature. 2014 Sep 25;513(7519):555-8. doi: 10.1038/nature13575. Epub 2014 Jul 27.
                                PMID: 25079329   Free PMC Article   🔍 Scott Harrison (71)
                                Similar articles   💬 1 comment   PubMed

                            ☐ Decoupled visually-guided reaching in optic ataxia: differences in motor control between canonical   See more...
                            5. and non-canonical orientations in space.
                                Granek JA, Pisella L, Stemberger J, Vighetto A, Rossetti Y, Sergio LE.
                                PLoS One. 2013 Dec 31;8(12):e86138. doi: 10.1371/journal.pone.0086138. eCollection 2013.
                                PMID: 24392035   Free PMC Article
                                Similar articles

                            ☐ The genetic architecture of biofilm formation in a clinical isolate of Saccharomyces cerevisiae.
                            6. **Granek JA**, Murray D, Kayrkçi Ö, Magwene PM.
                                Genetics. 2013 Feb;193(2):587-600. doi: 10.1534/genetics.112.142067. Epub 2012 Nov 19.
                                PMID: 23172850   Free PMC Article
                                Similar articles

                            ☐ The role of the caudal superior parietal lobule in updating hand location in peripheral vision: further
                            7. evidence from optic ataxia.
                                Granek JA, Pisella L, Blangero A, Rossetti Y, Sergio LE.
                                PLoS One. 2012;7(10):e46619. doi: 10.1371/journal.pone.0046619. Epub 2012 Oct 5.
                                PMID: 23071599   Free PMC Article
                                Similar articles

                            ☐ Pleiotropic signaling pathways orchestrate yeast development.
                            8. **Granek JA**, Kayıkçı Ö, Magwene PM.
                                Curr Opin Microbiol. 2011 Dec;14(6):676-81. doi: 10.1016/j.mib.2011.09.004. Epub 2011 Sep 28. Review.
                                PMID: 21962291   Free PMC Article
                                Similar articles

PubMed.gov
US National Library of Medicine
National Institutes of Health

PubMed ⊙   granek JA   ⊗   Search

Create RSS   Create alert   Advanced   Help

Article types   Summary ⊙   20 per page ⊙   Sort by Most Recent ⊙   Send to: ⊙   Filters: Manage Filters
Clinical Trial
Review   Find related data
Customize ...   Search results   Database: Select ⊙

Text availability   Items: 17   Find items
Abstract
Free full text
Full text   ☐   Evidence for distinct brain networks in the control of rule-based motor behavior.
                            1. Granek JA, Sergio LE.   Search details
PubMed   J Neurophysiol. 2015 Aug;114(2):1298-309. doi: 10.1152/jn.00233.2014. Epub 2015 Jul 1.
Commons   PMID: 26133796   granek JA[Author]
Reader comments   Similar articles
Trending articles
                            ☐   Rapid mapping of insertional mutations to probe cell wall regulation in Cryptococcus neoformans.
Publication dates   2. Esher SK, Granek JA, Alspaugh JA.
5 years   Fungal Genet Biol. 2015 Sep;82:9-21. doi: 10.1016/j.fgb.2015.06.003. Epub 2015 Jun 23.
10 years   PMID: 26112692   Search   See more...
Custom range...   Similar articles
                                                                                               Recent Activity
Species   ☐   Integrating chemical mutagenesis and whole-genome sequencing as a platform for forward and
Humans   3. reverse genetic analysis of Chlamydia.   Turn Off Clear
Other Animals   Kokes M, Dunn JD, Granek JA, Nguyen BD, Barker JR, Valdivia RH, Bastidas RJ.
                                Cell Host Microbe. 2015 May 13;17(5):716-25. doi: 10.1016/j.chom.2015.03.014. Epub 2015 Apr 23. 🔍 granek JA (17)
Clear all   PMID: 25920978   Free PMC Article   PubMed
                                Similar articles
Show additional filters   🔍 granek J (20)
                            ☐   Antifungal drug resistance evoked via RNAi-dependent epimutations.   PubMed
                            4. Calo S, Shertz-Wall C, Lee SC, Bastidas RJ, Nicolás FE, Granek JA, Mieczkowski P, Torres-Martinez 📄 Global patterns of 16S rRNA diversity at a
                                S, Ruiz-Vázquez RM, Cardenas ME, Heitman J.   depth of millions of sequences per sa. PubMed
                                Nature. 2014 Sep 25;513(7519):555-8. doi: 10.1038/nature13575. Epub 2014 Jul 27.
                                PMID: 25079329   Free PMC Article   🔍 Scott Harrison (71)
                                Similar articles   💬 1 comment   PubMed

                                                                                                               See more...
                            ☐   Decoupled visually-guided reaching in optic ataxia: differences in motor control between canonical
                            5. and non-canonical orientations in space.
                                Granek JA, Pisella L, Stemberger J, Vighetto A, Rossetti Y, Sergio LE.
                                PLoS One. 2013 Dec 31;8(12):e86138. doi: 10.1371/journal.pone.0086138. eCollection 2013.
                                PMID: 24392035   Free PMC Article
                                Similar articles

                            ☐   The genetic architecture of biofilm formation in a clinical isolate of Saccharomyces cerevisiae.
                            6. Granek JA, Murray D, Kayrkçi Ö, Magwene PM.
                                Genetics. 2013 Feb;193(2):587-600. doi: 10.1534/genetics.112.142067. Epub 2012 Nov 19.
                                PMID: 23172850   Free PMC Article
                                Similar articles

                            ☐   The role of the caudal superior parietal lobule in updating hand location in peripheral vision: further
                            7. evidence from optic ataxia.
                                Granek JA, Pisella L, Blangero A, Rossetti Y, Sergio LE.
                                PLoS One. 2012;7(10):e46619. doi: 10.1371/journal.pone.0046619. Epub 2012 Oct 5.
                                PMID: 23071599   Free PMC Article
                                Similar articles

                            ☐   Pleiotropic signaling pathways orchestrate yeast development.
                            8. Granek JA, Kayıkçı Ö, Magwene PM.
                                Curr Opin Microbiol. 2011 Dec;14(6):676-81. doi: 10.1016/j.mib.2011.09.004. Epub 2011 Sep 28. Review.
                                PMID: 21962291   Free PMC Article
                                Similar articles

NCBI   Resources ⊙   How To ⊙   Sign in to NCBI

PubMed.gov
US National Library of Medicine
National Institutes of Health

PubMed ⌄   granek JA   ⊗ Search

Create RSS   Create alert   Advanced   Help

Article types   Summary ⌄   20 per page ⌄   Sort by Most Recent ⌄   Send to: ⌄   Filters: Manage Filters
Clinical Trial
Review   Find related data   ▲
Customize ...   Search results   Database: Select ⌄

Text availability   Items: 17   Find items
Abstract
Free full text   Search details   ▲
Full text   ☐ Evidence for distinct brain networks in the control of rule-based motor behavior.
                           1. Granek JA, Sergio LE.   granek JA[Author]
PubMed   J Neurophysiol. 2015 Aug;114(2):1298-309. doi: 10.1152/jn.00233.2014. Epub 2015 Jul 1.
Commons   PMID: 26133796
Reader comments   Similar articles
Trending articles
                           ☐ Rapid mapping of insertional mutations to probe cell wall regulation in Cryptococcus neoformans.
Publication dates   2. Esher SK, Granek JA, Alspaugh JA.
5 years   Fungal Genet Biol. 2015 Sep;82:9-21. doi: 10.1016/j.fgb.2015.06.003. Epub 2015 Jun 23.   Search   See more...
10 years   PMID: 26112692
Custom range...   Similar articles   Recent Activity   ▲
                                                                                                         Turn Off Clear
Species   ☐ Integrating chemical mutagenesis and whole-genome sequencing as a platform for forward and
Humans   3. reverse genetic analysis of Chlamydia.   🔍 granek JA (17)
Other Animals   Kokes M, Dunn JD, Granek JA, Nguyen BD, Barker JR, Valdivia RH, Bastidas RJ.   PubMed
                               Cell Host Microbe. 2015 May 13;17(5):716-25. doi: 10.1016/j.chom.2015.03.014. Epub 2015 Apr 23.
Clear all   PMID: 25920978   Free PMC Article   🔍 granek J (20)
                               Similar articles   PubMed
Show additional filters
                           ☐ Antifungal drug resistance evoked via RNAi-dependent epimutations.   📄 Global patterns of 16S rRNA diversity at a
                           4. Calo S, Shertz-Wall C, Lee SC, Bastidas RJ, Nicolás FE, Granek JA, Mieczkowski P, Torres-Martinez   depth of millions of sequences per sa. PubMed
                               S, Ruiz-Vázquez RM, Cardenas ME, Heitman J.
                               Nature. 2014 Sep 25;513(7519):555-8. doi: 10.1038/nature13575. Epub 2014 Jul 27. 🔍 Scott Harrison (71)
                               PMID: 25079329   Free PMC Article   PubMed
                               Similar articles   💬 1 comment
                                                                                                                          See more...
                           ☐ Decoupled visually-guided reaching in optic ataxia: differences in motor control between canonical
                           5. and non-canonical orientations in space.
                               Granek JA, Pisella L, Stemberger J, Vighetto A, Rossetti Y, Sergio LE.
                               PLoS One. 2013 Dec 31;8(12):e86138. doi: 10.1371/journal.pone.0086138. eCollection 2013.
                               PMID: 24392035   Free PMC Article
                               Similar articles

                           ☐ The genetic architecture of biofilm formation in a clinical isolate of Saccharomyces cerevisiae.
                           6. Granek JA, Murray D, Kayrkçi Ö, Magwene PM.
                               Genetics. 2013 Feb;193(2):587-600. doi: 10.1534/genetics.112.142067. Epub 2012 Nov 19.
                               PMID: 23172850   Free PMC Article
                               Similar articles

                           ☐ The role of the caudal superior parietal lobule in updating hand location in peripheral vision: further
                           7. evidence from optic ataxia.
                               Granek JA, Pisella L, Blangero A, Rossetti Y, Sergio LE.
                               PLoS One. 2012;7(10):e46619. doi: 10.1371/journal.pone.0046619. Epub 2012 Oct 5.
                               PMID: 23071599   Free PMC Article
                               Similar articles

                           ☐ Pleiotropic signaling pathways orchestrate yeast development.
                           8. Granek JA, Kayıkçı Ö, Magwene PM.
                               Curr Opin Microbiol. 2011 Dec;14(6):676-81. doi: 10.1016/j.mib.2011.09.004. Epub 2011 Sep 28. Review.
                               PMID: 21962291   Free PMC Article
                               Similar articles

NCBI   Resources ⊙   How To ⊙   Sign in to NCBI

PubMed.gov   PubMed ◊   granek JA   ⊗   Search
US National Library of Medicine
National Institutes of Health   Create RSS   Create alert   Advanced   Help

Article types   Summary ▾   20 per page ▾   Sort by Most Recent ▾   Send to: ▾   Filters: Manage Filters
Clinical Trial
Review   **Search results**   **Find related data**
Customize ...   Database: Select ◊
                                  Items: 17   Find items
Text availability
Abstract
Free full text   ☐   Evidence for distinct brain networks in the control of rule-based motor behavior.   **Search details**
Full text   1.   **Granek JA**, Sergio LE.   granek JA[Author]
                                     J Neurophysiol. 2015 Aug;114(2):1298-309. doi: 10.1152/jn.00233.2014. Epub 2015 Jul 1.
PubMed   PMID: 26133796
Commons   Similar articles
Reader comments
Trending articles   ☐   Rapid mapping of insertional mutations to probe cell wall regulation in Cryptococcus neoformans.
                                 2.   Esher SK, **Granek JA**, Alspaugh JA.   Search   See more...
Publication dates   Fungal Genet Biol. 2015 Sep;82:9-21. doi: 10.1016/j.fgb.2015.06.003. Epub 2015 Jun 23.
5 years   PMID: 26112692
10 years   Similar articles   **Recent Activity**
Custom range...   Turn Off   Clear
                                 ☐   Integrating chemical mutagenesis and whole-genome sequencing as a platform for forward and   🔍 granek JA (17)
Species   3.   reverse genetic analysis of Chlamydia.   PubMed
Humans   Kokes M, Dunn JD, **Granek JA**, Nguyen BD, Barker JR, Valdivia RH, Bastidas RJ.
Other Animals   Cell Host Microbe. 2015 May 13;17(5):716-25. doi: 10.1016/j.chom.2015.03.014. Epub 2015 Apr 23.   🔍 granek J (20)
                                     PMID: 25920978   Free PMC Article   PubMed
Clear all   Similar articles
                                                                                                              📄 Global patterns of 16S rRNA diversity at a
Show additional filters   ☐   Antifungal drug resistance evoked via RNAi-dependent epimutations.   depth of millions of sequences per sa. PubMed
                                 4.   Calo S, Shertz-Wall C, Lee SC, Bastidas RJ, Nicolás FE, **Granek JA**, Mieczkowski P, Torres-Martinez
                                     S, Ruiz-Vázquez RM, Cardenas ME, Heitman J.   🔍 Scott Harrison (71)
                                     Nature. 2014 Sep 25;513(7519):555-8. doi: 10.1038/nature13575. Epub 2014 Jul 27.   PubMed
                                     PMID: 25079329   Free PMC Article
                                     Similar articles   💬 1 comment   See more...

                                 ☐   Decoupled visually-guided reaching in optic ataxia: differences in motor control between canonical
                                 5.   and non-canonical orientations in space.
                                     Granek JA, Pisella L, Stemberger J, Vighetto A, Rossetti Y, Sergio LE.
                                     PLoS One. 2013 Dec 31;8(12):e86138. doi: 10.1371/journal.pone.0086138. eCollection 2013.
                                     PMID: 24392035   Free PMC Article
                                     Similar articles

                                 ☐   The genetic architecture of biofilm formation in a clinical isolate of Saccharomyces cerevisiae.
                                 6.   **Granek JA**, Murray D, Kayrkçi Ö, Magwene PM.
                                     Genetics. 2013 Feb;193(2):587-600. doi: 10.1534/genetics.112.142067. Epub 2012 Nov 19.
                                     PMID: 23172850   Free PMC Article
                                     Similar articles

                                 ☐   The role of the caudal superior parietal lobule in updating hand location in peripheral vision: further
                                 7.   evidence from optic ataxia.
                                     Granek JA, Pisella L, Blangero A, Rossetti Y, Sergio LE.
                                     PLoS One. 2012;7(10):e46619. doi: 10.1371/journal.pone.0046619. Epub 2012 Oct 5.
                                     PMID: 23071599   Free PMC Article
                                     Similar articles

                                 ☐   Pleiotropic signaling pathways orchestrate yeast development.
                                 8.   **Granek JA**, Kayıkçı Ö, Magwene PM.
                                     Curr Opin Microbiol. 2011 Dec;14(6):676-81. doi: 10.1016/j.mib.2011.09.004. Epub 2011 Sep 28. Review.
                                     PMID: 21962291   Free PMC Article
                                     Similar articles

Metagenomics

Metagenomics

Amplicon

Shotgun Metagenome

Shotgun Metatranscriptome

Metagenomics

	Information
Amplicon	Who is Present
Shotgun Metagenome	What Genes are Present
Shotgun Metatranscriptome	What is Happening

Metagenomics

	Information	Analogy
Amplicon	Who is Present	Name
Shotgun Metagenome	What Genes are Present	CV
Shotgun Metatranscriptome	What is Happening	Twitter Feed

Metagenomics

	Information	Analogy	Target Size
Amplicon	Who is Present	Name	100bp - 1kb
Shotgun Metagenome	What Genes are Present	CV	100kb - 100Mb
Shotgun Metatranscriptome	What is Happening	Twitter Feed	100kb - 100Mb

Metagenomics

	Information	Analogy	Target Size	Discovery?
Amplicon	Who is Present	Name	100bp - 1kb	+/-
Shotgun Metagenome	What Genes are Present	CV	100kb - 100Mb	++
Shotgun Metatranscriptome	What is Happening	Twitter Feed	100kb - 100Mb	++

Metagenomics

	Information	Analogy	Target Size	Discovery?	Cost
Amplicon	Who is Present	Name	100bp - 1kb	+/-	Low
Shotgun Metagenome	What Genes are Present	CV	100kb - 100Mb	++	High
Shotgun Metatranscriptome	What is Happening	Twitter Feed	100kb - 100Mb	++	High

Metagenomics

	Information	Analogy	Target Size	Discovery?	Cost
Amplicon	Who is Present	Name	100bp - 1kb	+/-	Low
Shotgun Metagenome	What Genes are Present	CV	100kb - 100Mb	++	High
Shotgun Metatranscriptome	What is Happening	Twitter Feed	100kb - 100Mb	++	High

Amplicon Sequencing

PCR amplify and sequence a marker gene

Amplicon Sequencing

PCR amplify and sequence a marker gene

	Marker Gene

Amplicon Sequencing

PCR amplify and sequence a marker gene

	Marker Gene
Bacteria	16s rRNA

Amplicon Sequencing

PCR amplify and sequence a marker gene

	Marker Gene
Bacteria	16s rRNA
Fungi	18s or ITS rRNA

Amplicon Sequencing

PCR amplify and sequence a marker gene

	Marker Gene
Bacteria	16s rRNA
Fungi	18s or ITS rRNA
Archaea	16s rRNA

Amplicon Sequencing

PCR amplify and sequence a marker gene

	Marker Gene
Bacteria	16s rRNA
Fungi	18s or ITS rRNA
Archaea	16s rRNA
Protozoa	18s rRNA

Amplicon Sequencing

PCR amplify and sequence a marker gene

	Marker Gene
Bacteria	16s rRNA
Fungi	18s or ITS rRNA
Archaea	16s rRNA
Protozoa	18s rRNA
Viruses	?????

Metagenomics

	Information	Analogy	Target Size	Cost	Discovery?
Amplicon	Who is Present	Name	100bp - 1kb	Low	+/-
Shotgun Metagenome	What Genes are Present	CV	100kb - 100Mb	High	++
Shotgun Metatranscriptome	What is Happening	Twitter Feed	100kb - 100Mb	High	++

Sequencing Details
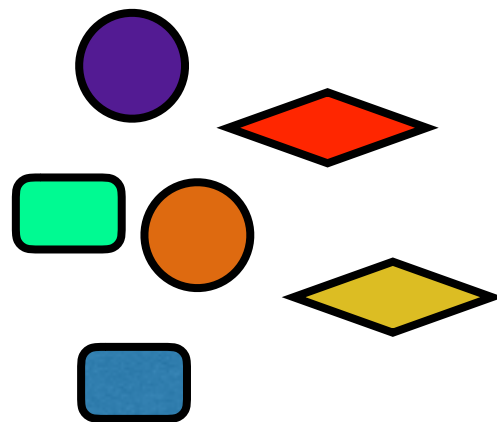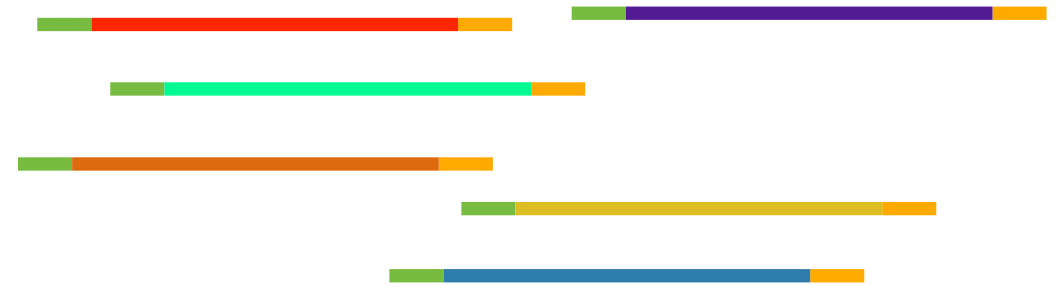
Single-End

TCGAAAAG
AGCTTTTCATTCTGACTGCAACGGGCAATATGTCTCTGTGTGGA

Paired-End

TCGAAAAG
AGCTTTTCATTCTGACTGCAACGGGCAATATGTCTCTGTGTGGA

Paired-End
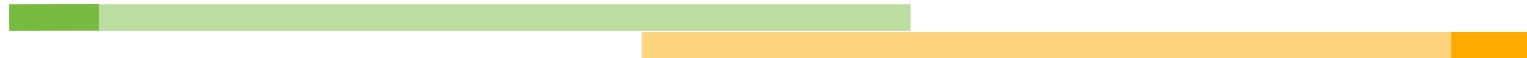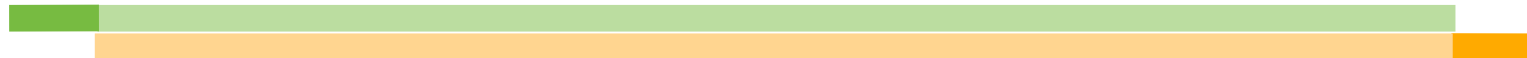
Multiplexing (Barcodes)
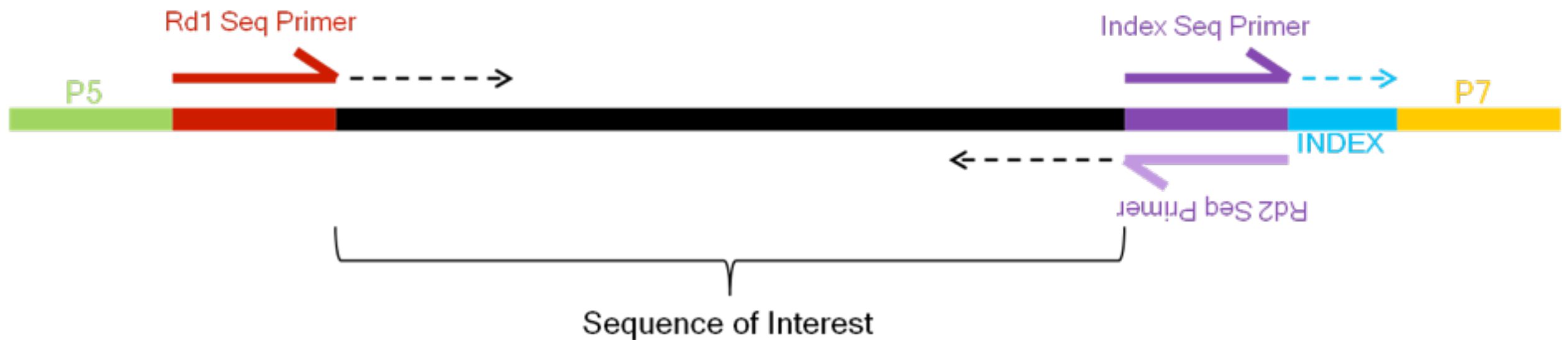
HiSeq vs. MiSeq

- Same technology! Same libraries!

- Differences:

  - Run-time (MiSeq is faster)

  - Maximum read length (MiSeq is longer)

  - Data yield (HiSeq is higher)

  - Cost (HiSeq is cheaper per base)

Illumina Video

https://www.youtube.com/watch?v=HMyCqWhwB8E

FASTQ

- FASTA with Quality

- https://en.wikipedia.org/wiki/FASTQ_format

File Formats

FASTQ: Format

- ## FASTA Format

>NC_007779.1 Escherichia coli str. K-12 substr. W3110, complete genome
AGCTTTTCATTCTGACTGCAACGGGCAATATGTCTCTGTGTGGATTAAAAAAAGAGTGTCTGATAGCAGCTTCTGAACTG
GTTACCTGCCGTGAGTAAATTAAAATTTTATTGACTTAGGTCACTAAATACTTTAACCAATATAGGCATAGCGCACAGAC
AGATAAAAATTACAGAGTACACAACATCCATGAAACGCATTAGCACCACCATTACCACCACCATCACCATTACCACAGGT
AACGGTGCGGGCTGACGCGTACAGGAAACACAGAAAAAAGCCCGCACCTGACAGTGCGGGCTTTTTTTTTCGACCAAAGG
TAACGAGGTAACAACCATGCGAGTGTTGAAGTTCGGCGGTACATCAGTGGCAAATGCAGAACGTTTTCTGCGTGTTGCCG
ATATTCTGGAAAGCAATGCCAGGCAGGGGCAGGTGGCCACCGTCCTCTCTGCCCCCGCCAAAATCACCAACCACCTGGTG
GCGATGATTGAAAAAACCATTAGCGGCCAGGATGCTTTACCCAATATCAGCGATGCCGAACGTATTTTTGCCGAACTTTT
GACGGGACTCGCCGCCGCCCAGCCGGGGGTTCCCGCTGGCGCAATTGAAAACTTTCGTCGATCAGGAATTTGCCCAAATAA
AACATGTCCTGCATGGCATTAGTTTGTTGGGGCAGTGCCCGGATAGCATCAACGCTGCGCTGATTTGCCGTGGCGAGAAA

- ## FASTQ Format

@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0

CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG

+

AAABBFFFFFFFFGGGGGGGGGGGGGGHHHHHHHHHGHGHGHHHHHGHHHGGGGGHHHHGGGGGGGGHHHGHHFFHHHHHGHGGGGGGGGGGGGHHHHHHHHHHHGGG

FASTQ: Format

- **FASTA Format**

>NC_007779.1 Escherichia coli str. K-12 substr. W3110, complete genome

AGCTTTTCATTCTGACTGCAACGGGCAATATGTCTCTGTGTGGATTAAAAAAAGAGTGTCTGATAGCAGCTTCTGAACTG
GTTACCTGCCGTGAGTAAATTAAAATTTTATTGACTTAGGTCACTAAATACTTTAACCAATATAGGCATAGCGCACAGAC
AGATAAAAATTACAGAGTACACAACATCCATGAAACGCATTAGCACCACCATTACCACCACCATCACCATTACCACAGGT
AACGGTGCGGGCTGACGCGTACAGGAAACACAGAAAAAAGCCCGCACCTGACAGTGCGGGCTTTTTTTTTCGACCAAAGG
TAACGAGGTAACAACCATGCGAGTGTTGAAGTTCGGCGGTACATCAGTGGCAAATGCAGAACGTTTTCTGCGTGTTGCCG
ATATTCTGGAAAGCAATGCCAGGCAGGGGCAGGTGGCCACCGTCCTCTCTGCCCCCGCCAAAATCACCAACCACCTGGTG
GCGATGATTGAAAAAACCATTAGCGGCCAGGATGCTTTACCCAATATCAGCGATGCCGAACGTATTTTTGCCGAACTTTT
GACGGGACTCGCCGCCGCCCAGCCGGGGTTCCCGCTGGCGCAATTGAAAACTTTCGTCGATCAGGAATTTGCCCAAATAA
AACATGTCGCATGGCATTAGTTTGTTGGGGCAGTGCCCGGATAGCATCAACGCTGCGCTGATTTGCCGTGGCGAGAAA

- **FASTQ Format**

@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0

CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG

+

AAABBFFFFFFFFGGGGGGGGGGGGGHHHHHHHHGHGHGHHHHHGHHHGGGGGHHHGGGGGGGHHHGHHFFHHHHHGHGGGGGGGGGGGGHHHHHHHHHHGGG

FASTQ: Format

- **FASTA Format**

>NC_007779.1 Escherichia coli str. K-12 substr. W3110, complete genome
AGCTTTTCATTCTGACTGCAACGGGCAATATGTCTCTGTGTGGATTAAAAAAAGAGTGTCTGATAGCAGCTTCTGAACTG
GTTACCTGCCGTGAGTAAATTAAAATTTTATTGACTTAGGTCACTAAATACTTTAACCAATATAGGCATAGCGCACAGAC
AGATAAAAATTACAGAGTACACAACATCCATGAAACGCATTAGCACCACCATTACCACCACCATCACCATTACCACAGGT
AACGGTGCGGGCTGACGCGTACAGGAAACACAGAAAAAAGCCCGCACCTGACAGTGCGGGCTTTTTTTTTCGACCAAAGG
TAACGAGGTAACAACCATGCGAGTGTTGAAGTTCGGCGGTACATCAGTGGCAAATGCAGAACGTTTTCTGCGTGTTGCCG
ATATTCTGGAAAGCAATGCCAGGCAGGGGCAGGTGGCCACCGTCCTCTCTGCCCCCGCCAAAATCACCAACCACCTGGTG
GCGATGATTGAAAAAACCATTAGCGGCCAGGATGCTTTACCCAATATCAGCGATGCCGAACGTATTTTTGCCGAACTTTT
GACGGGACTCGCCGCCGCCCAGCCGGGGTTCCCGCTGGCGCAATTGAAAACTTTCGTCGATCAGGAATTTGCCCAAATAA
AACATGTCCTGCATGGCATTAGTTTGTTGGGGCAGTGCCCGGATAGCATCAACGCTGCGCTGATTTGCCGTGGCGAGAAA

Sequence

- **FASTQ Format**

@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAABBFFFFFFFFGGGGGGGGGGGGGGHHHHHHHGHGHGHHHHHGHHHGGGGGHHHHGGGGGGGHHHGHHFFHHHHHGHGGGGGGGGGGGHHHHHHHHHHHGGG

FASTQ: Format

- **FASTA Format**

```
>NC_007779.1 Escherichia coli str. K-12 substr. W3110, complete genome
AGCTTTTCATTCTGACTGCAACGGGCAATATGTCTCTGTGTGGATTAAAAAAAGAGTGTCTGATAGCAGCTTCTGAACTG
GTTACCTGCCGTGAGTAAATTAAAATTTTATTGACTTAGGTCACTAAATACTTTAACCAATATAGGCATAGCGCACAGAC
AGATAAAAATTACAGAGTACACAACATCCATGAAACGCATTAGCACCACCATTACCACCACCATCACCATTACCACAGGT
AACGGTGCGGGCTGACGCGTACAGGAAACACAGAAAAAAGCCCGCACCTGACAGTGCGGGCTTTTTTTTTCGACCAAAGG
TAACGAGGTAACAACCATGCGAGTGTTGAAGTTCGGCGGTACATCAGTGGCAAATGCAGAACGTTTTCTGCGTGTTGCCG
ATATTCTGGAAAGCAATGCCAGGCAGGGGCAGGTGGCCACCGTCCTCTCTGCCCCCGCCAAAATCACCAACCACCTGGTG
GCGATGATTGAAAAAACCATTAGCGGCCAGGATGCTTTACCCAATATCAGCGATGCCGAACGTATTTTTGCCGAACTTTT
GACGGGACTCGCCGCCGCCCAGCCGGGGGTTCCCGCTGGCGCAATTGAAAACTTTCGTCGATCAGGAATTTGCCCAAATAA
AACATGTCCTGCATGGCATTAGTTTGTTGGGGCAGTGCCCGGATAGCATCAACGCTGCGCTGATTTGCCGTGGCGAGAAA
```

- **FASTQ Format**

```
@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAABBFFFFFFFFGGGGGGGGGGGGGHHHHHHHGHGHGHHHHGHHHGGGGGHHHHGGGGGGGHHHGHHFFHHHHHGHGGGGGGGGGGHHHHHHHHHHGGG
```

Quality Score

FASTQ: Format

- **FASTA Format**

>NC_007779.1 Escherichia coli str. K-12 substr. W3110, complete genome
AGCTTTTCATTCTGACTGCAACGGGCAATATGTCTCTGTGTGGATTAAAAAAAGAGTGTCTGATAGCAGCTTCTGAACTG
GTTACCTGCCGTGAGTAAATTAAAATTTTATTGACTTAGGTCACTAAATACTTTAACCAATATAGGCATAGCGCACAGAC
AGATAAAAATTACAGAGTACACAACATCCATGAAACGCATTAGCACCACCATTACCACCACCATCACCATTACCACAGGT
AACGGTGCGGGCTGACGCGTACAGGAAACACAGAAAAAAGCCCGCACCTGACAGTGCGGGCTTTTTTTTTCGACCAAAGG
TAACGAGGTAACAACCATGCGAGTGTTGAAGTTCGGCGGTACATCAGTGGCAAATGCAGAACGTTTTCTGCGTGTTGCCG
ATATTCTGGAAAGCAATGCCAGGCAGGGGCAGGTGGCCACCGTCCTCTCTGCCCCCGCCAAAATCACCAACCACCTGGTG
GCGATGATTGAAAAAACCATTAGCGGCCAGGATGCTTTACCCAATATCAGCGATGCCGAACGTATTTTTGCCGAACTTTT
GACGGGACTCGCCGCCGCCCAGCCGGGGGTTCCCGCTGGCGCAATTGAAAACTTTCGTCGATCAGGAATTTGCCCAAATAA
AACATGTCCTGCATGGCATTAGTTTGTTGGGGCAGTGCCCGGATAGCATCAACGCTGCGCTGATTTGCCGTGGCGAGAAA

- **FASTQ Format**

@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAABBFFFFFFFFGGGGGGGGGGGGGHHHHHHHHGHGHGHHHHHGHHHGGGGGHHHHGGGGGGGHHHGHHFFHHHHHGHGGGGGGGGGGHHHHHHHHHHHGGG

```
@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAABBFFFFFFFGGGGGGGGGGGGGHHHHHHHHGHGHGHHHHHGHHHGGGGGHHHGGGGGGGGHHHGHHFFHHHHHHGHGGGGGGGGGGGHHHHHHHHHHHGGG
@M00698:36:000000000-AFBEL:1:1101:15928:1413 1:N:0:0
GTAAAGTCCTGAGTGATACCGGCAACTTTTACCCCCAGTCCCACTTTCGAACCGGCAAACATATCGGCAAAAGAGGCCGTGCCTGATTTAAAGCCGTAGGT
+
1>AAAFFFFFFFFGGGGGGGGGGCEGGGHHHHHFHHHHGGGHHHHHHHHGHFA0FEGGGGGHGGHHHHEECEEHFEFH/E/E/>EGGHBHHHDGHHHHC<?E/1
@M00698:36:000000000-AFBEL:1:1101:15876:1413 1:N:0:0
CCGGAACAATCGCTGGCAGGCTTTTTACGTCCGGGTTATAGCGAGAGCCGTCTCAATATTCATCAGCCGCTTTGGCTCGCGGGTGTCGATTGTCGCGT
+
ABCCCCDFFFFFGGGGGGGGGGGGGHHHHHHGHCGGGFFHHHHHGGHGCHGCGGGGHHHHHHHHHHHHGGGGGHHHGGGGGG<CHHGHGHEHDDGGF
@M00698:36:000000000-AFBEL:1:1101:15340:1413 1:N:0:0
CCACTAACAAACTAGCCTGATTAAGTTTTAACGCTTCAACCCCAGGCAGGGCTTCCACGCGATCTCTTTTGGGTTTGACCTCTCTTGATCCCCGTCCTAAG
+
AABABFFFFFFCGGGGGGGGGGGGFHHGFHHGFHGGGGGHHHHGGGGFHGGFGGHHHHCFGFFEFCGHHFHHHHGGAFGGFFFFHHHHHHHHHHHGEEGGGHHB
@M00698:36:000000000-AFBEL:1:1101:16045:1413 1:N:0:0
GTAGCATTATCAGAGAGTTGCCATTCACGCATTGGCTTAACCGCGCGCAGACCATCAACAGTCACTTTGGCGTCAAAGACATTAGGCGTGCAGTATTTTTT
+
?ABCCFFFFFFFGGGGGGGGGGGGHHHHHHHHGGGHHHHHHHHHGGGGGGGGGGGHHHHHHHHHHHHHHHHHHHHGGGGGHHHHHHHHHHHHHGGGGHHGHHHHHHG
@M00698:36:000000000-AFBEL:1:1101:17191:1414 1:N:0:0
TCGGCACCAATATAGGTAACGCATGGTTCACCGTCTTCAGCTACGGCGGCGATTTTGGTCAGGATCGGTGCTACCAATTCATAGGCTTCTTTCTGGCCAC
+
ABAABBBBFBFFGGGGGGGGGGGGGGHHHHHHHGHGHGHHHHHHHGGGGGGGGGGHGHGGHHHHHHHHGGGGGHHHHHHHHHHHHFHGHHHHHHHHHHHBFHHG
@M00698:36:000000000-AFBEL:1:1101:16186:1414 1:N:0:0
TCAGGGTCGTCCGTGGAGCAAACATAGCCCTGAGGCTTATTGAGCATGAAGTAACGTGGACCGTGTTGCTGCGCCAGCGGGTTGCCATCGTAAGCGACAT
+
BCCCCFFFCCCCGGGGGGGGGGGHHHGHGHHHHHHGGHHHHHHHHHHHHHHHHHHHGHHGGHHGGHHHHHHHHHGGGGGGGGGGGGGHHHHHGHHHFHGGGGG
@M00698:36:000000000-AFBEL:1:1101:15394:1415 1:N:0:0
ACTTCTTGAAGGTACGTTGCAGGCTGGCACTTCTGCCGTTTCTGATAAGTTGCTTGATTTGGTTGGACTTGGTGGCAAGTCTGCCGCTGATAAAGGAAAGG
+
@BBCCFFFFFFFGGGGGGGGGGGGGHHDGHHHHHHHHHGEGHGHHHGHHHHHHHHHHEHHHHHHHGGHHHHHHHEGGHHGHHHHHHGGGGFHHHHHHHHHHHH
@M00698:36:000000000-AFBEL:1:1101:14326:1417 1:N:0:0
TGGCGCAACTAACAGAACGTCTTGCGTTTTGTTGGCGAAGCCGCTGGTGTTTGTAAATTTATTAGTGATCGGCGTAGCGTTACGGGTTTCACCGTAGTTCG
+
ABCCCCCCCFFFGGGGGGGGGGGGHHGGGGGHHHHHHGGGGGHGGGGGFGFFHHHHHHHGHHHHHHHHHHGHGGGGGFFAE>EGGGGGEGGHHHHGGHGGHHG
@M00698:36:000000000-AFBEL:1:1101:15479:1417 1:N:0:0
GCCCCCCTCACTCTGACTTTAGTGTGCGCCTTTTGCCTGCGCCACGATCTCCTCGACATTTTCCGGCATTGGTTCATACGGCGTTTCTTTCGCCGTTGG
+
@BCCCCCCCFCFGGGGGGGGGGGGGHHHHGGGGGHHHHHHHGGGGGGGGHHHHHHGGGGGHHHHHGGGGGHHHHHHHHHHHGGGGGGHHHHHGGGGGGGGH
@M00698:36:000000000-AFBEL:1:1101:16850:1418 1:N:0:0
CGTTTTCTTCATCGCGCTCTTTGCTGCCTAACAGCGTGCGCCAGCCTGCTTCAGCAAGAAAACGCGCTTTAGCGACAAATTTGCCTTTGGCAATGTCCAGT
+
AAACCCFFFFFFGGGGGGGGGGGGGHHHHHHHHHHHHHGGGGGGGGGGGGHHHHHHHHHHHHHHHGGGGGGGHGHGGGGGFHHHHHHHHGHHHHHHHHHHHHG
@M00698:36:000000000-AFBEL:1:1101:16255:1418 1:N:0:0
CCGGCTTGCTGGTTGCAGCCGTTGCTGTACTTGATGTCAGGCGTGCCGGTGCCGTATTTTCAAACGGTGATGCCGGACGCGACTCTACGGGTTTGACATCG
+
AAABCCCCCFFFGGGGGGGGGGGGGHHHHHHHHHHHHHHHHHGGGGHGGGGGHGFGHHHHHHHHHGGGHGHHHHGGGGGGGGGGGGHHHGGGCGGGHHHHH?
@M00698:36:000000000-AFBEL:1:1101:17071:1419 1:N:0:0
CACGCCACCTTTATCCAGTTTGCGGCTTTGCGAAGTGGCGAGCCACAGCGCGTTTTCTTGCTGGCTATAAGCCATTTCGTAGGCACCTTTACCTACCGCTT
+
AABBBBBBFFFFGGGGGGGGGGHGGGGGGHHHGGGGFHHGGGGGGGHHHHHGGGFGGGHHHHHHHHGHHHHHHHHHHHHHHGGHGHGHHHFHHHHHHGGGG
@M00698:36:000000000-AFBEL:1:1101:15606:1420 1:N:0:0
CGGGTTTTTAACTTTCAGCCACGGGCCACCGTCGATCAGTTCACCGCCAAACTCTTCACGCGCCAGCTGGTAGCCCCAGTCTTTAAACGCTCCTTCGGTGA
+
BBCDBBCCDCFFGGGGGGGGGGGGGGGGGHGGGGGHGHHHHHHHGGGGGHHHHHHHHHHGGGGGGHHHHHHHHHGGGHHHHHHHHHHGGGGGHHHHGFGG
@M00698:36:000000000-AFBEL:1:1101:15945:1421 1:N:0:0
TCATTGTTGCGCGGTATTCGCGCCCGTTGGTCGAGTAGCAGAAGGGGATTTTAAACCGTTGTTTGCCGCTGGTGTCCTGCCAGCTGGTTTCATACTCTGG
+
?ABABFFFFBBBFGEEGGGGGGGGGGGGHGHCHGCGGGHGHHHHHHGGGGHGHHHHHGHGEGDGHHHHHGGG?EB2GGHGHHHGHGHHHHGHHFHHHHHGGH
@M00698:36:000000000-AFBEL:1:1101:16329:1421 1:N:0:0
GCCCCGACAGCTGTATGCATAGCGATAAATTCCAGCAGGCCGGGACGCCGGTCTATTTTGCCCCAGAGTGTCAATGTTAGACTTGACGGACATTGTGCAG
+
3ABCCCCCCCCFGGGGGGGGGGHGGHGGHHHHHHHHHHHGGGGGGGGGGGGGGGGGHHHHHHHHGGGHHHHHHHGHHHHHHHHHHHHGGGGGHHHHHHHH
@M00698:36:000000000-AFBEL:1:1101:16360:1422 1:N:0:0
```

FASTQ: Format

FASTQ: Read Files

Combined_R1.fastq.gz
@M00698:36:000000000-AFBEL:1:1101:14738:1412 1:N:0:0
TTACGCTAACAGGCGGTAGCCTGGCAGGGTCAGGAAATCAATTAACTCATCGGAAGTGGTGATCTGTTCCATCAAGCGTGCGGCATCGTCAAAACGCCC
+
ABBBABBBAFFFGGGGGGGGGGGHGGHGGGCG2GF3FFGHHHHHHGGFGHEHHGGGEHHHHAGGHHGHHHFFDHFHHHGEGGGG@F@H?GHH/GBEFGGG
@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAABBFFFFFFFGGGGGGGGGGGGHHHHHHHHGHGHGHHHHGHHHGGGGGHHHHGGGGGGGHHHGHHFFHHHHHGHGGGGGGGGGGHHHHHHHHHHHHHGGG

Combined_R2.fastq.gz
@M00698:36:000000000-AFBEL:1:1101:14738:1412 2:N:0:0
GGAAGATGCGGCGACGGCTGAAATTTCCCGTACCTCGATCTGGCAGTGGATCCATCATCAAAAAACGTTGAGCAATGGCAAACCGGTGACCAAAGCCTTGT
+
ABBABFFFFDBDGCG??FFGGGHGHFEG3EAEGGFHAE3GFBGGHGGGHHCFGHFGBGHFHHDFEGGHFHEFHHHH3BFGF0GFEGGGGGHHA/FGHFHHH
@M00698:36:000000000-AFBEL:1:1101:16483:1412 2:N:0:0
GCTTCTTCCGTACTCATGCGGGCATTGAGCAAGCGATCAGCCGTGGCCTGGCGTATGCGCCATATGCTGACCTGGTCTGGTGTGAAACCTCCACGCCGGAT
+
CCCCCFFFFBFFGGGGGGGGGCECGHHHHHHHHHGGHGGGGHGGCGCHHGFHGGGGHHGGGGGHHHHHHGHHHHHHHHHHHHHEHGHHHGHHHHGGGGGGG

Combined_I1.fastq.gz
@M00698:36:000000000-AFBEL:1:1101:14738:1412 1:N:0:0
AGTTCC
+
CCCCDF
@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CCTGTC
+
A11>>1

#SampleID Barc...

#Example mapp... ...e effects of

#exercise and ...

PC.... AGCACGA... ...354

PC.355 AGCAC... ...D._355

PC.355 ACAGA... ...D._356

PC.481 ACCAG... ...D._481

PC.593 AGCAG... ...D._593

PC.607 AACTG... ...D._607

PC.634 ACAGA...

PC.635 ACCGC... ...635

PC.636 ACGGT... ...636

#SampleID
BarcodeSequence
LinkerPrimerSequence
Treatment DOB Description
PC.... AGCACGAGCCTA

Metadata Example

```
#Example mapping file for the QIIME analysis package. These 9 samples are from a study of the
#effects of exercise and diet on mouse cardiac physiology (Crawford, et al, PNAS, 2009).
#
#
#SampleID    BarcodeSequence    LinkerPrimerSequence      Treatment    DOB         Description
PC.354       AGCACGAGCCTA       YATGCTGCCTCCCGTAGGAGT      Control      20061218    Control_mouse__I.D._354
PC.355       AGCACGAGCCTA       YATGCTGCCTCCCGTAGGAGT      Control      20061218    Control_mouse__I.D._355
PC.356       ACAGACCACTCA       YATGCTGCCTCCCGTAGGAGT      Control      20061126    Control_mouse__I.D._356
PC.481       ACCAGCGACTAG       YATGCTGCCTCCCGTAGGAGT      Control      20070314    Control_mouse__I.D._481
PC.593       AGCAGCACTTGT       YATGCTGCCTCCCGTAGGAGT      Control      20071210    Control_mouse__I.D._593
PC.607       AACTGTGCGTAC       YATGCTGCCTCCCGTAGGAGT      Fast_2       20071112    Fasting_mouse__I.D._607
PC.634       ACAGAGTCGGCT       YATGCTGCCTCCCGTAGGAGT      Fast         20080116    missing_description
PC.635       ACCGCAGAGTCA       YATGCTGCCTCCCGTAGGAGT      Fast         20080116    Fasting_mouse__I.D._635
PC.636       ACGGTGAGTGTC       YATGCTGCCTCCCGTAGGAGT      Fast         20080116    Fasting_mouse__I.D._636
```

Multiplexing (Barcodes)

STRUCTURE DETAILS

Rd1 Seq Primer

Index Seq Primer

P5

P7

INDEX

Rd2 Seq Primer

Sequence of Interest

Metadata Example

```
#Example mapping file for the QIIME analysis package. These 9 samples are from a study of the
#effects of exercise and diet on mouse cardiac physiology (Crawford, et al, PNAS, 2009).
#
#
#SampleID    BarcodeSequence    LinkerPrimerSequence       Treatment  DOB       Description
PC.354       AGCACGAGCCTA       YATGCTGCCTCCCGTAGGAGT       Control    20061218  Control_mouse__I.D._354
PC.355       AGCACGAGCCTA       YATGCTGCCTCCCGTAGGAGT       Control    20061218  Control_mouse__I.D._355
PC.356       ACAGACCACTCA       YATGCTGCCTCCCGTAGGAGT       Control    20061126  Control_mouse__I.D._356
PC.481       ACCAGCGACTAG       YATGCTGCCTCCCGTAGGAGT       Control    20070314  Control_mouse__I.D._481
PC.593       AGCAGCACTTGT       YATGCTGCCTCCCGTAGGAGT       Control    20071210  Control_mouse__I.D._593
PC.607       AACTGTGCGTAC       YATGCTGCCTCCCGTAGGAGT       Fast_2     20071112  Fasting_mouse__I.D._607
PC.634       ACAGAGTCGGCT       YATGCTGCCTCCCGTAGGAGT       Fast       20080116  missing_description
PC.635       ACCGCAGAGTCA       YATGCTGCCTCCCGTAGGAGT       Fast       20080116  Fasting_mouse__I.D._635
PC.636       ACGGTGAGTGTC       YATGCTGCCTCCCGTAGGAGT       Fast       20080116  Fasting_mouse__I.D._636
```

Illumina Sequencing

From Library to Data

Library Preparation

DNA Fragment

200-1000 bp

+

Adapters

=

Sequencing "Library"
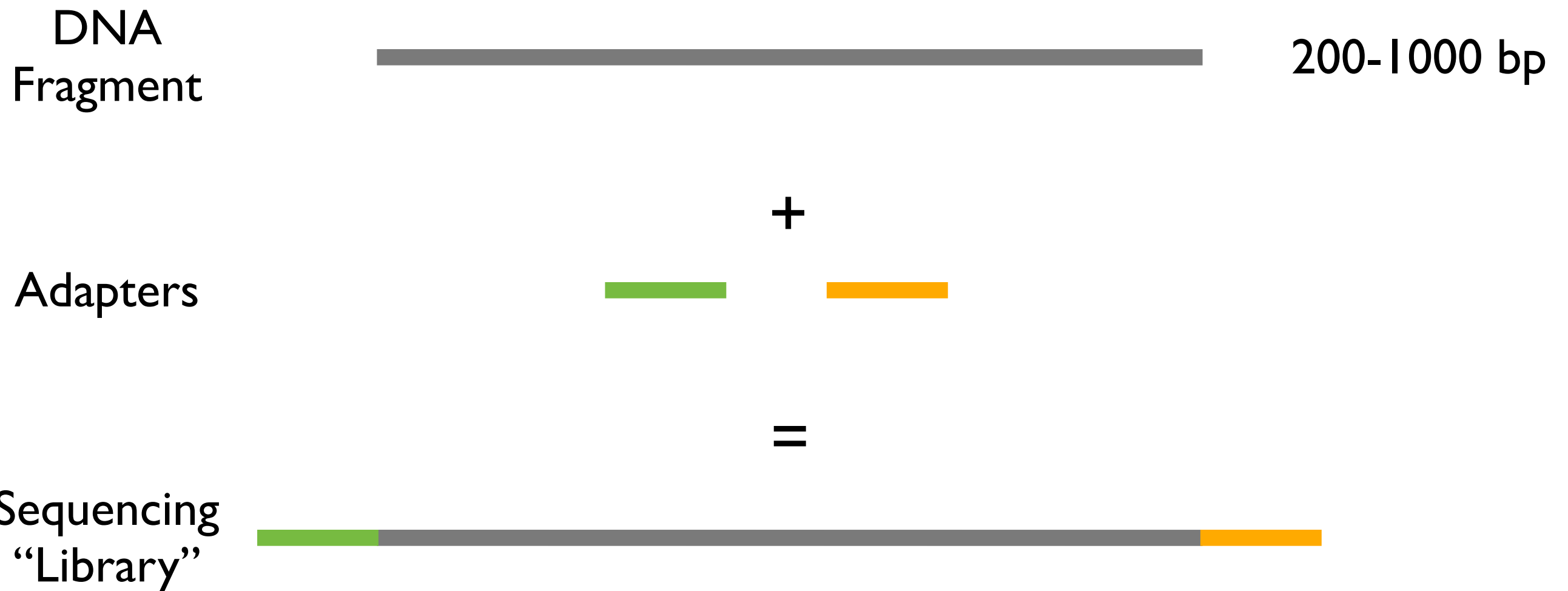
Sequencing

AGCTTTTCATTCTGACTGCAACGGGCAATATGTCTCTGTGTGGA

Sequencing

AGCTTTTCATTCTGACTGCAACGGGCAATATGTCTCTGTGTGGA

Sequencing

Sequencing
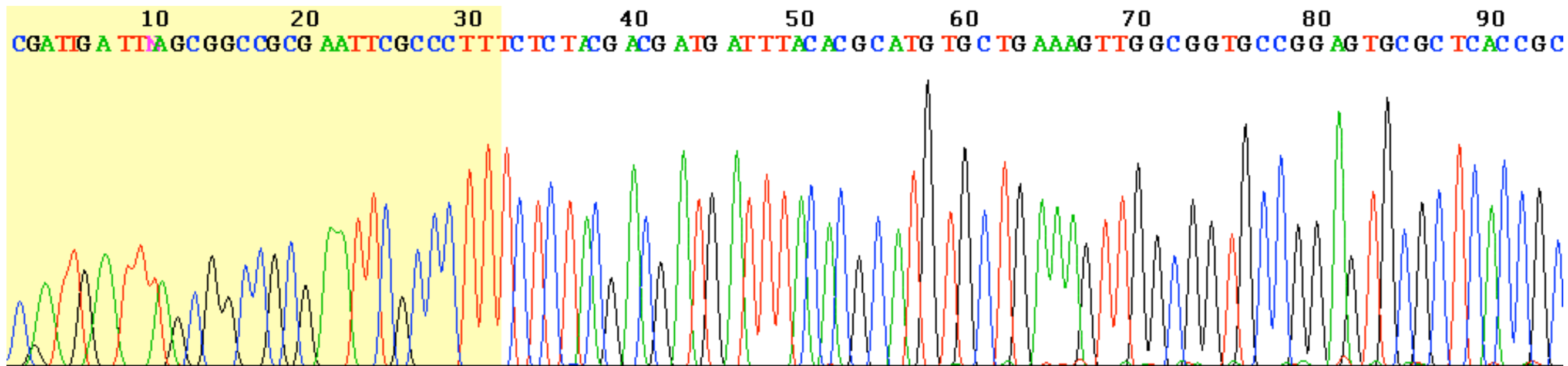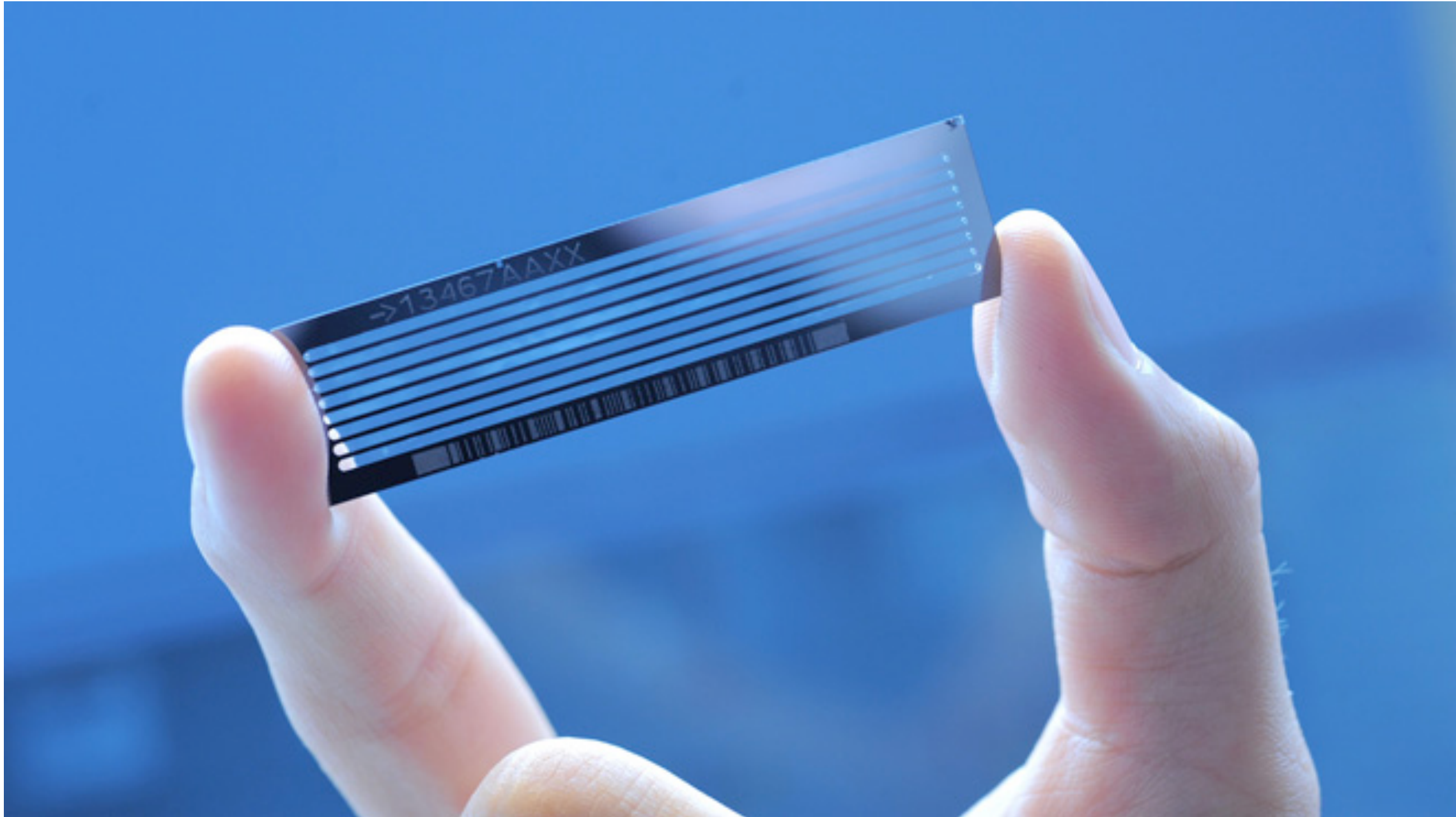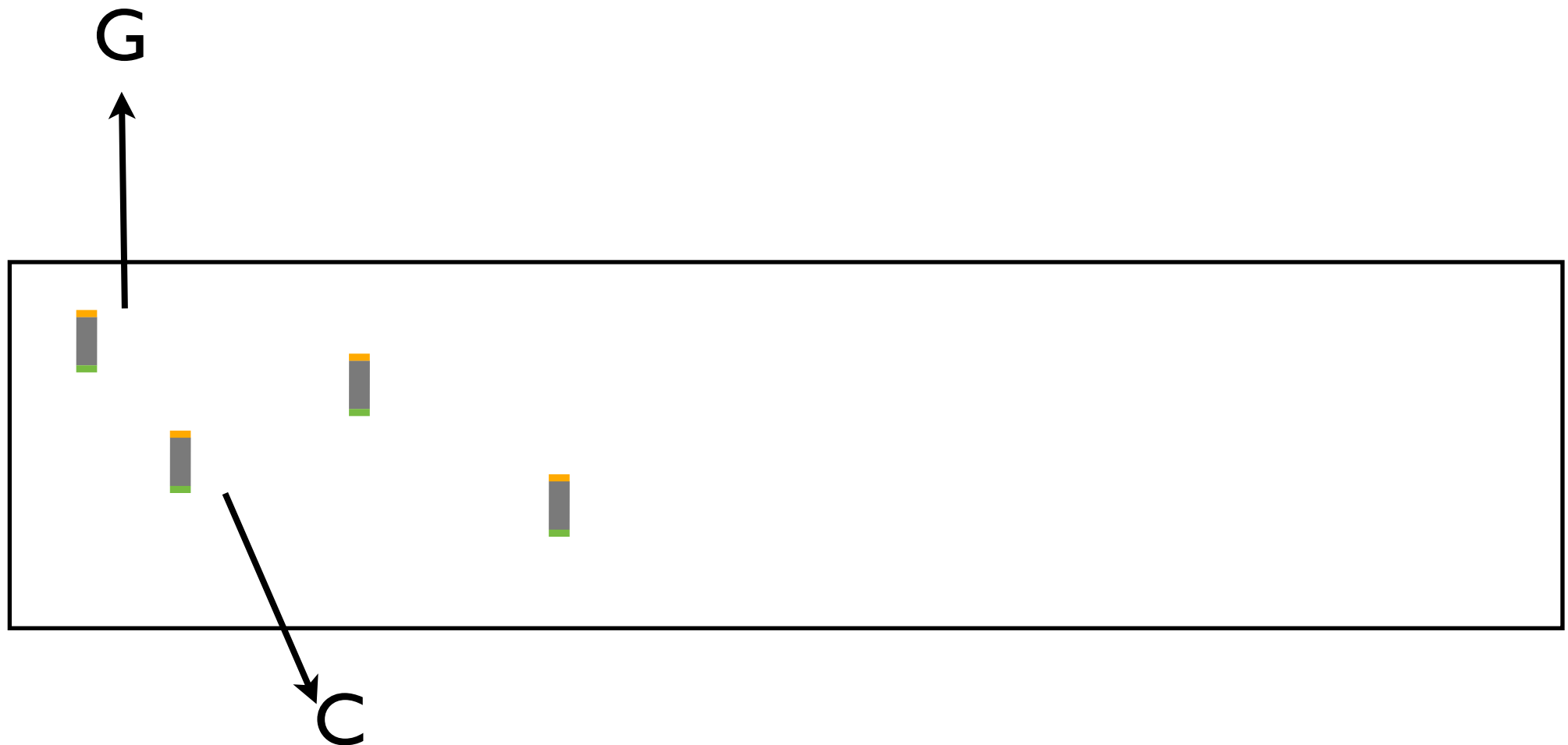
T
AGCTTTTCATTCTGACTGCAACGGGCAATATGTCTCTGTGTGGA

Sequencing

Sequencing

T C
AGCTTTTCATTCTGACTGCAACGGGCAATATGTCTCTGTGTGGA

Sequencing

T CG ●
AGCTTTTCATTCTGACTGCAACGGGCAATATGTCTCTGTGTGGA

Dye-terminator Sanger Sequencing
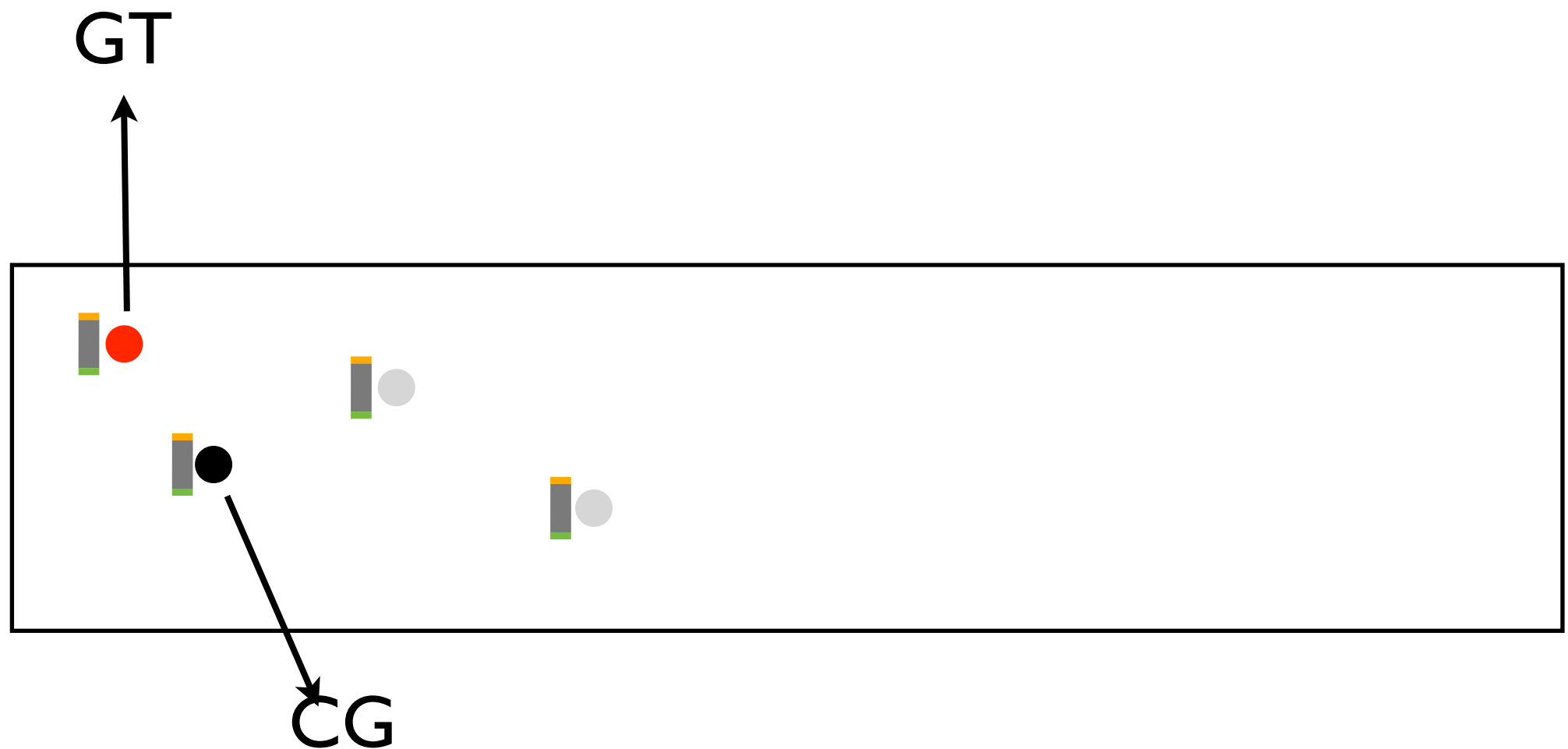
A Flow Cell
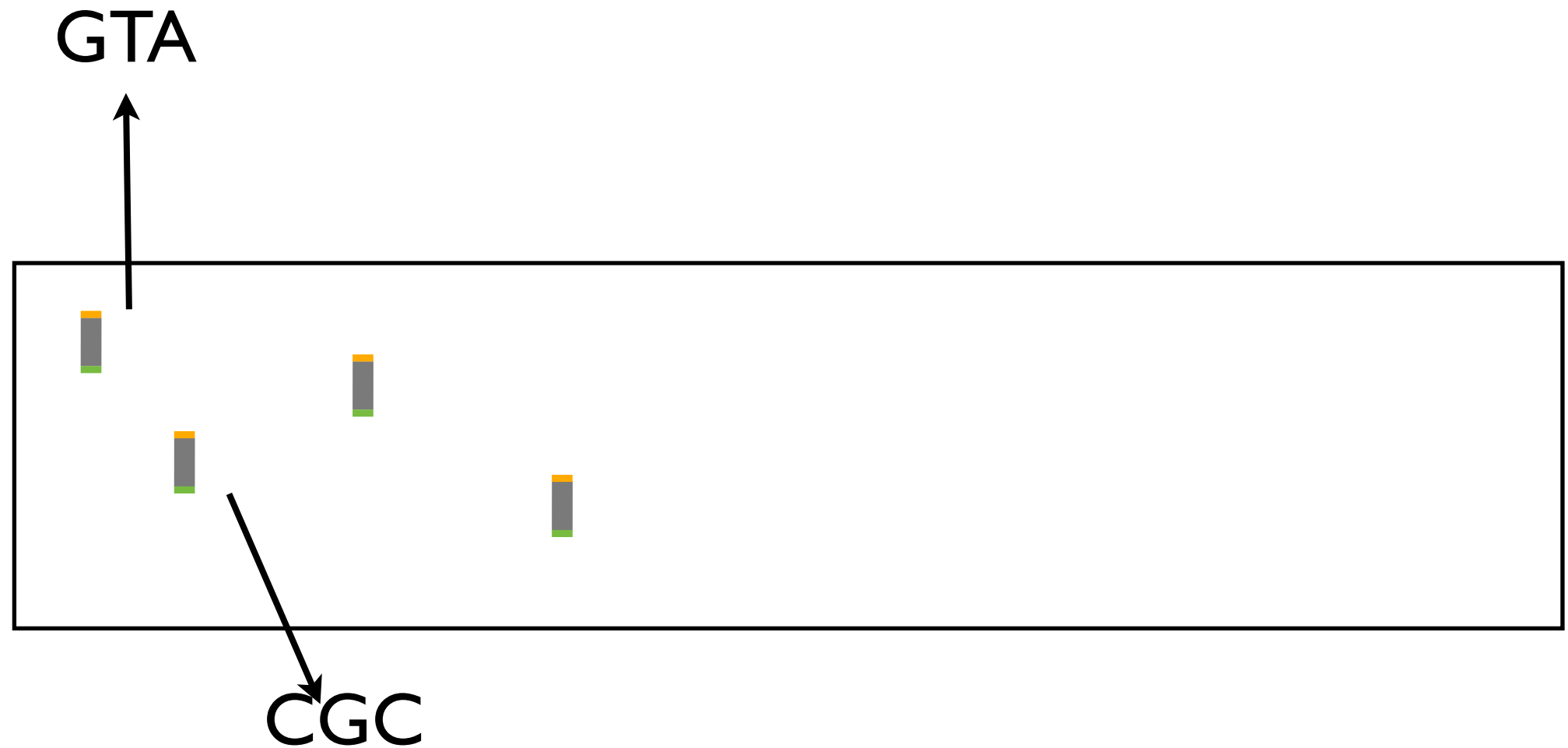
Parallel Sequencing

Parallel Sequencing

Parallel Sequencing

GT

CG
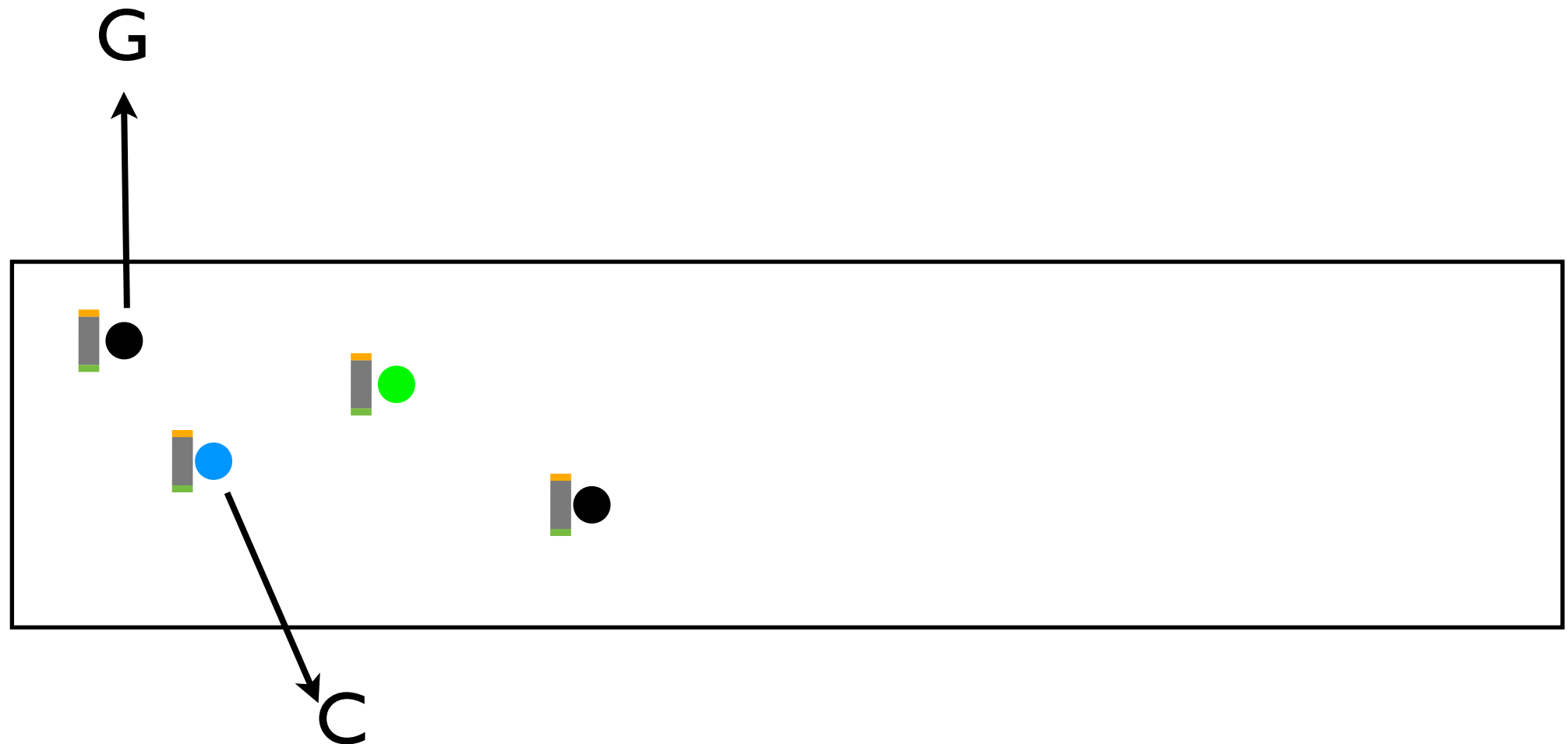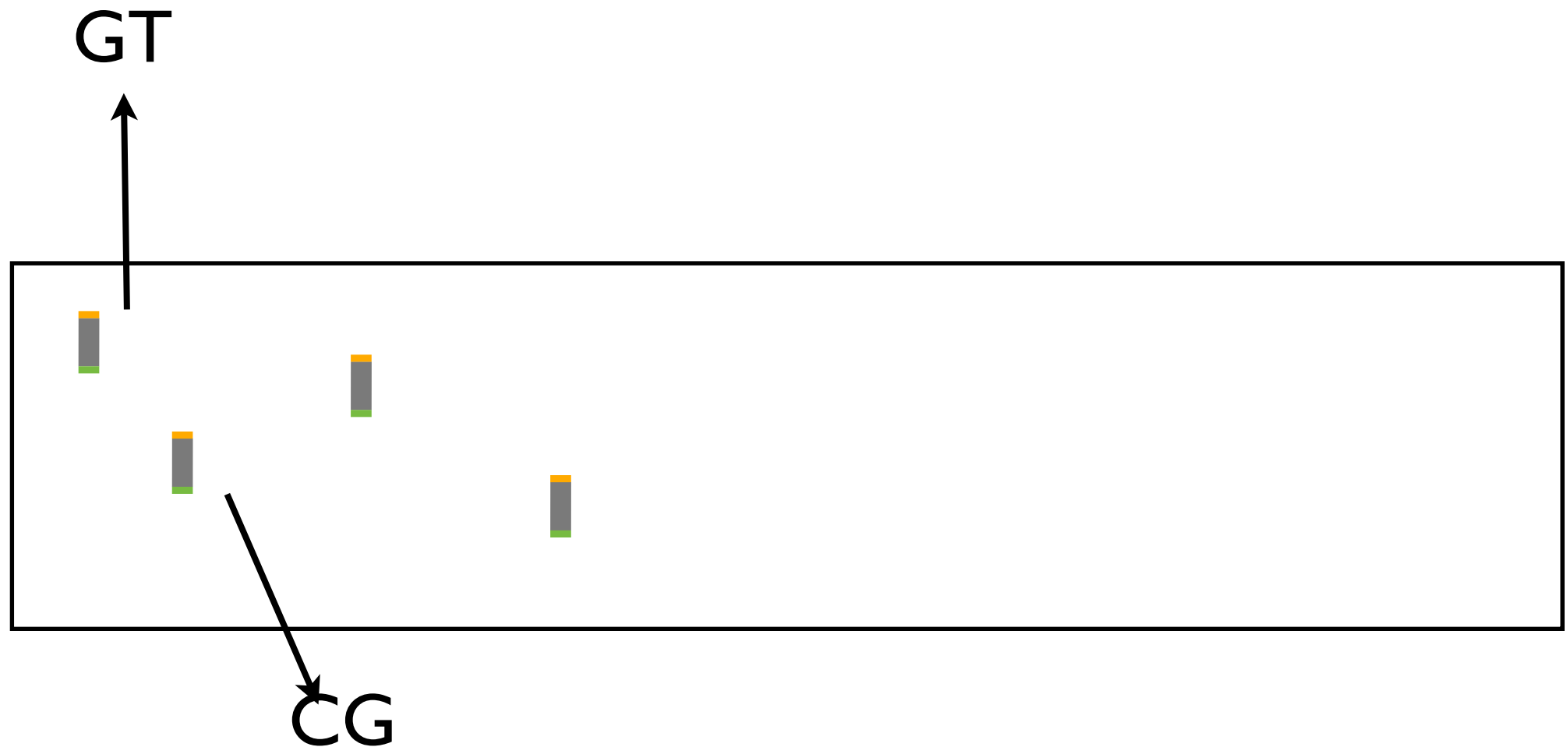
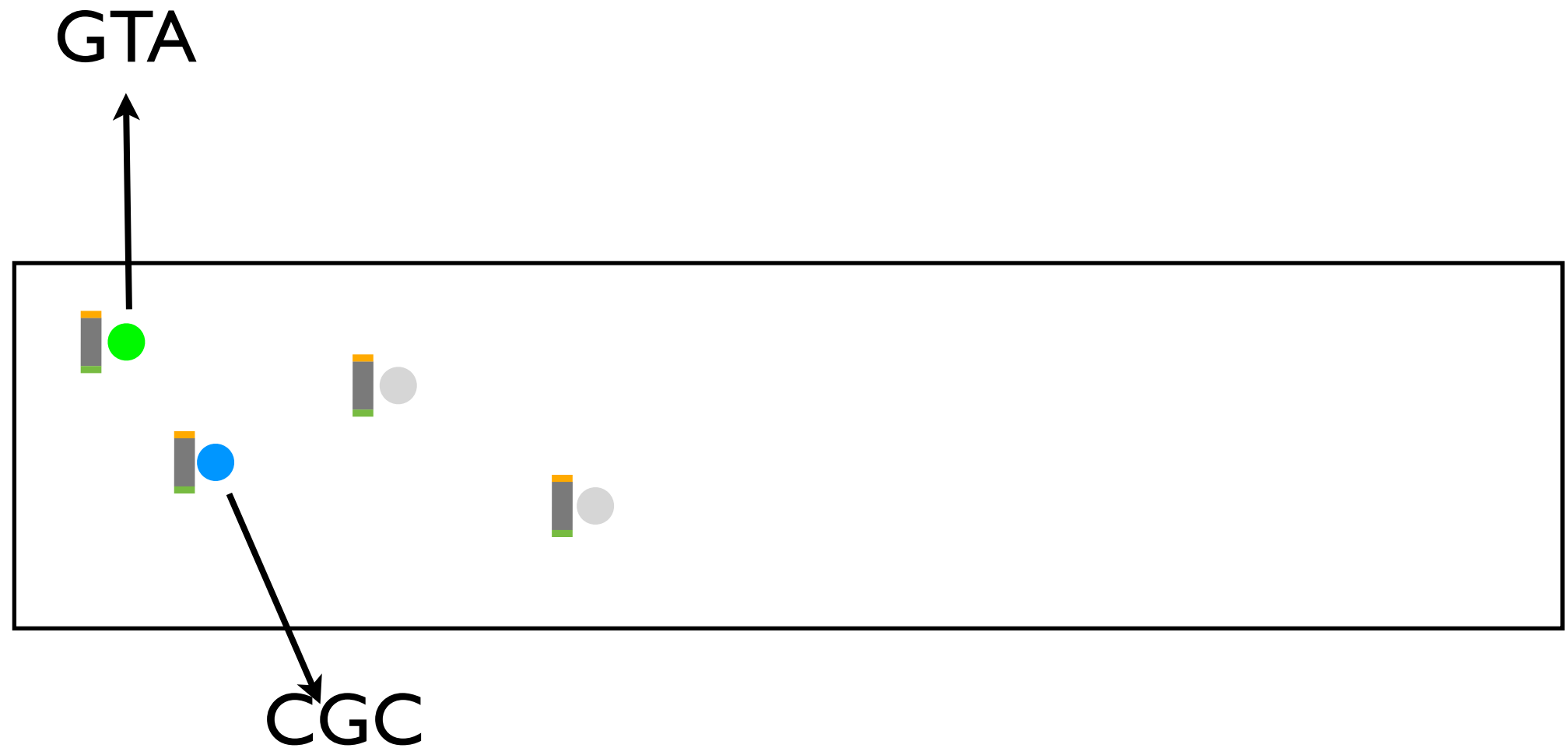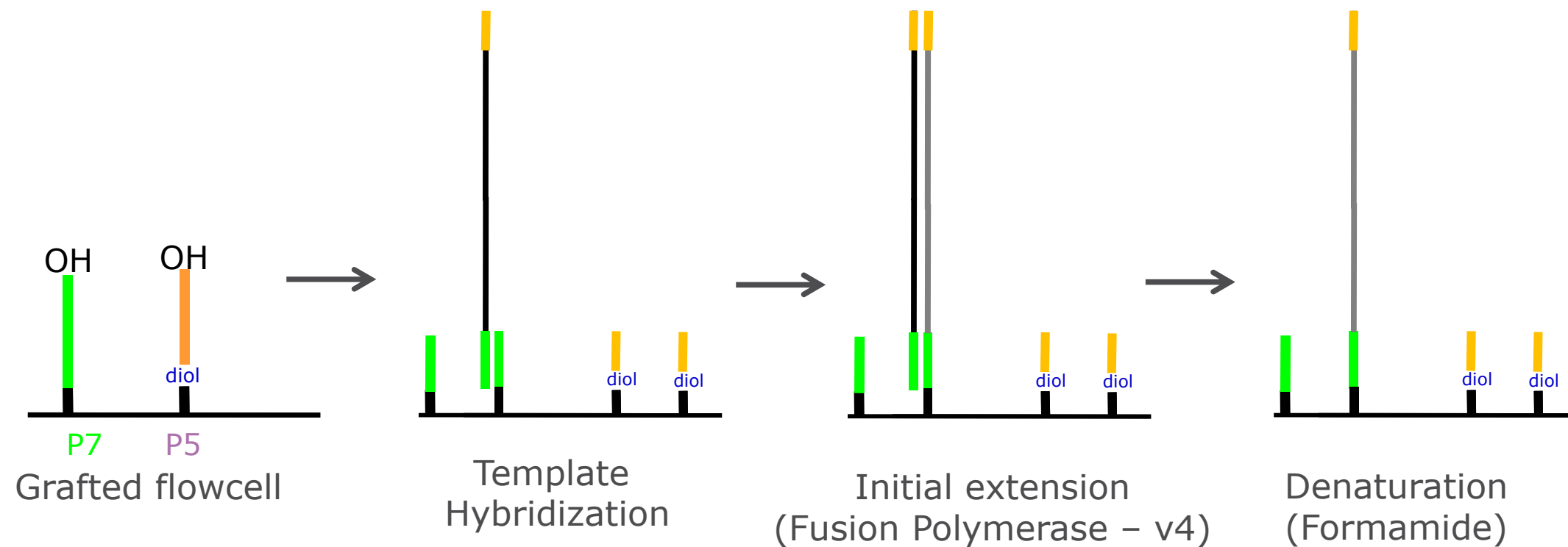# Parallel Sequencing

Parallel Sequencing

Parallel Sequencing

Cluster generation – hybridization and amplification

OH   OH

P7   P5
Grafted flowcell

Template
Hybridization

Initial extension
(Fusion Polymerase – v4)

Denaturation
(Formamide)

illumina®

Cluster generation – hybridization and amplification

1st cycle Denaturation (Formamide)

1st cycle Annealing

1st cycle Extension (*Bst* Polymerase)

2nd cycle Denaturation (Formamide)

2nd cycle extension

2nd cycle annealing

n=35 total

illumina®