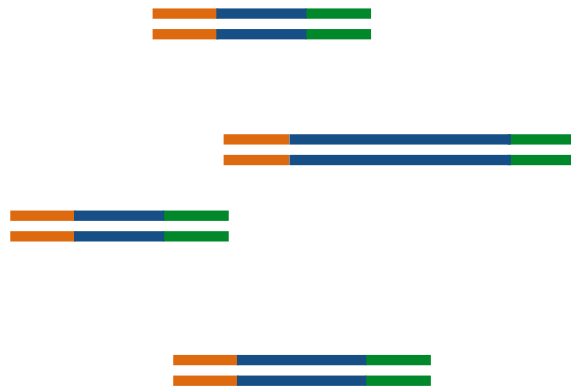


Sample Preparation

The Pof Workshop
7/18/16

[https://www.youtube.com/watch?
v=HMyCqWhwB8E](https://www.youtube.com/watch?v=HMyCqWhwB8E)

Desired input for Illumina sequencers



DNA fragments of interest
with **adaptors**
10nM concentration
< 1.5kb in length



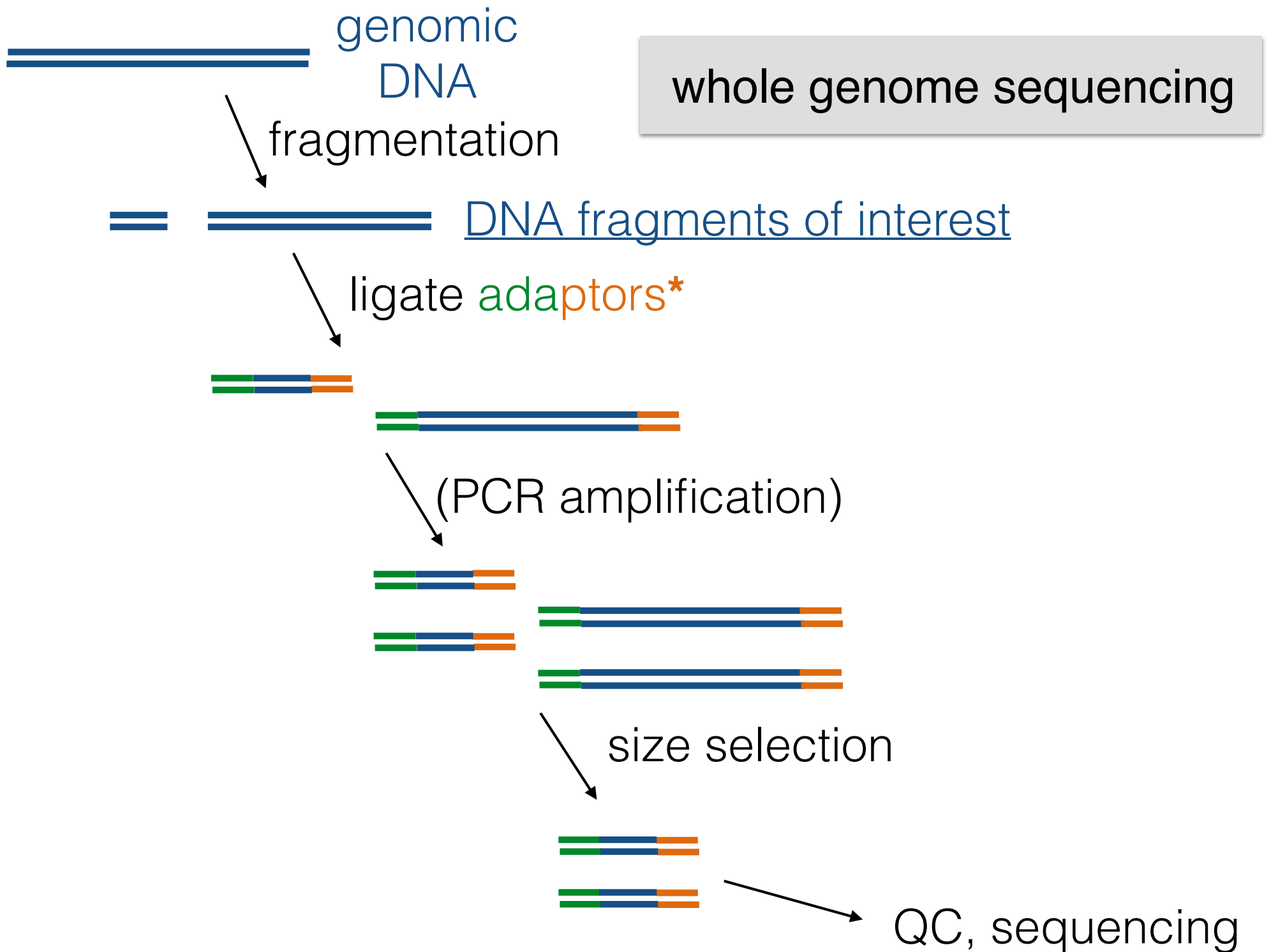
genomic
DNA

The diagram illustrates the process of whole genome sequencing. It begins with a representation of genomic DNA as two parallel blue horizontal lines. An arrow points from this DNA to a collection of smaller DNA fragments. Each fragment consists of a blue segment flanked by orange and green segments, representing the addition of sequencing adaptors. A red square with a white question mark is positioned near the arrow, indicating a step in the process. To the right, a grey box contains the text 'whole genome sequencing'. Below the fragments, text specifies the characteristics of the DNA fragments of interest: they have a 10nM concentration and are less than 1.5kb in length.

whole genome sequencing



DNA fragments of interest
with adaptors
10nM concentration
< 1.5kb in length



genome

ATAC seq

tagmentation



(PCR amplification)



QC, sequencing

cell or tissue

ChIP-Seq

PFA fix



fragmentation



antibody
pull-down

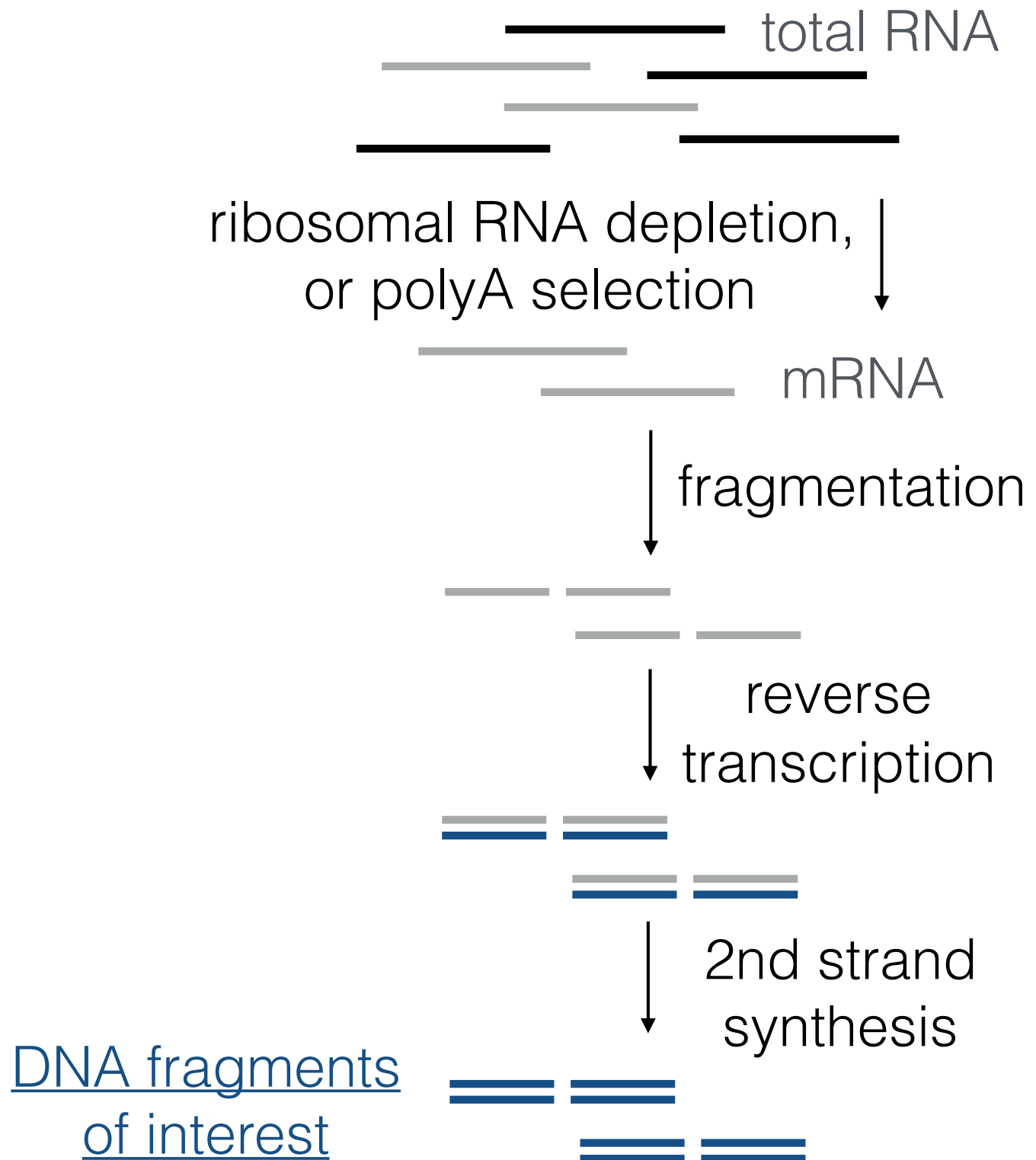


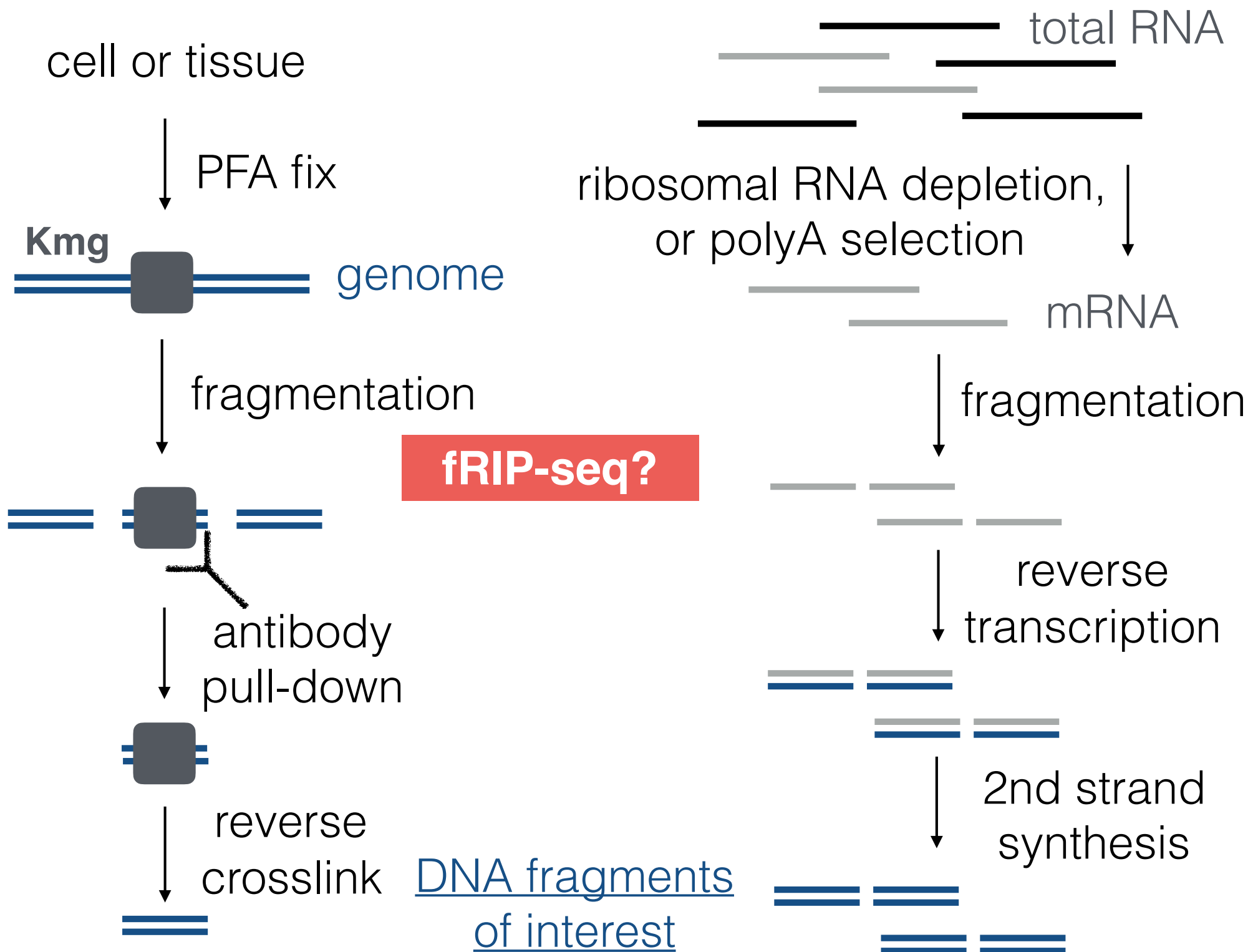
reverse
crosslink

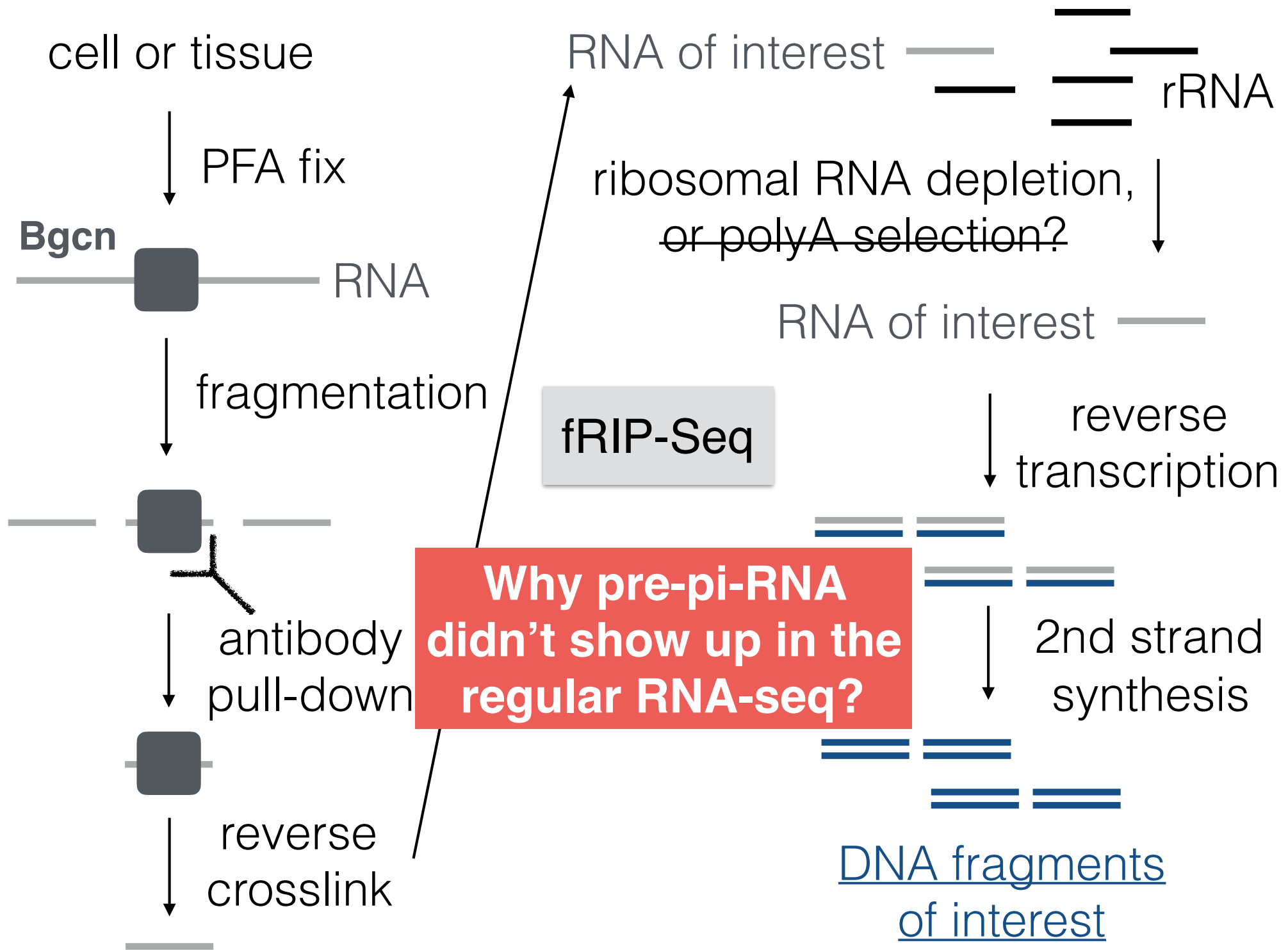


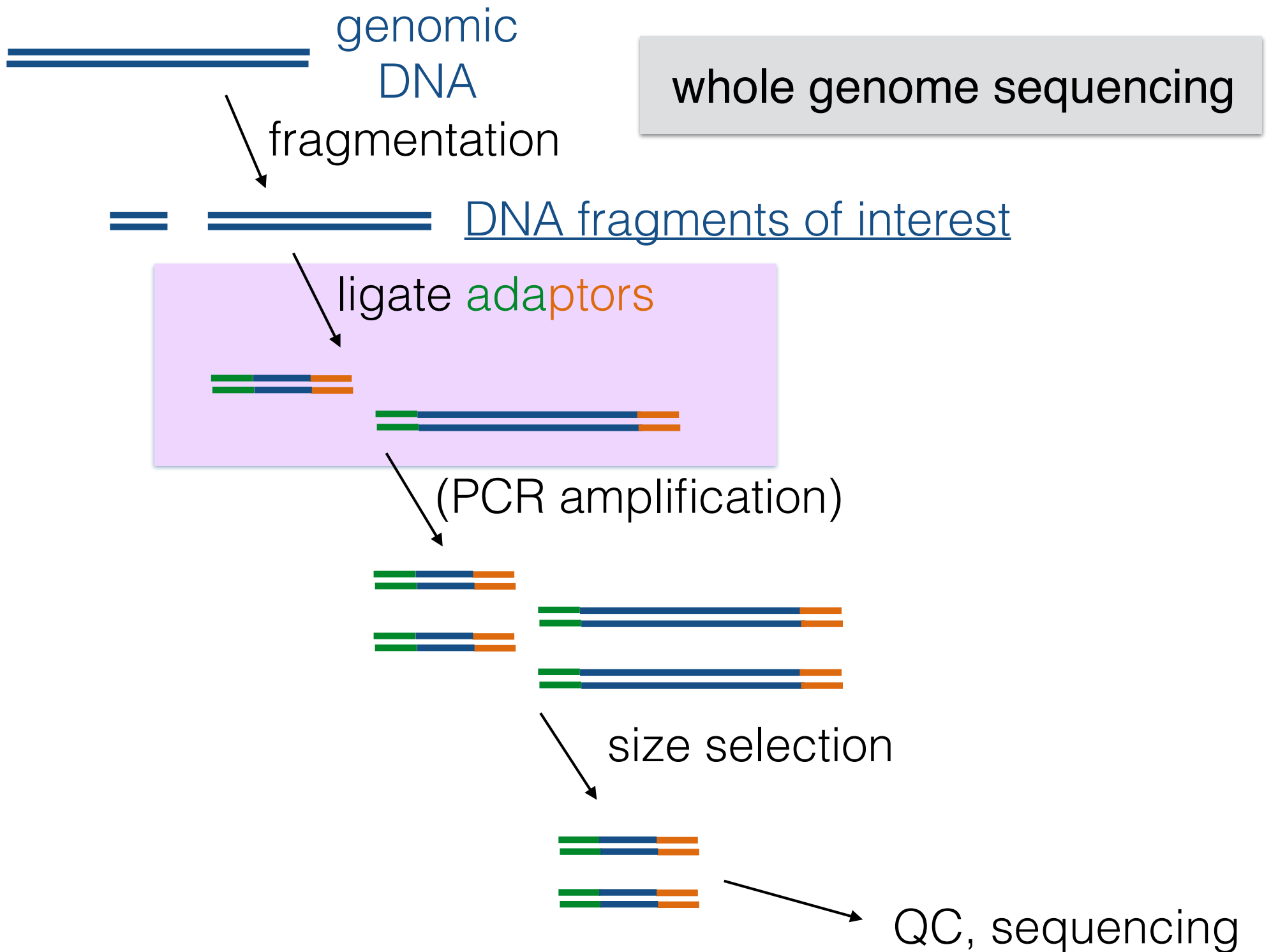
DNA fragments
of interest

RNA-Seq









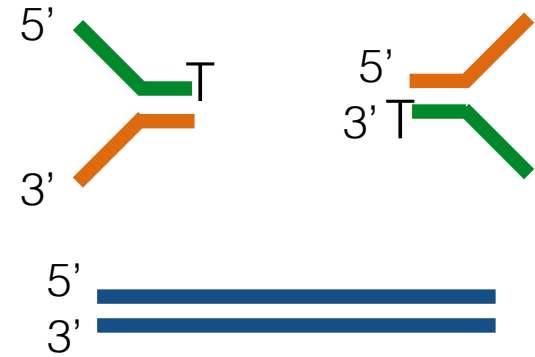
adaptor ligation



↓ blunt end
ligation



All can PCR, align to the flow cell,
amplify, but only the last one can be
sequenced



↓ end repair
A-tailing



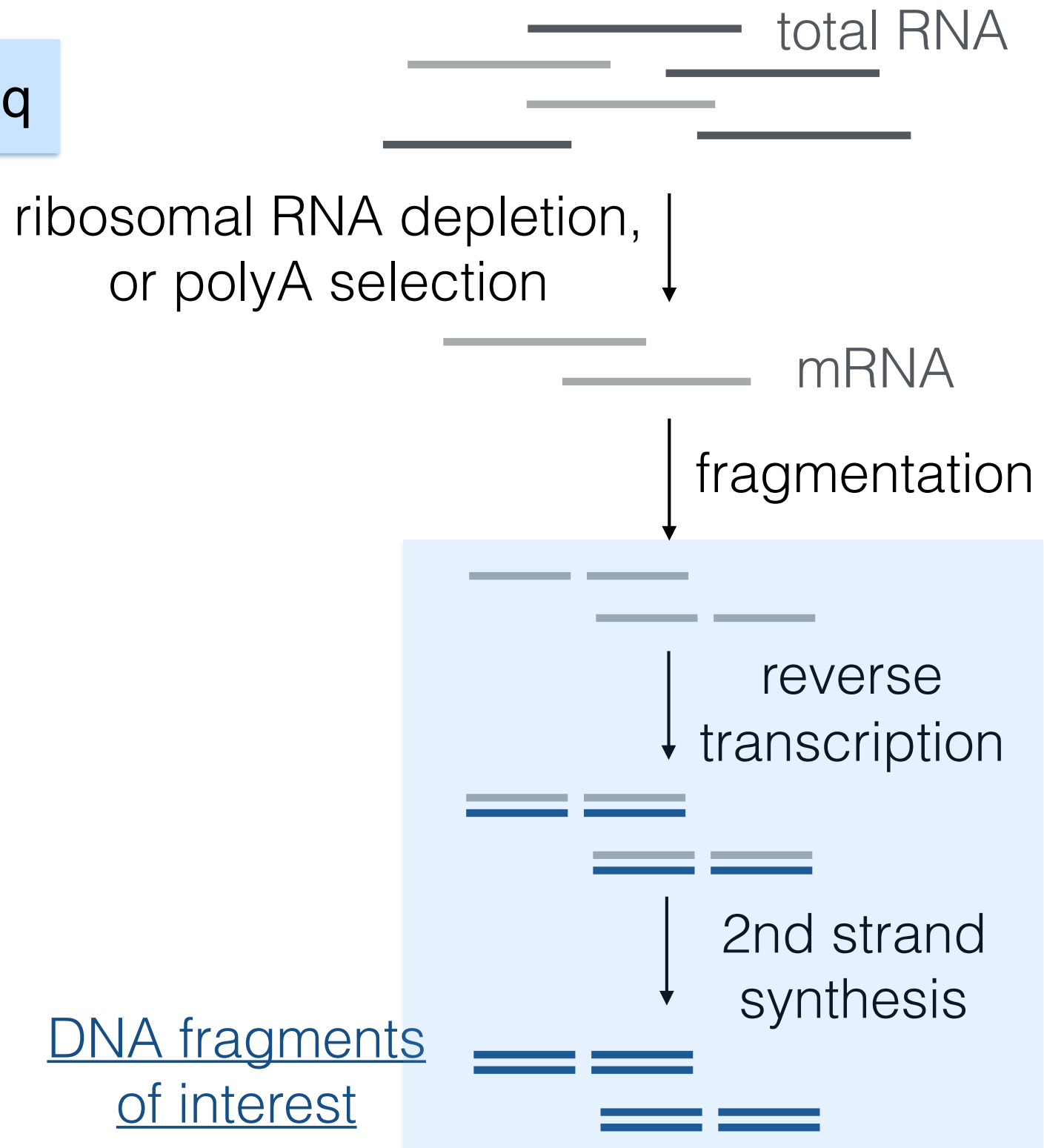
↓ ligate



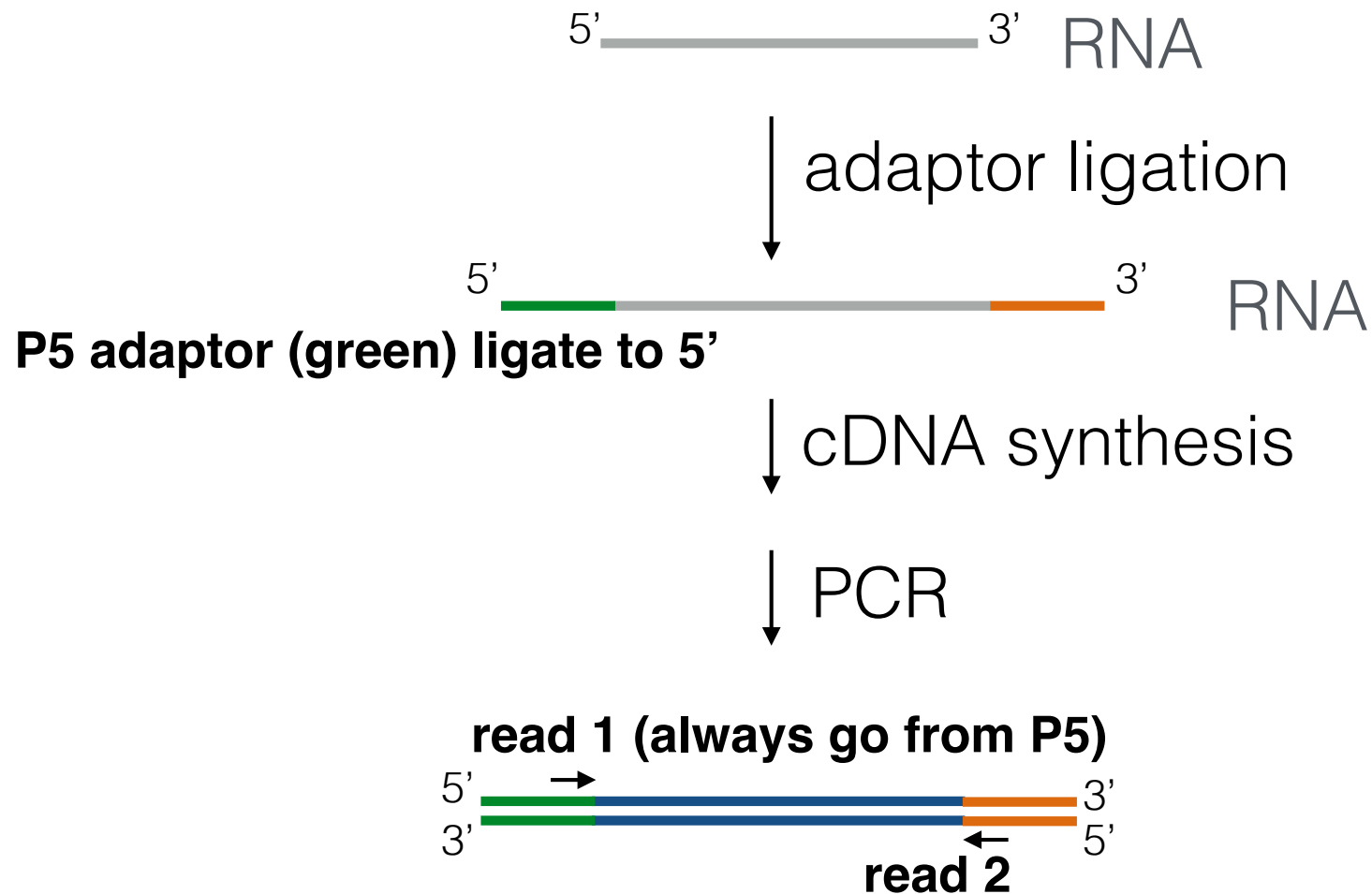
↓ PCR



Stranded RNA-Seq



Stranded RNA-Seq: Illumina kit



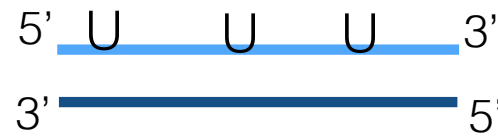
forward strand got sequenced

Stranded RNA-Seq: KAPA kit (we use)

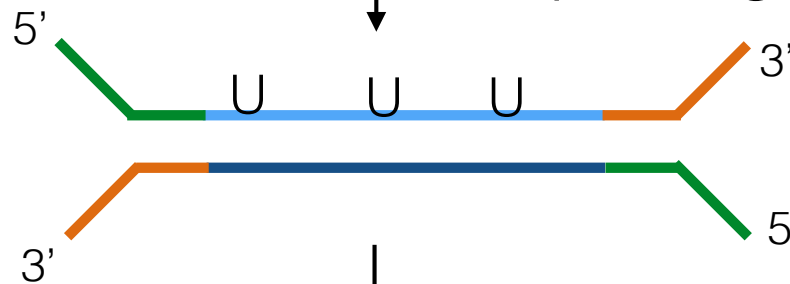
1st strand synthesis



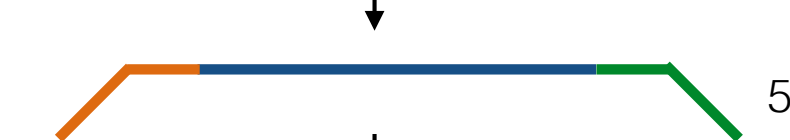
2nd strand synthesis



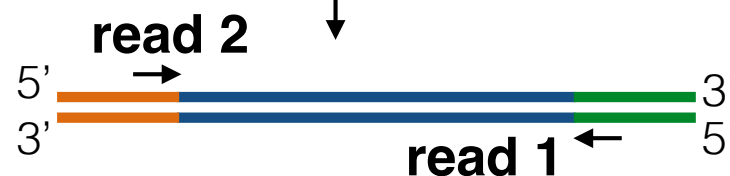
adaptor ligation



dUTP strand degradation



PCR



reverse
strand got
sequenced