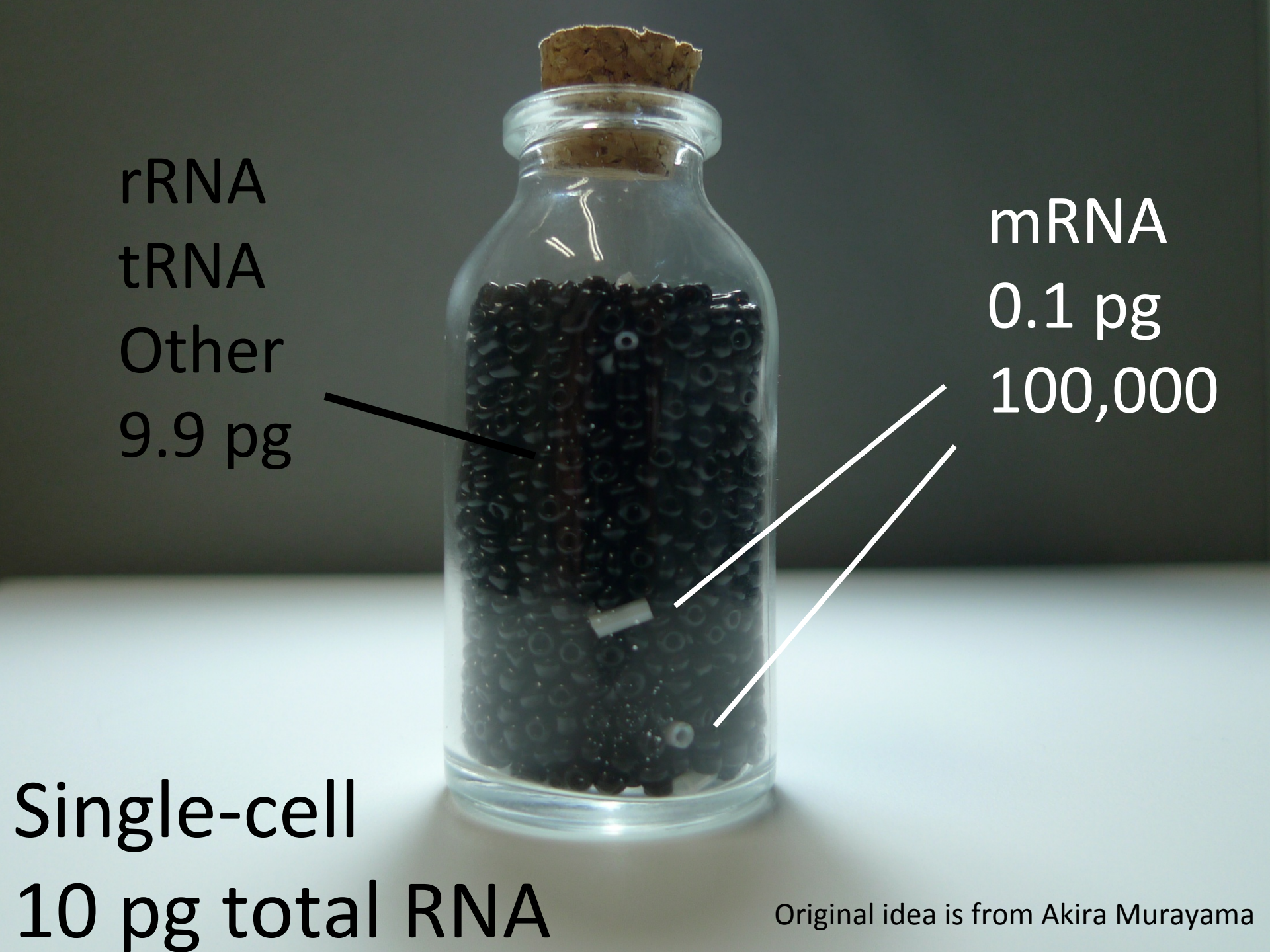
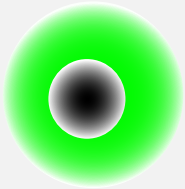

rRNA
tRNA
Other
9.9 pg
mRNA
0.1 pg
100,000
Single-cell
10 pg total RNA
Original idea is from Akira Murayama

1細胞RNA-SeqにはcDNAの増幅が必要となる



Single cell

10pg Total RNA

1%

mRNA

5' AAAAAAAAAA 3'

100,000 molecules

(If average length of mRNA is 1000bp)

99%

rRNA tRNA pRNA mtRNA ncRNA

Need

Whole-Transcript-amplification

over 10,000-fold amplification

Detectors

>100ng Total RNA

(>10,000 cell)

Microarray mRNA-seq

1細胞トランスクリプトーム解析方法

Poly-A tailing (Target: all cDNA)

Reproducibility and sensitivity

Brady et al. 1990 → Microarray: Kurimoto et al. 2006 → Seq (Solid): Tang et al. 2009 → Microarray, Seq (Illumina), Quartz-Seq/Chip

Template switching (Target: full length cDNA)

SMARTer system

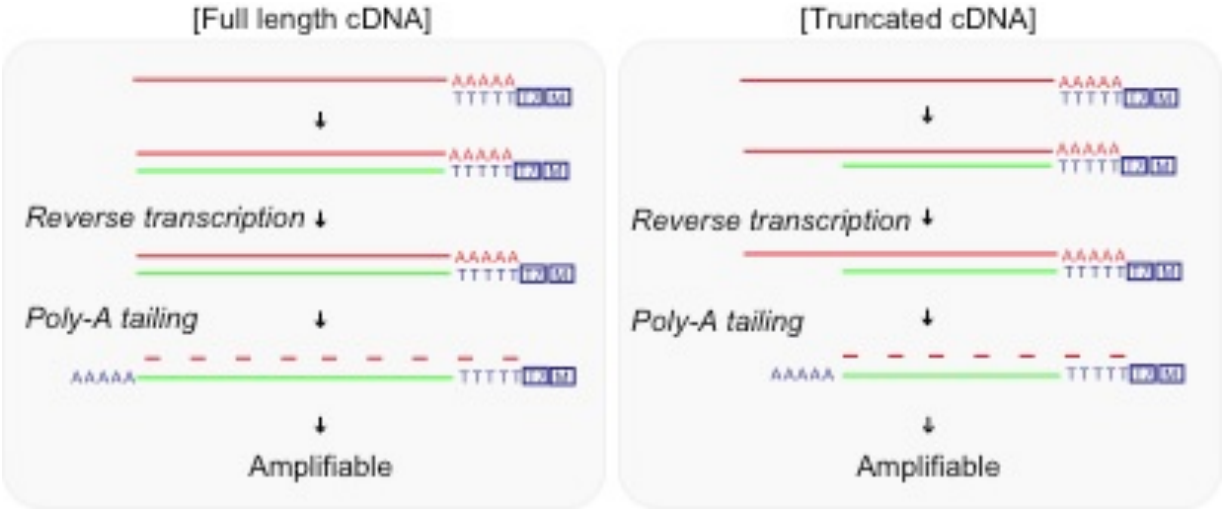
- Seq (RPKM, Illumina): Smart-Seq 2012 — *Transcript Coverage*
- Seq (tag count, Illumina): STRT-Seq 2011 — *High-throughput (5' end)*

In vitro transcription-based

Seq (tag count, Illumina): CEL-Seq 2012 — *High-throughput (3' end)*

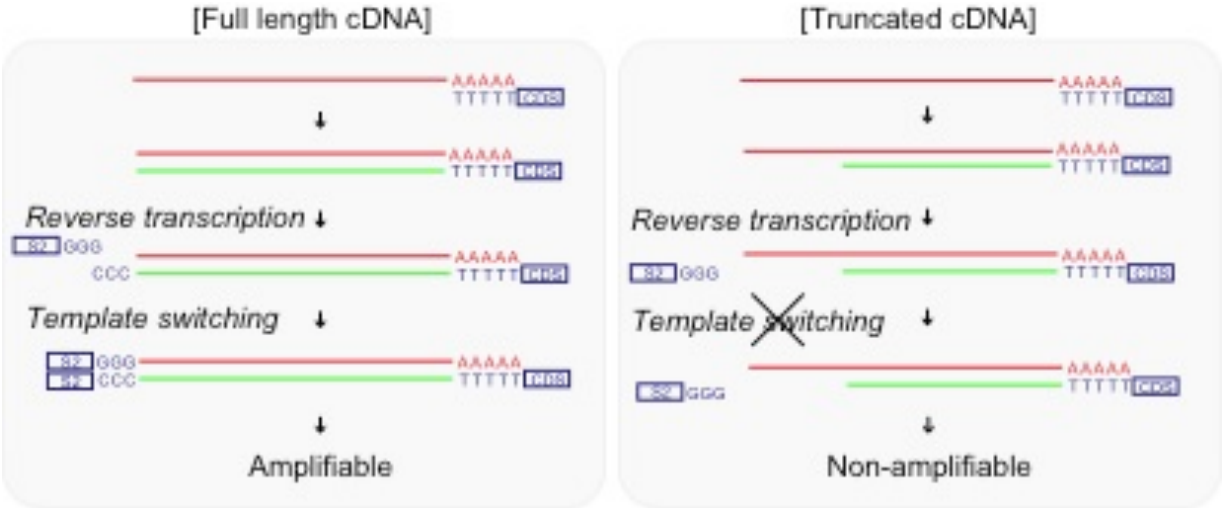
1細胞トランスクリプトーム解析方法

Poly-A tailing (Target: all cDNA)



TruncateなcDNAも対象となる。

Template switching (Target: full length cDNA)

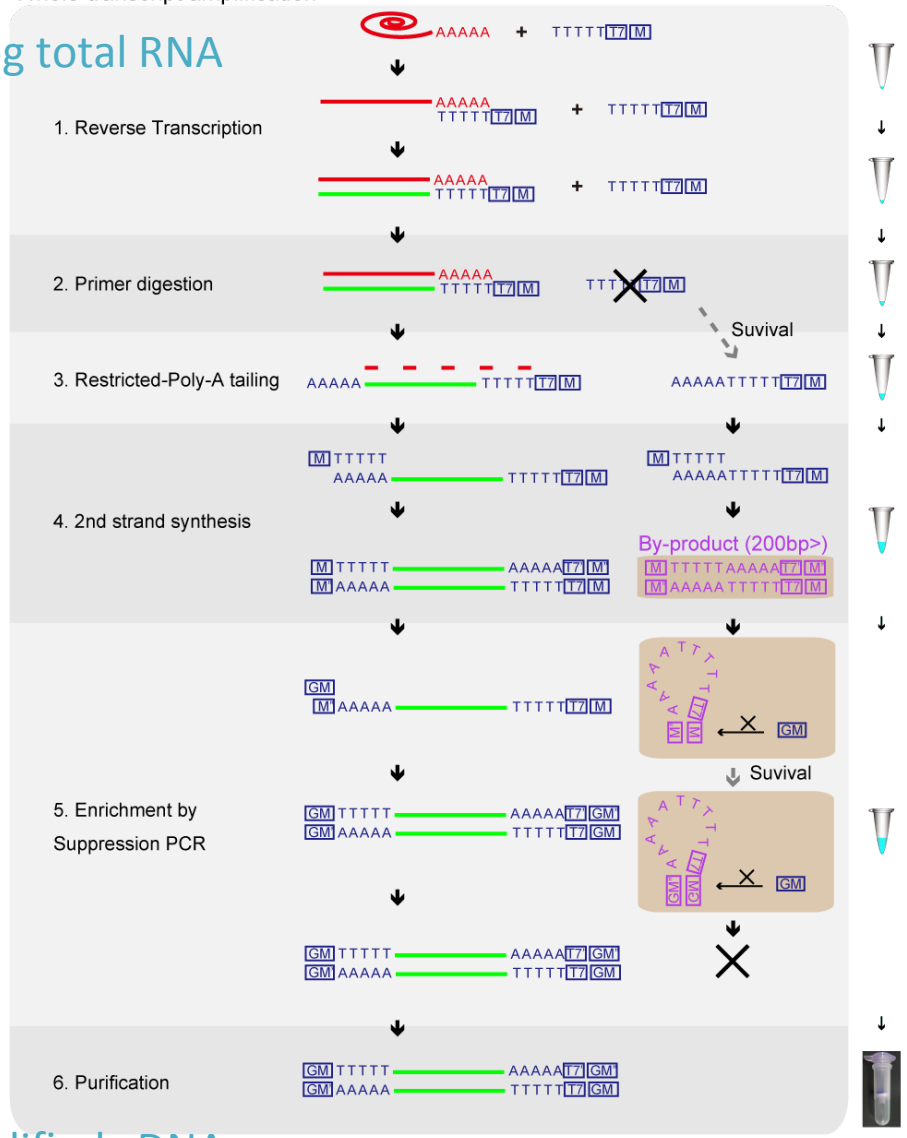


Quartz-Seq法の概要

WTA

Whole-transcript amplification

10 pg total RNA

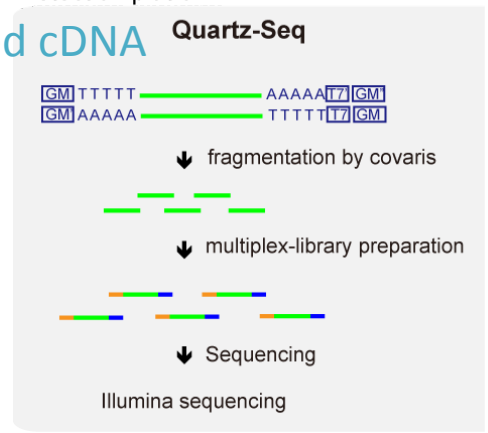


Amplified cDNA

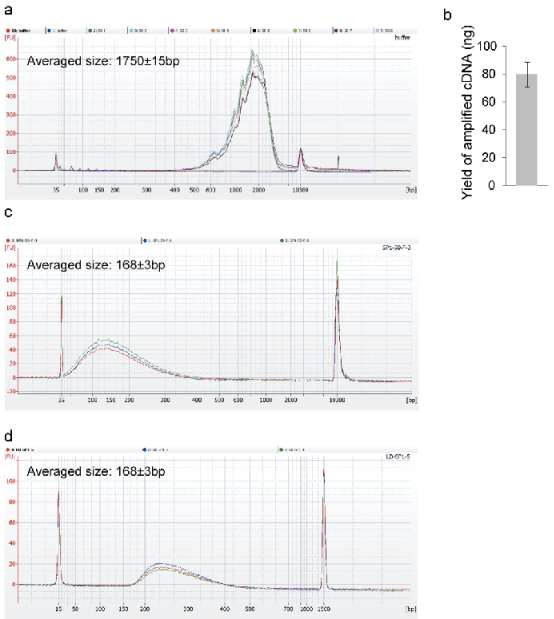
LIMprep

Detection platform

Amplified cDNA

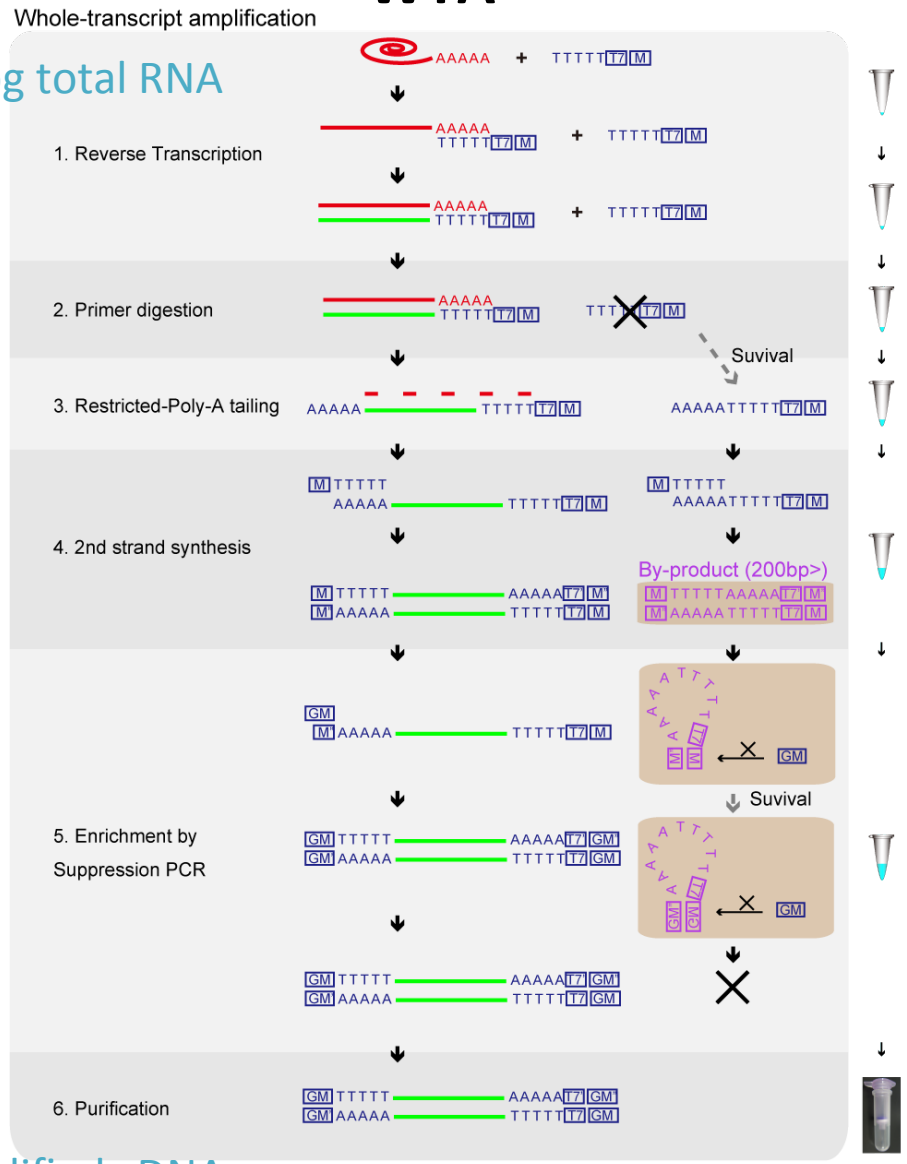


Sequence Library DNA

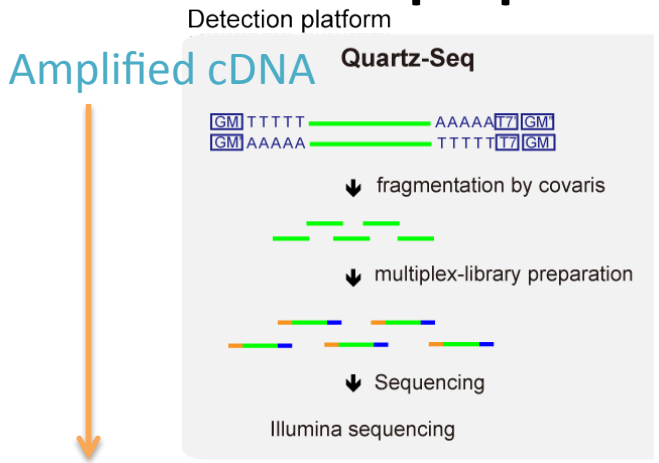


Quartz-Seq法の概要

WTA



LIMprep



株式会社日本ジェネティクス「KAPAエンジニア酵素を利用した微量サンプルからのマルチプレックスNGSライブラリー調製の成功事例」

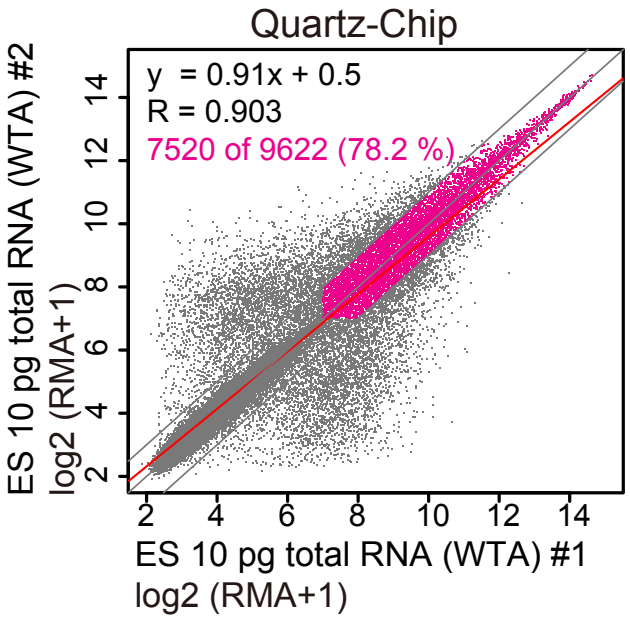
微量DNAからのマルチプレックス可能なシーケンスライブラリ作製がコマーシャル品で対応してなかったので立ち上げた。

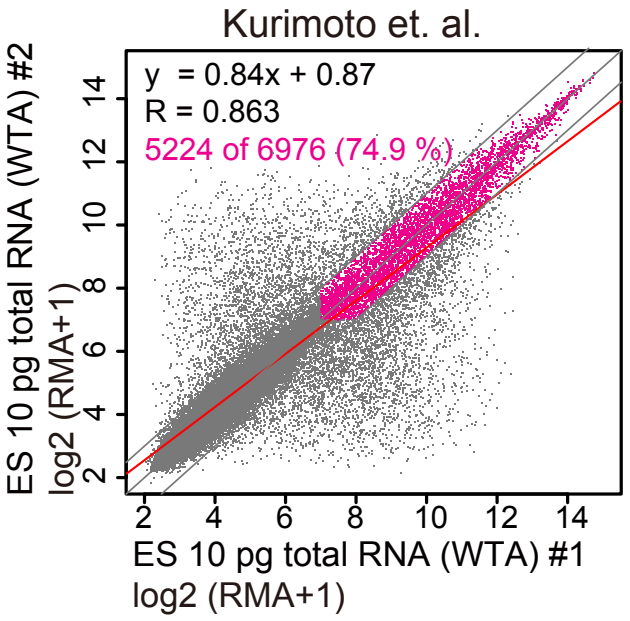
1細胞トランスクリプトーム解析方法

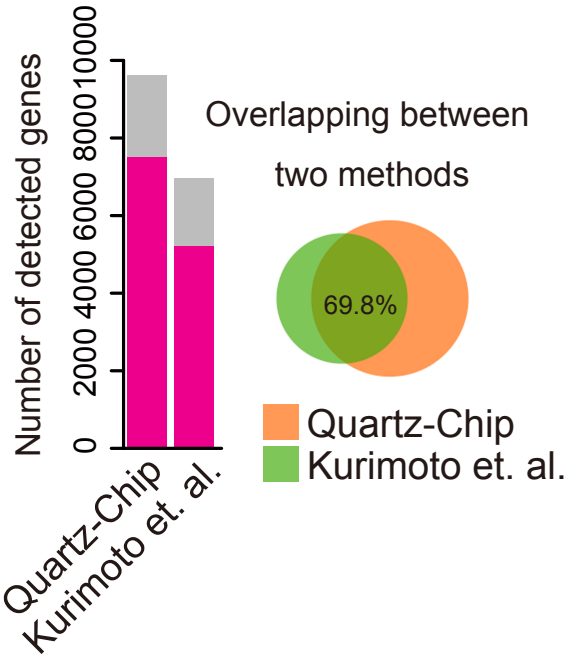
Poly-A tailing (Target: all cDNA)

Reproducibility and sensitivity

Brady et al. 1990 → Microarray: Kurimoto et al. 2006 → Seq (Solid): Tang et al. 2009 → Microarray, Seq (Illumina): Quartz-Seq/Chip



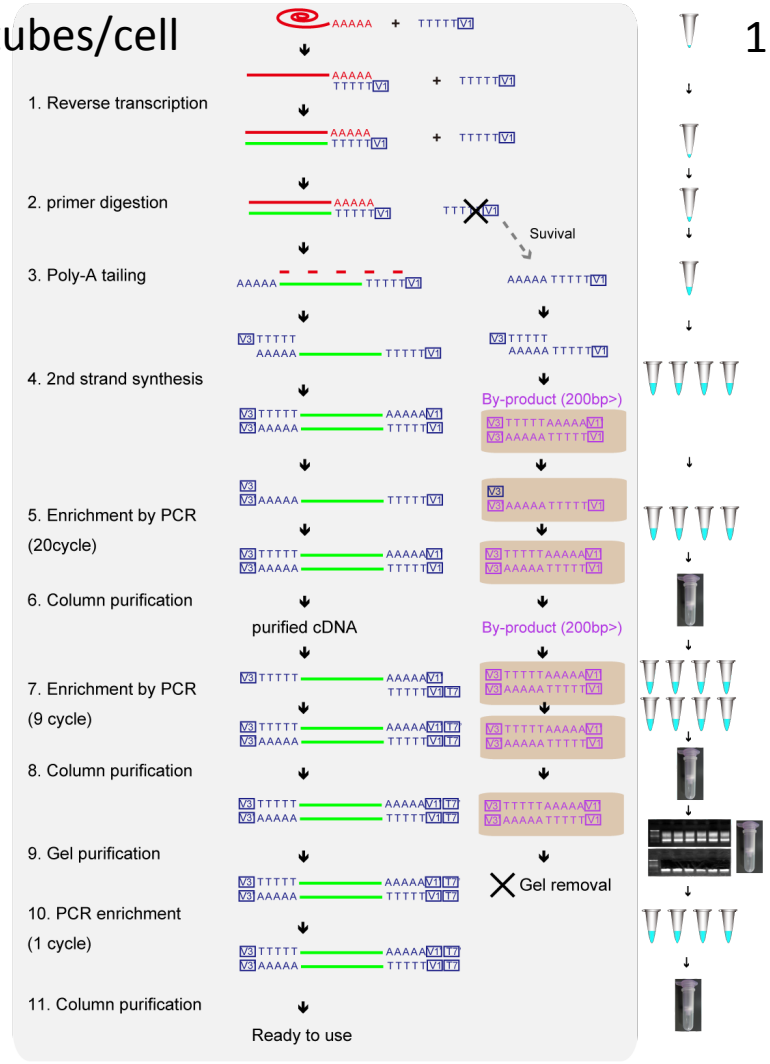


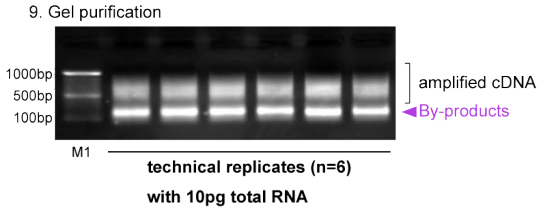


Kurimoto et al. 2006

Whole-transcript amplification for Kurimoto et al. method

17 PCR tubes/cell

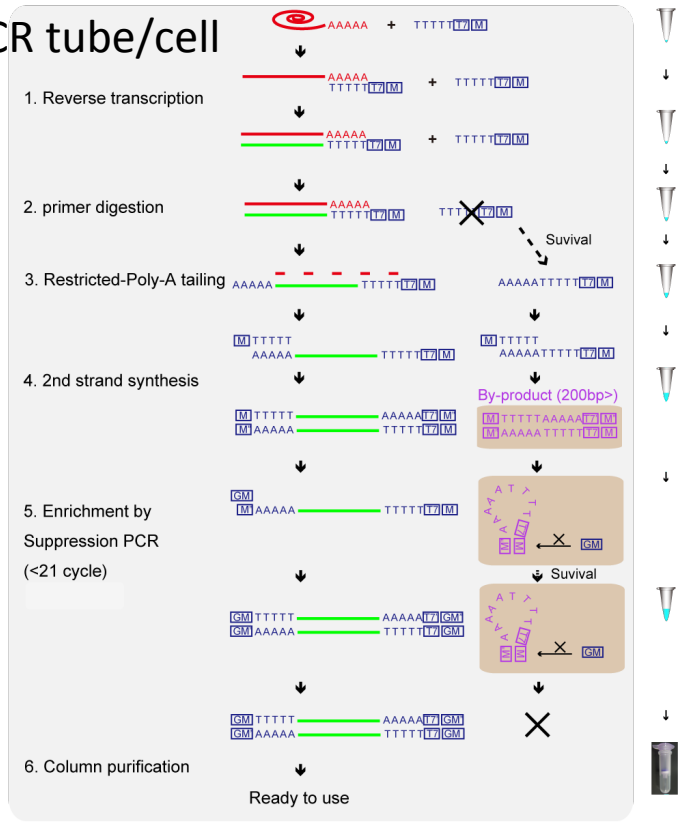


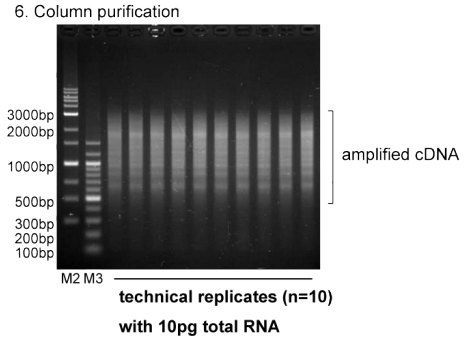


Quartz-Seq 2013

Whole-transcript amplification for Quartz-Seq/Chip

1 PCR tube/cell





Single-tube での高効率なcDNA増幅のために

1. キャリーオーバー

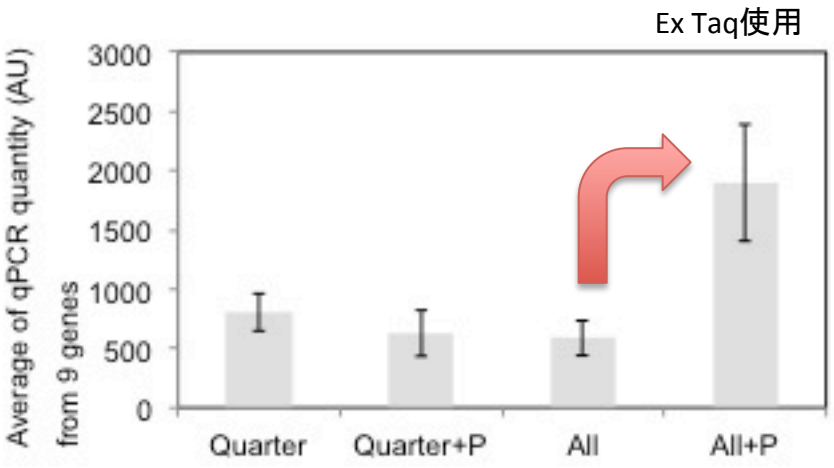
2. PCR byproduct

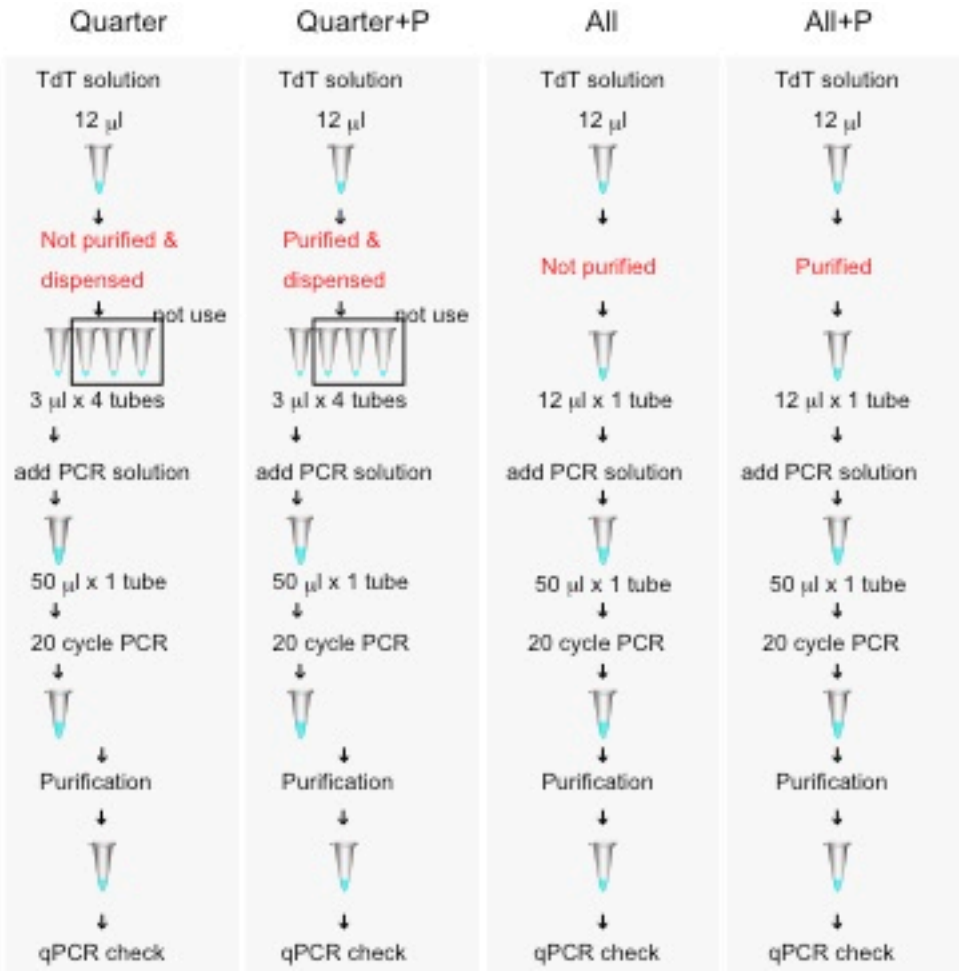
Single-tube での高効率なcDNA増幅のために

1. キャリーオーバー
2. PCR byproduct

キャリーオーバーによりcDNAの増幅効率の阻害

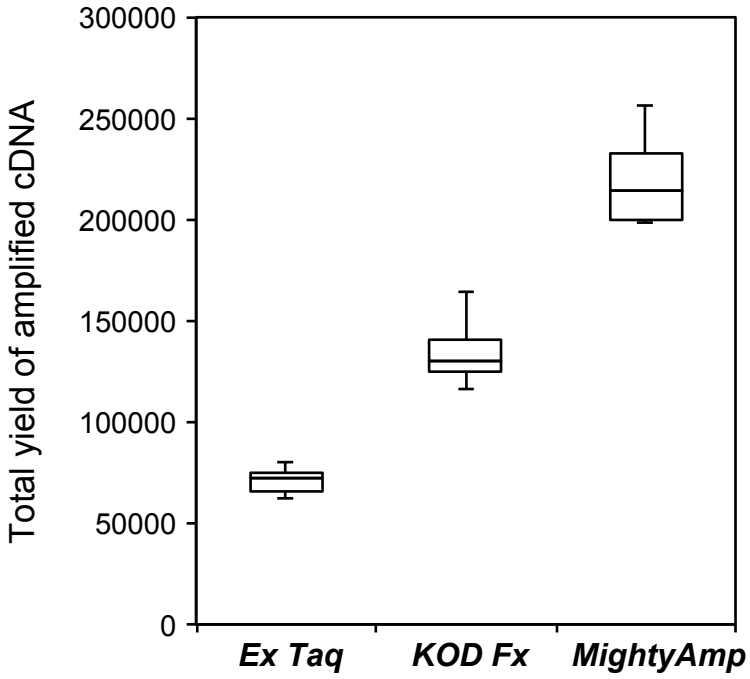
cDNA増幅直前のサンプル ⇒ 持ち込みの程度を変えて増幅 ⇒ 評価





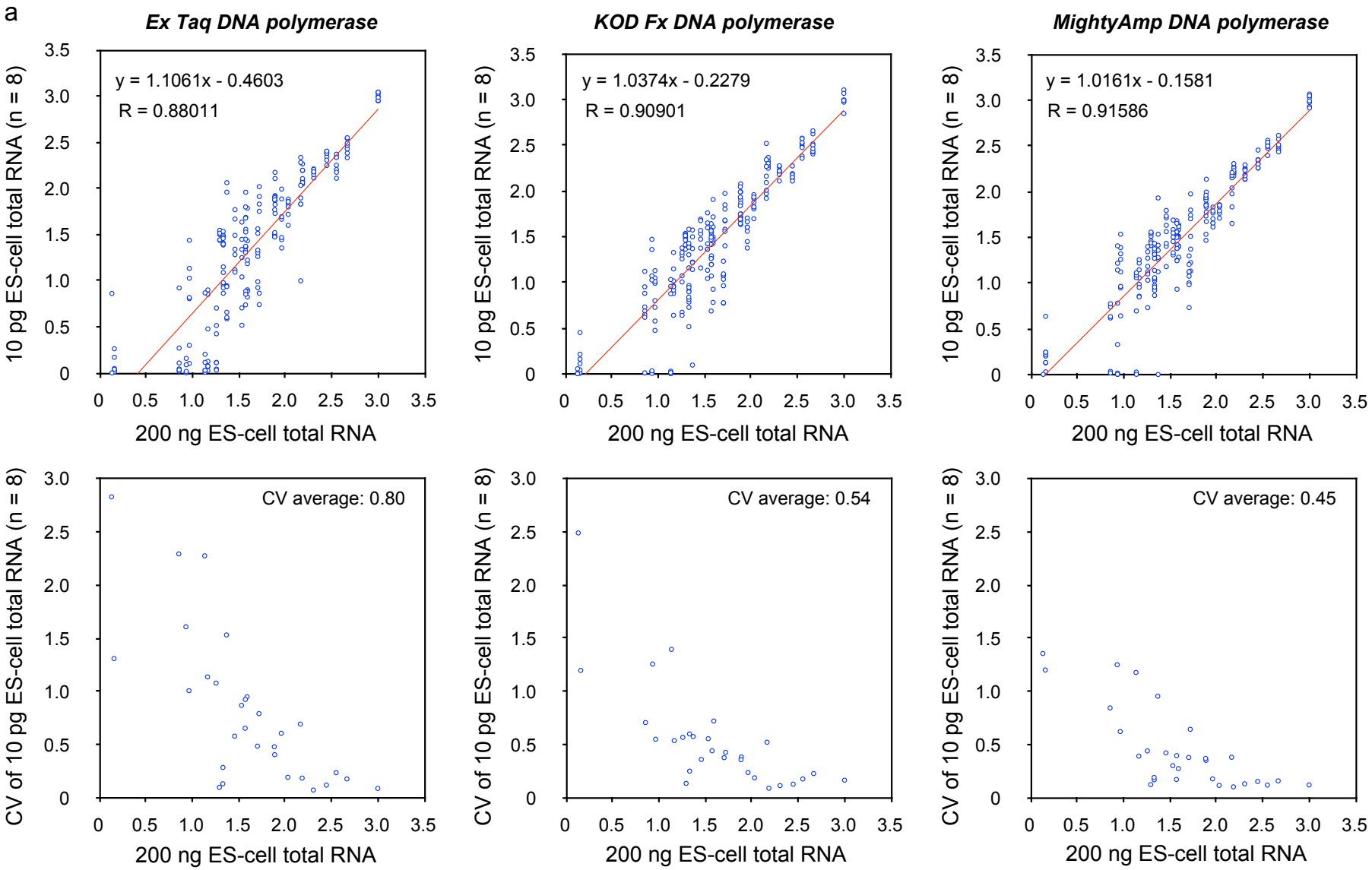
キャリオーバーを減らすために、
Quartz-Seqでは増幅直前までの
反応系のスケールを1/2.27に。

最適なPCR酵素の選択
～収量の改善～



クルードなサンプルに強いPCR酵素の使用で、収量の改善があった。

最適なPCR酵素の選択
～定量性の改善～



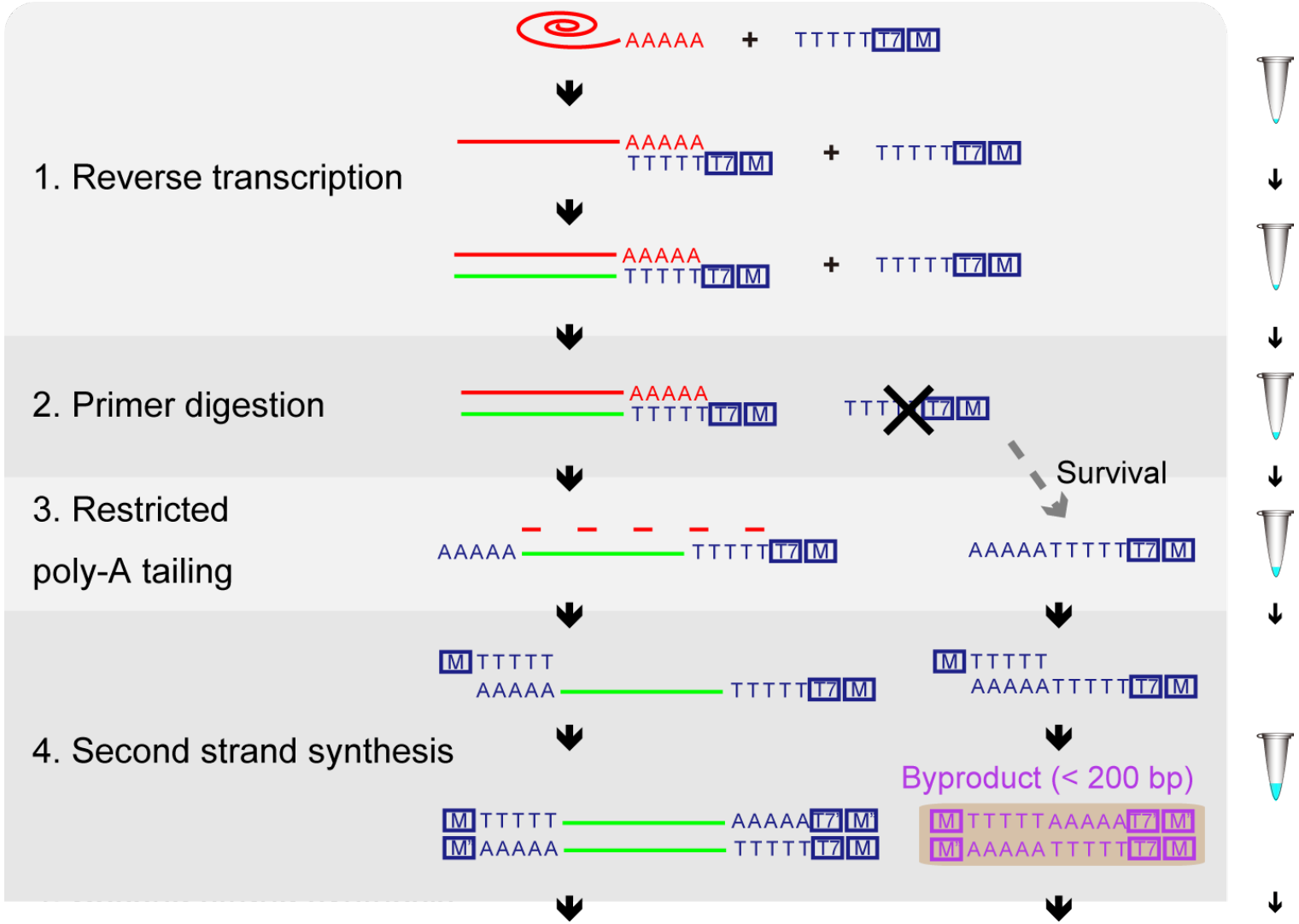
Single-tube での高効率なcDNA増幅のために

1. キャリーオーバー

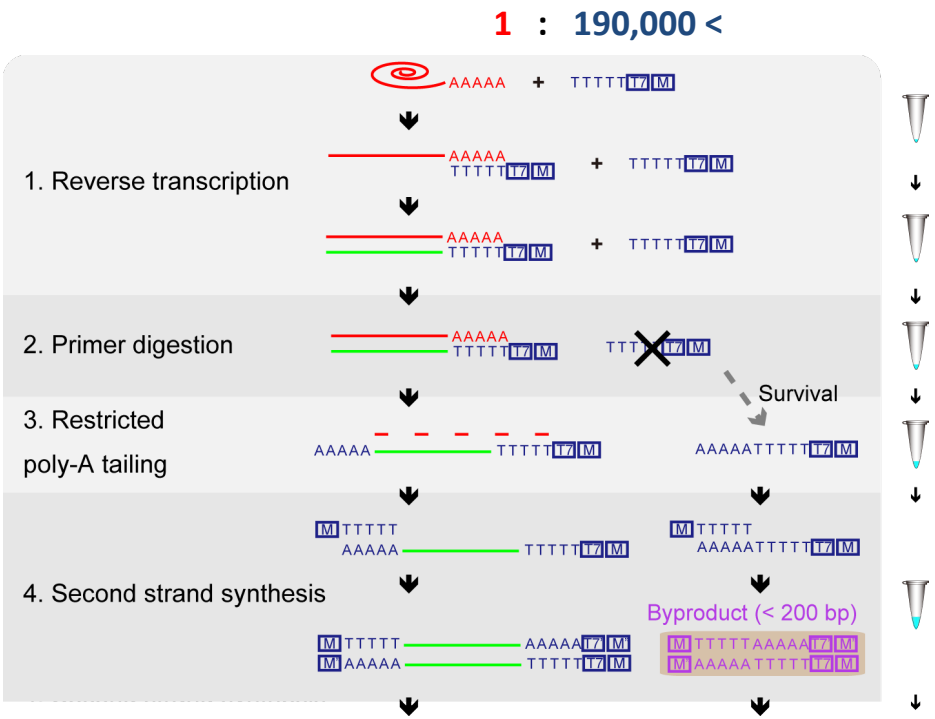
2. PCR byproduct

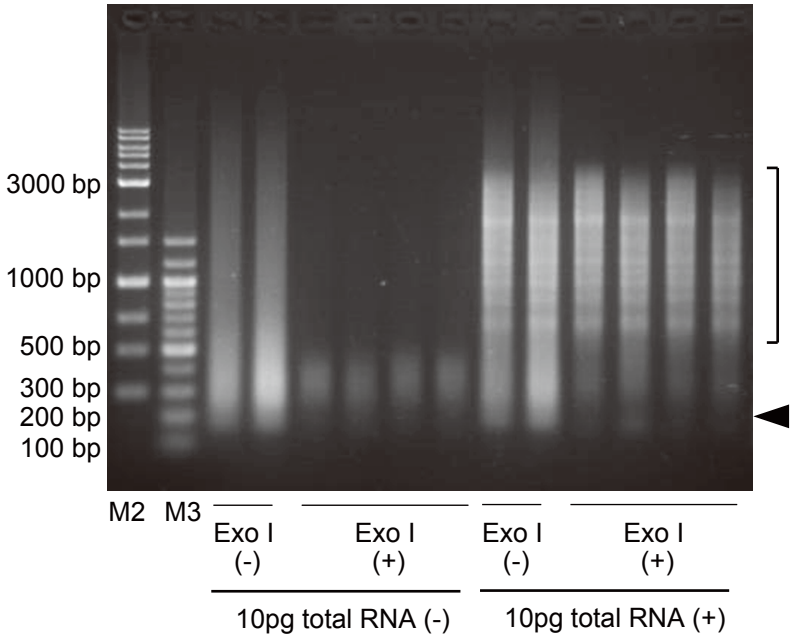
PCR byproduct
～できるメカニズム～

1 : 190,000 <

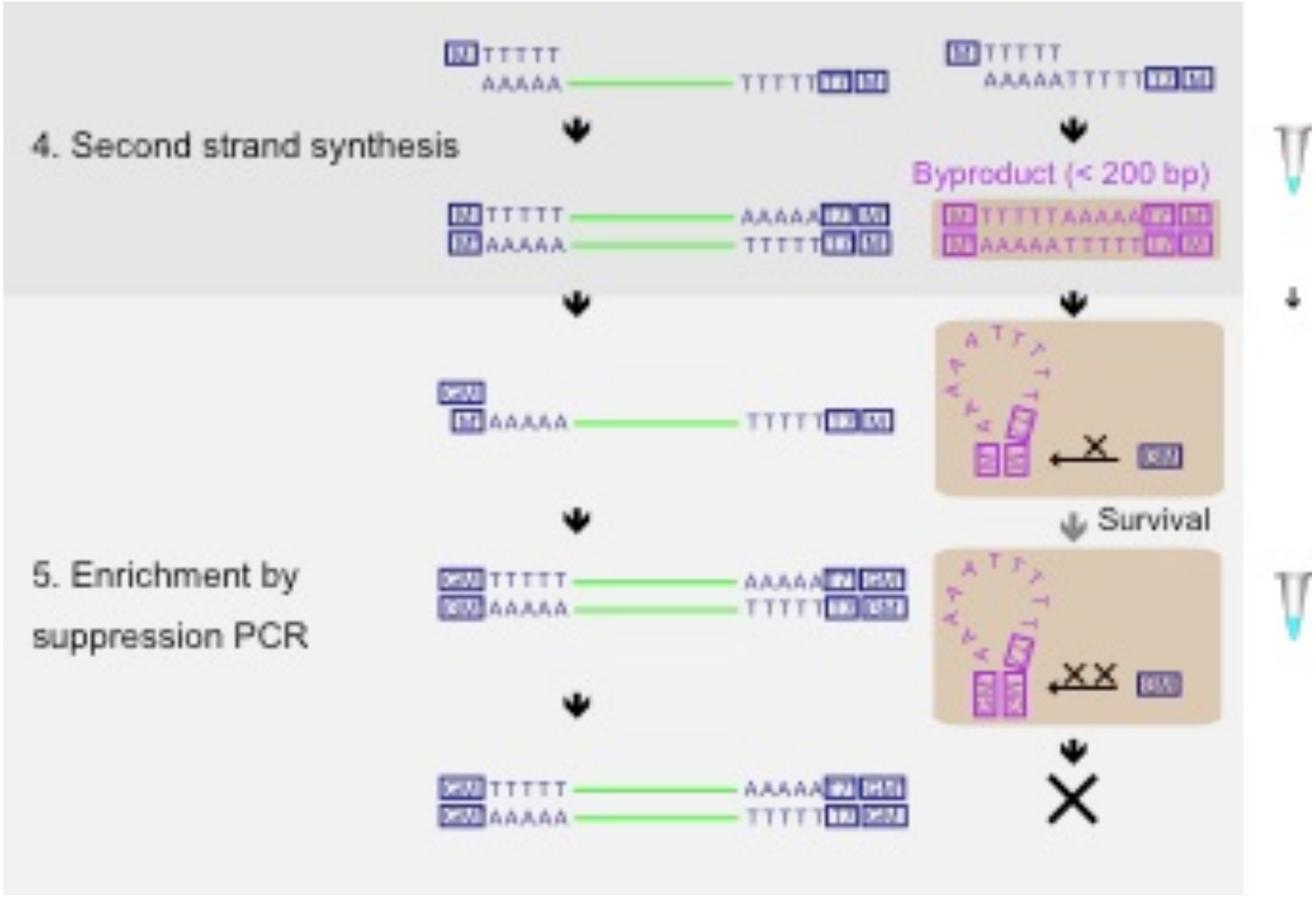


Byproduct
～プライマー分解だけでは足りない～

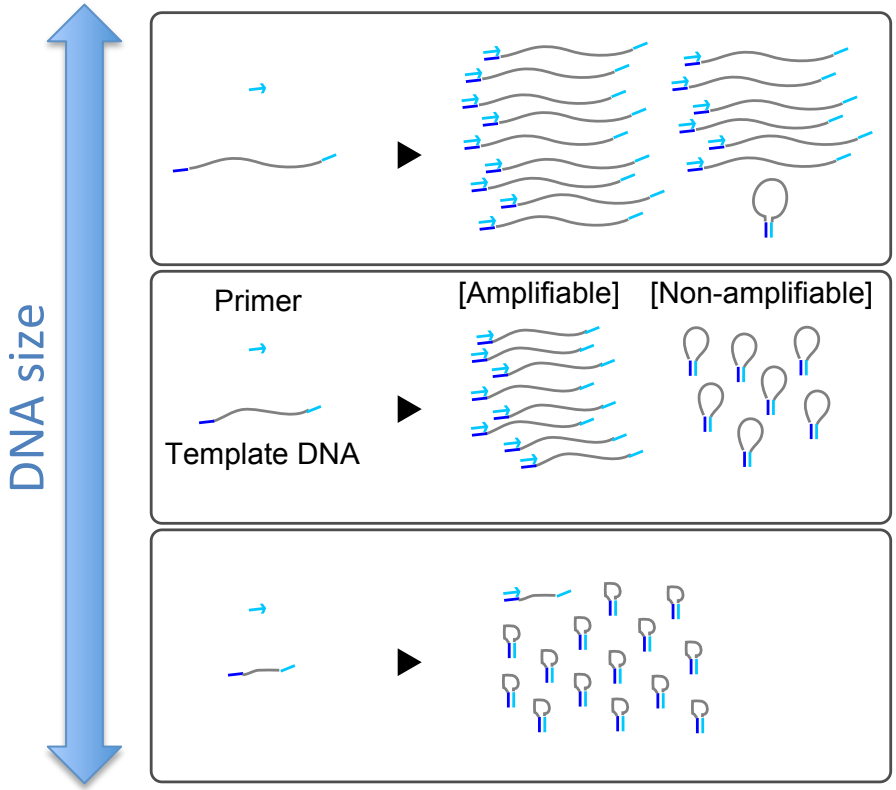


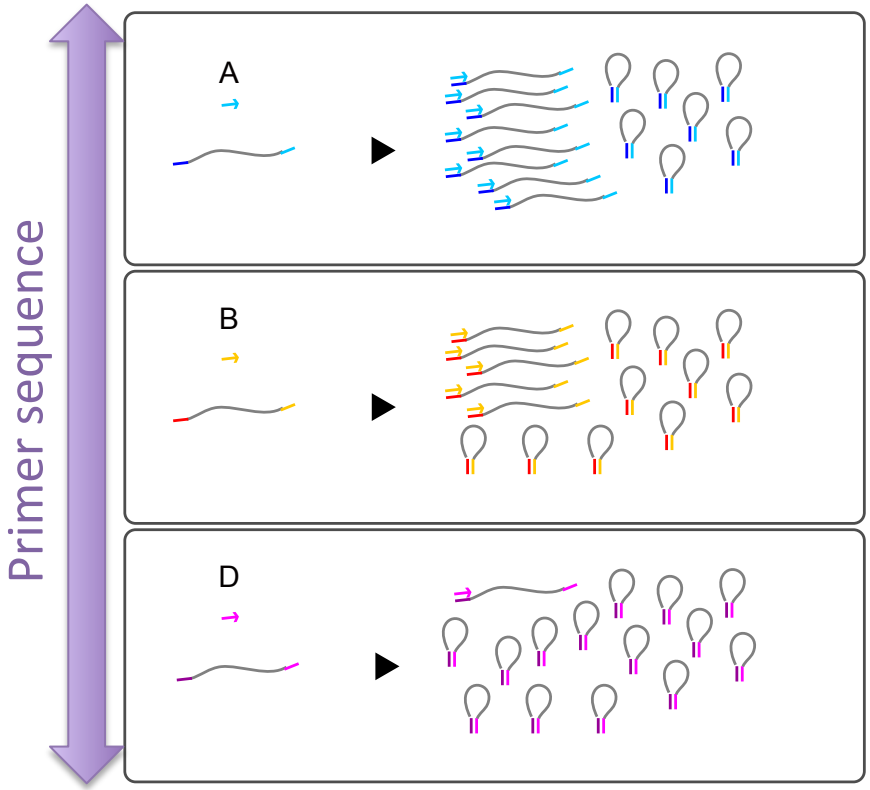


Byproduct
～Suppression PCRによるbyproduct増幅抑制～

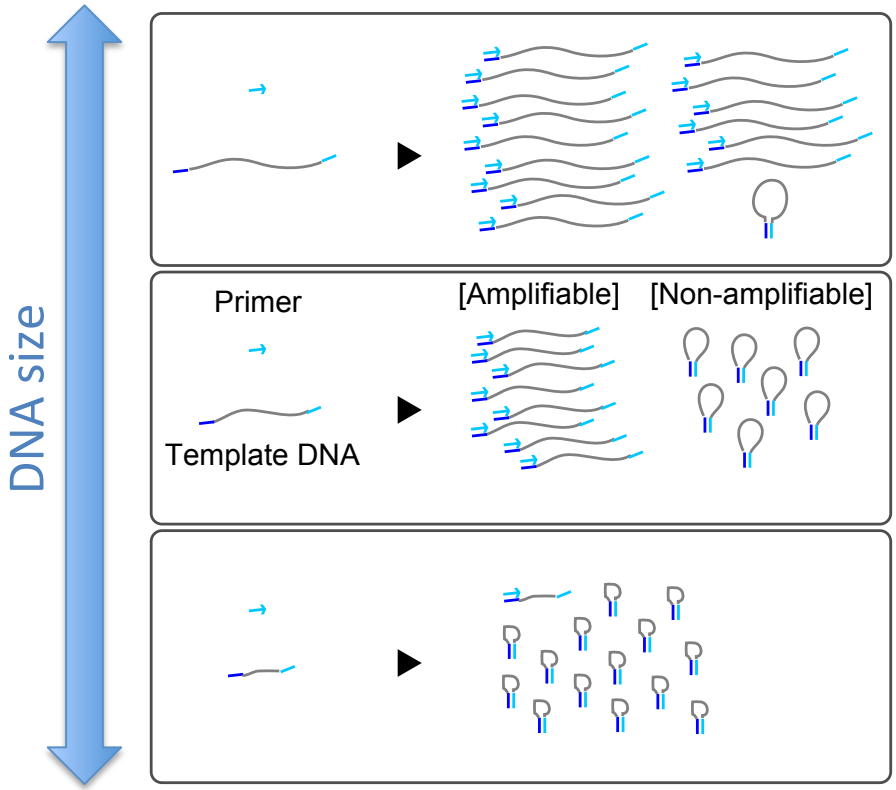


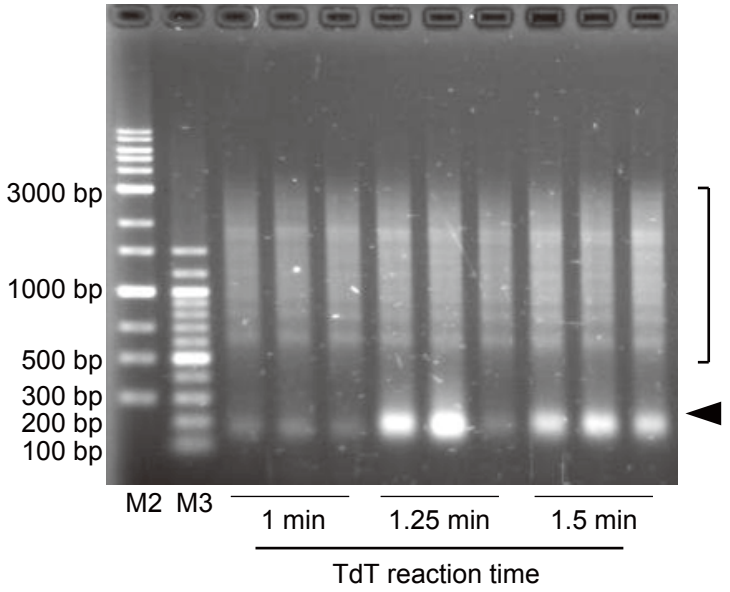
Byproduct
～Suppression PCRのパラメーター～

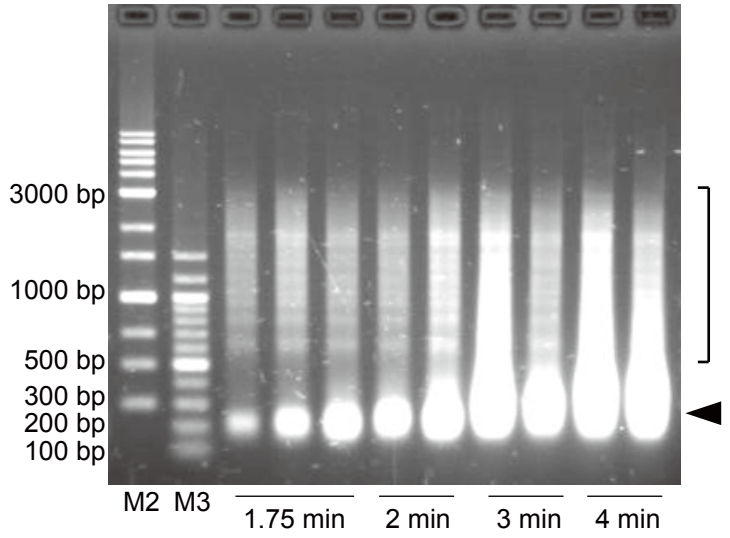




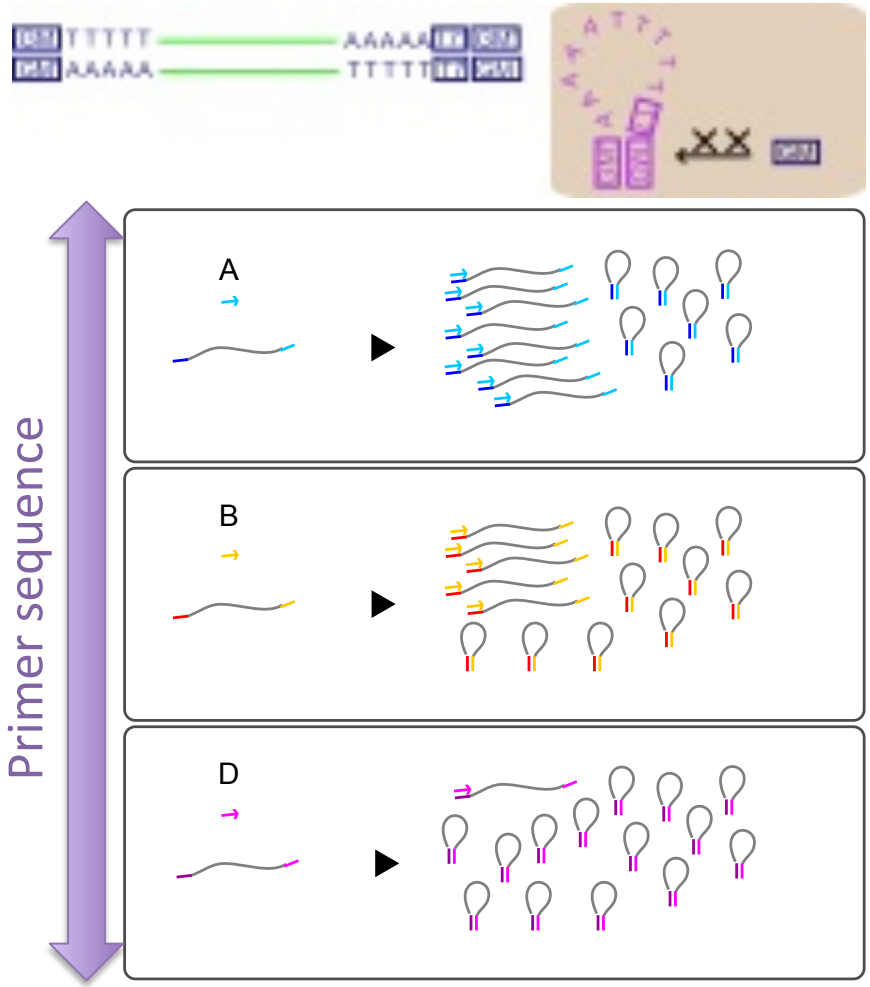
Byproduct
～DNAのサイズ効果～



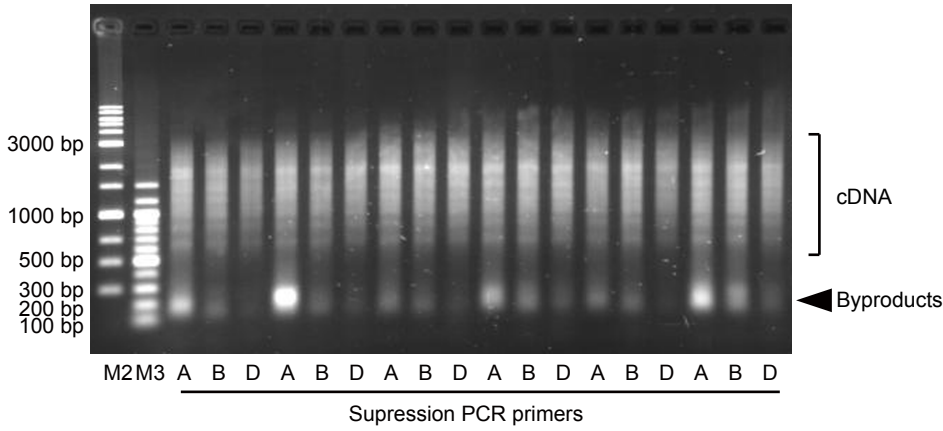


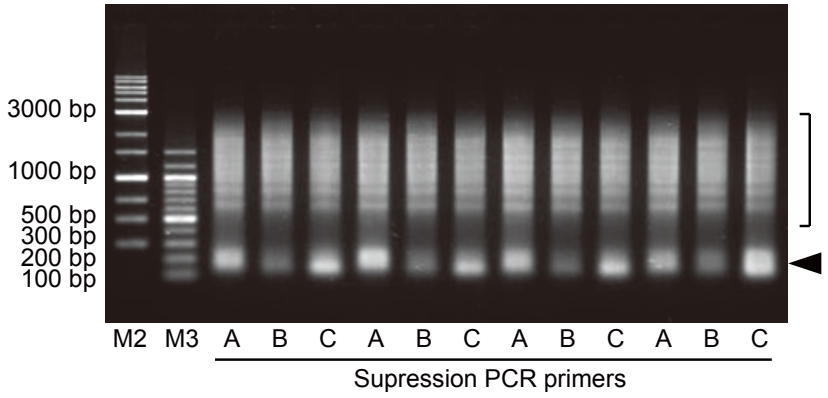


Byproduct
～Suppression PCRプライマーの効果～

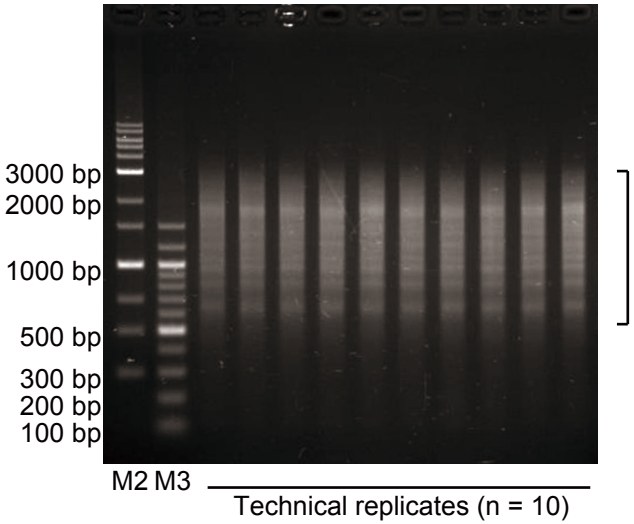


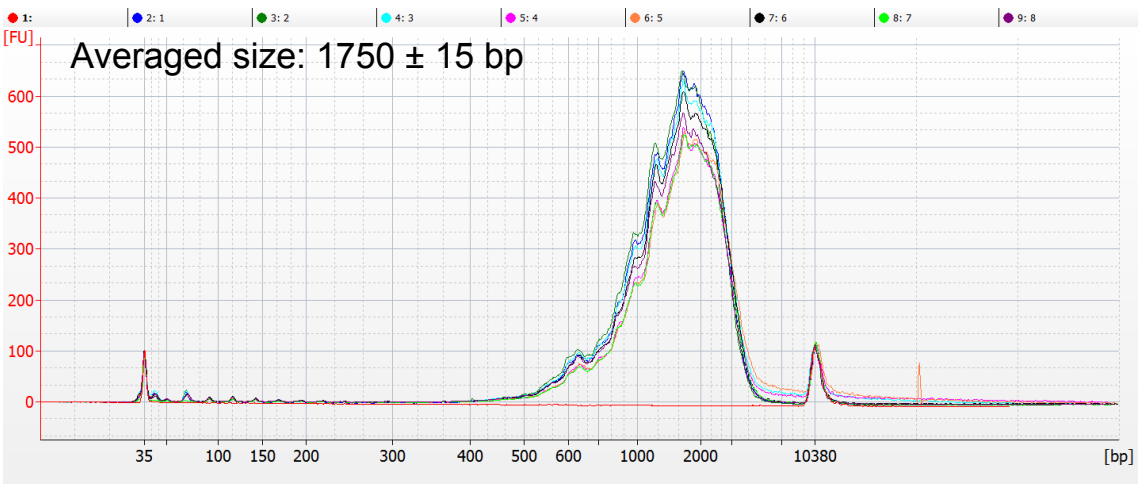
A: 5’ TATAGAATTCGCGGCCGCTCGCGAT 3’
B: 5’ GTATAGAATTCGCGGCCGCTCGCGAT 3’
C: 5’ CTATAGAATTCGCGGCCGCTCGCGAT 3’
D: 5’ TGTATAGAATTCGCGGCCGCTCGCGAT 3’



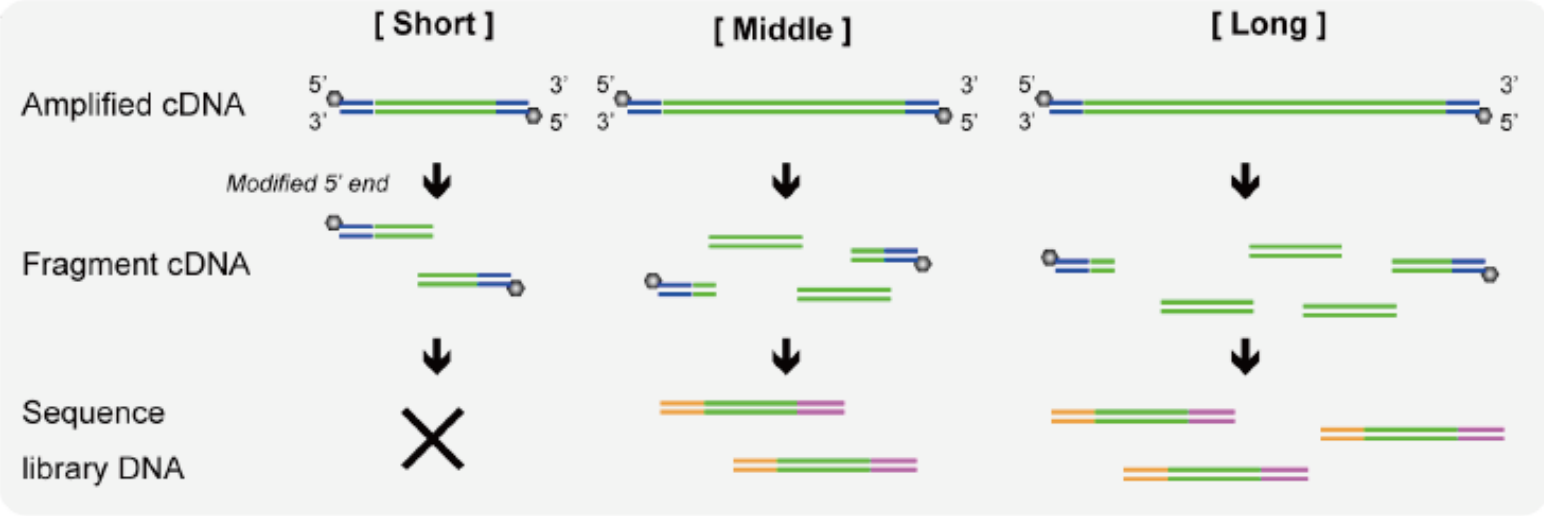


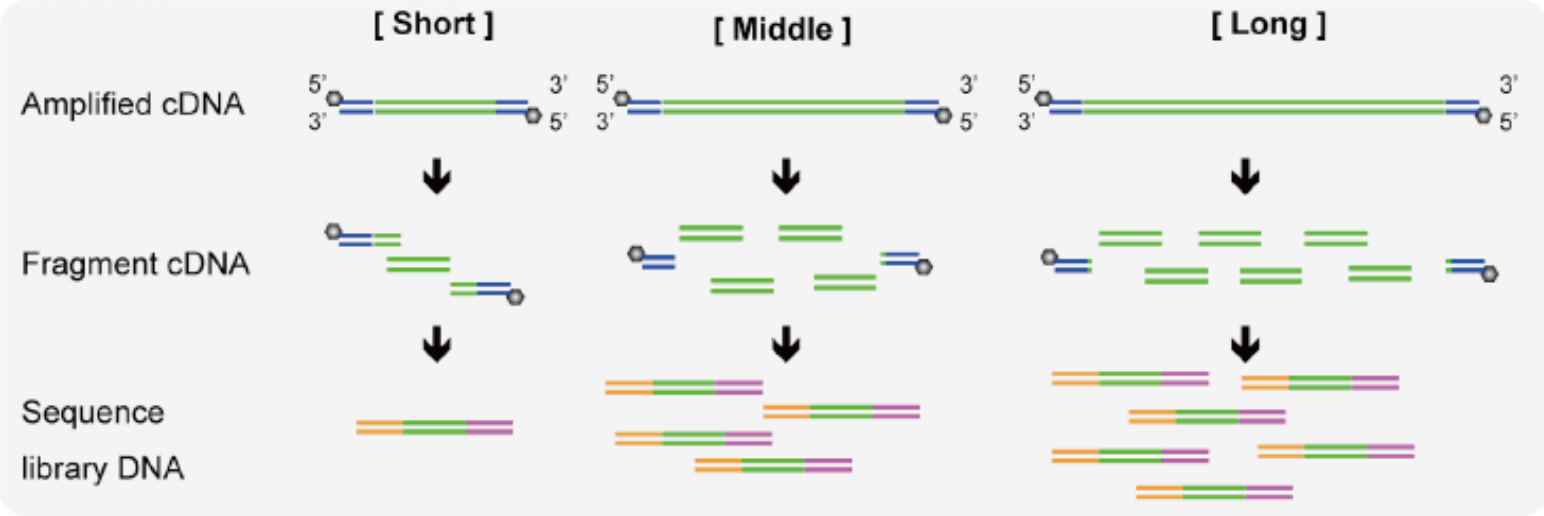
Byproduct
～複数の組み合わせでロバストに消す～





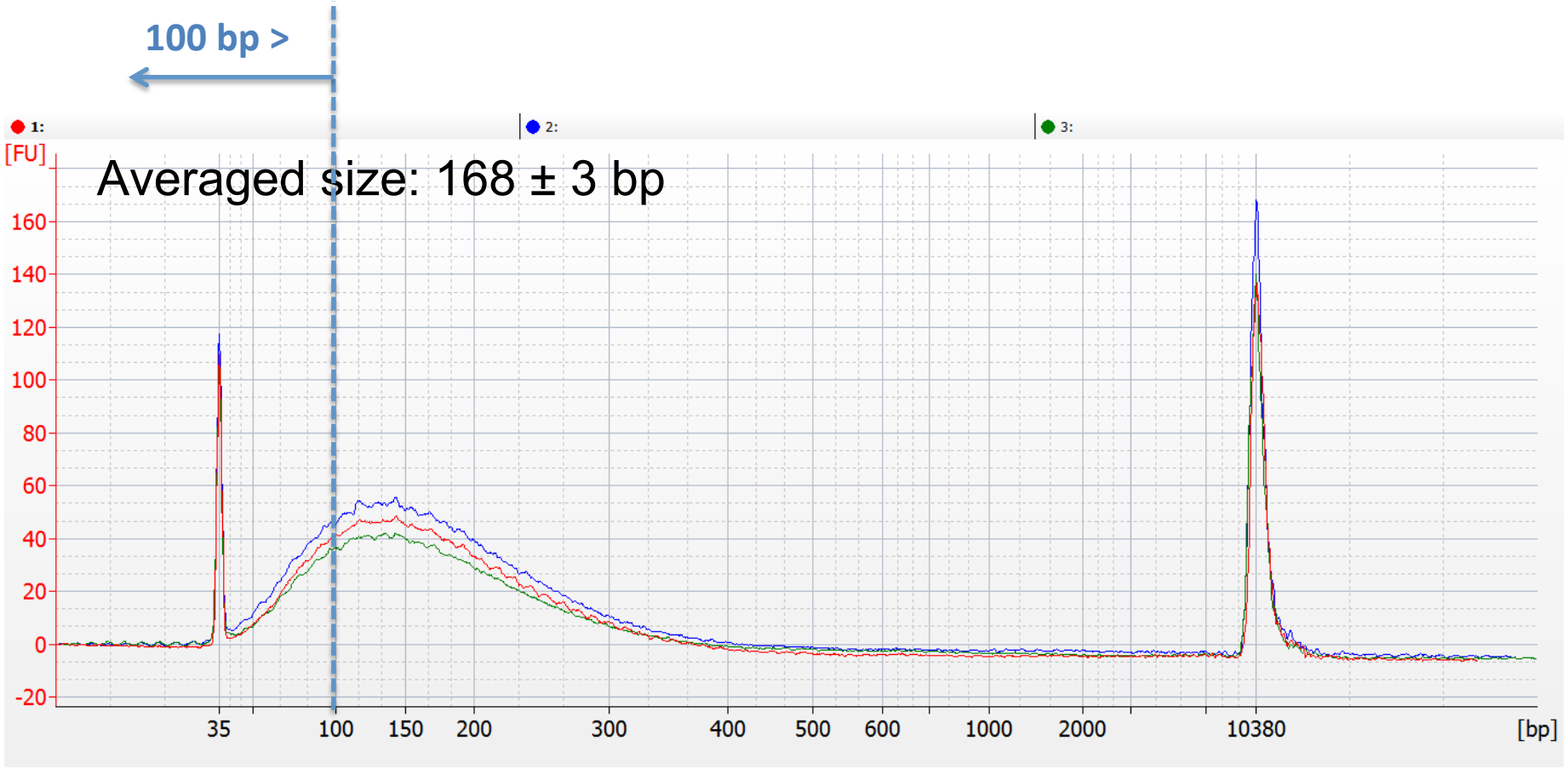
断片化DNAとライブラリ作製の関係





短いcDNAからは、有効なインサートが出にくい。

断片化DNAとライブラリ作製の関係



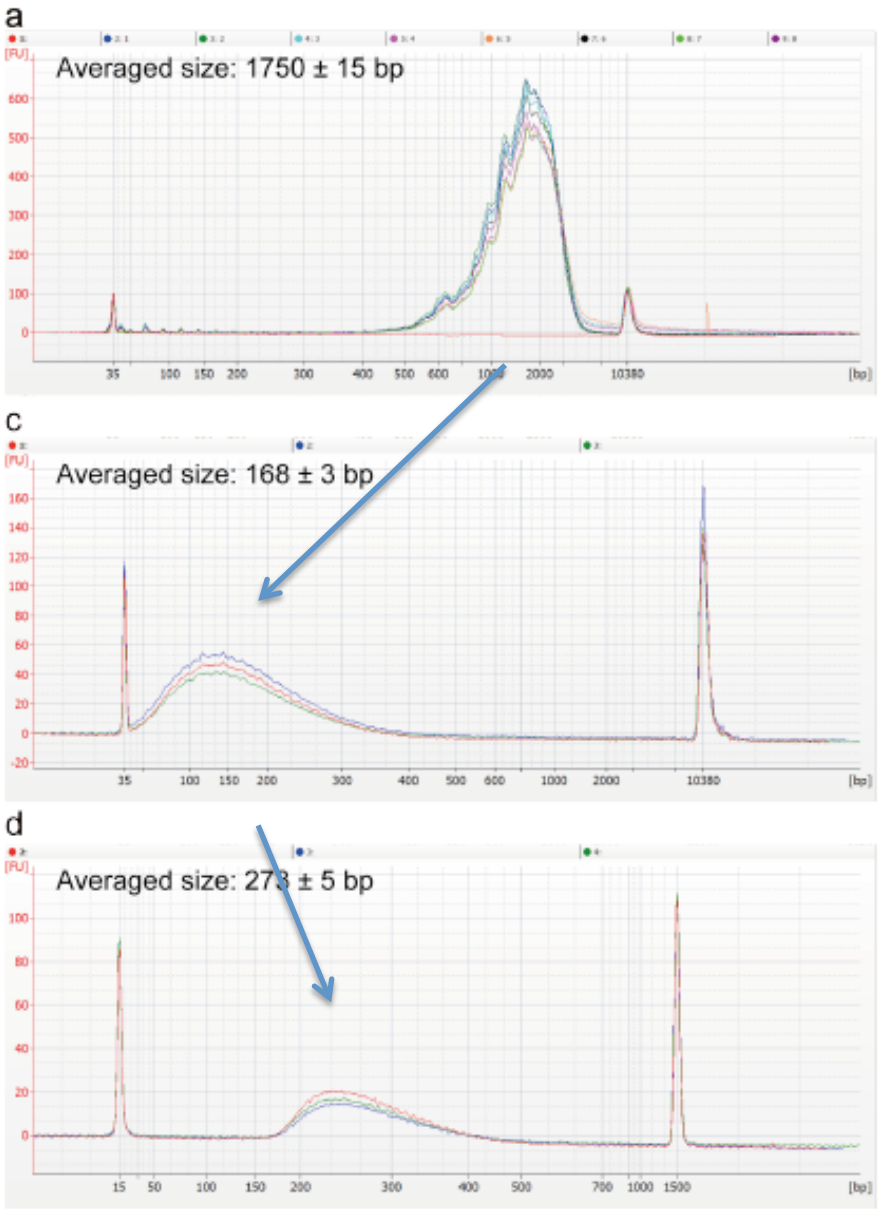
ライブラリ作製時に行うAmpure XPの精製により、100 bp以下は失われるので
Zymo DNA 5 などのカラムを使用する。もしくはPEGの濃度を変える。

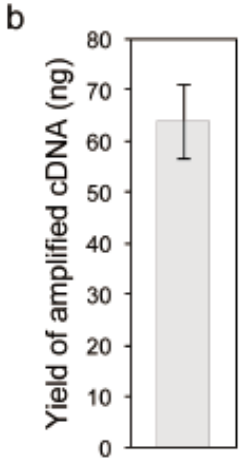
断片化DNAとライブラリ作製の関係

cDNA

断片化DNA

ライブラリDNA





RTのコントロール
～逆転写効率～